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The effect of dried ostrich meat in young rats' diet on selected hematological blood parameters

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The aim of the study was to investigate the effect of dried ostrich meat in the diet on the hematological blood parameters using the rat model. The study was carried out on 72 six-week-old male Wistar rats divided into 2 groups: rats fed for a month standard diet (SD) with iron level of 200 ppm and rats fed diet with 5ppm of iron (FeD) (n=36 rats in each group). Afterwards this period animals were classified as non- and iron deficient based on hemoglobin values and either of the 2 groups was further divided into 4 groups of 9 animals: receiving the SD, SD with ostrich meat, FeD or FeD with ostrich meat. Blood samples were collected to determine hematological parameters i.e. red blood cells (RBC), the hemoglobin (HGB), ferritin level and hematocrit (HCT). The ferritin and HCT levels in rats suffering from iron deficiency receiving SD with ostrich meat were significantly higher at the end of the experiment as compared to the beginning of it (91.90 ng/mL vs. 59.60 ng/mL (p=0.005) and 46.82 vs. 34.41% (p=0.05). Thus, ostrich meat may be a good source of heme iron and can be used as a supplement in diet of individuals with iron deficiencies.

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Two types of iron can be distinguished in food products: heme and non-heme iron. Heme iron is found mainly in red meat: beef, lamb, venison, as well as the more recently marketed, ostrich meat [Poławska *et al*. 2011] and in organs such as liver, brain, kidneys and heart [Uzel and Conrad 1998, Bermejo and Garcia-Lopez 2009] whereas, the latter is found in food of plant origin, for example: beans, lentils or nuts. Heme iron is better absorbed from the diet as compared to non-heme [Bermejo and Garcia-Lopez 2009]. Iron deficiency is associated with marked changes in blood counts. Deterioration of hemoglobin synthesis is caused by a decrease in the volume of erythrocytes. The newly formed blood cells are smaller in volume and often also abnormal in shape. The decrease of hemoglobin in the blood and serum is associated with the impairment of its formation. A reduced cell volume is characteristic of iron deficiency [Johnson-Wimbley and Graham 2011]. Iron levels can be determined by measuring the peripheral blood count, supplemented with tests assessing iron metabolism (iron level and ferritin concentration). The most important blood morphological parameters for diagnosis of iron deficiency are therefore: the number of red blood cells (RBC), hemoglobin (HBG), hematocrit (Ht), mean erythrocyte volume (MCV) and average hemoglobin content in the erythrocyte (MCH).

Ostrich meat is characterised by high content of iron (2.3- 4.0 mg/100 g) comparing to all other meat sources e.g. beef (1.7-2.0 mg/100 g), or chicken (0.4-0.7 mg/100 g) [Lombardi-Boccia *et al*. 2002, Karklina and Kivite 2007, Majewska *et al*. 2009]. Moreover, ostrich meat contains less sodium compared to other meats, as well as low intramuscular fat content and favourable fatty acids profile, which makes it a valuable meat of high nutritive and dietetic value [Horbańczuk *et al*. 1998, 2007, 2008, Sales and Horbanczuk 1998, Sales *et al*. 1999, Cooper and Horbańczuk 2004, Cooper *et al*. 2007, 2008, Kawka *et al.* 2007, Rybnik *et al*. 2007]. However, there is a lack of data regarding ostrich meat and its effect on the blood morphological indicators with the use of animal model. In the current study, we investigated the influence of the dried ostrich meat in the diet of young rats on their hematological blood parameters.

Material and methods

Animals

The study was carried out on 72 six-week-old male Wistar rats from the population maintained at the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences in Jastrzębiec. The animals were maintained in standard cages of the type IV with lids of the type IV made using non- ferrous metal (LID C III with divider (SS) (AnimaLab Company). All animals were kept at temperature 24±1.5°C under standard husbandry conditions with the 12 h of daylight and 12 h of darkness and with *ad libitum* access to food and water. All of the described experiments were

approved by the Local Ethical Commission. All procedures were performed according to the principles for the care and use of research animals and were approved by the III Local Bioethical Commission in Warsaw, Poland (No. 77/2015).

Diet

All animals (n=72) were divided into 2 groups of rats fed for a month with: standard diet (SD) with an iron level of 200 ppm and diet with 5ppm of iron (FeD) (n=36 rats in each group). After this period animals were classified as without and with iron deficiency based on haemoglobin values. Blood was drained from the tail vein. Hemoglobin (HGB) standard range for 8-16 week old male rats is 13.7 g/dL-17.6g/Dl [Giknis and Clifford 2008], therefore, rats with haemoglobin below the former level were classified as with iron deficiency. Either of the 2 groups (non-iron-deficient and iron-deficient) was further divided into 4 groups of 9 animals: receiving for four weeks the SD, SD with ostrich meat, FeD or FeD with ostrich meat. Two types of feed with different levels of iron were used in the experiment. Feed was produced by Altromin Spezialfutter GmbH & Co. Rats in dietary groups supplemented with ostrich meat received 20% of dried ostrich meat. Rats were kept in cages without metal pieces (to eliminate exogenous source of iron) and received water without iron ions.

Blood analysis

From all rats blood was collected twice; at the beginning and in the end of the experiment in each of the subgroups. From the collected blood we performed morphological analysis of blood cells as well as we determined iron and ferritin level. Tests were performed on fresh whole blood or serum blood. Samples were collected from rats in order to determine hematological parameters such as: red blood cells (RBC), the hemoglobin (HGB), iron and ferritin level, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), iron (Fe) and ferritin. The following hematological parameters: RBC, HGB, HCT, MCV and MCH were determined using veterinary analyzer (Abacus Vet 5, H-1097 Budapest, Hungary). Ferritin and iron levels were estimated using COBAS INTEGRA® 400 plus system (Roche Diagnostics Ltd., Rotkreuz, Switzerland).

Statistical analysis

A generalized linear mixed-model analysis (repeated measures ANOVA) was performed on all measured blood morphology parameters in order to determine the fixed effect of iron levels in rats, diet and the time of sample collection, as a repeated measure, as well as their interactions. There were no outliers present in the dataset. Normality and homogeneity of residual variance assumptions were checked using the Shapiro test and examination of the normal plot, and these were met by all variables under investigation. PROC GLIMMIX of SAS v 9.4 (SAS Institute Inc., Cary, NC, USA) including the Tukey's adjustment option was used to conduct the analysis. The validity of the models was tested using Akaike's information criterion. The least square means

for all significant effects in the models ($p \leq 0.05$) were computed using the LSMEANS option. For all analyses, results are reported as means and standard deviation (STD) of the mean.

Results and discussion

In developing countries over 50% of children and pregnant women suffer from iron deficiency [Uzel and Conrad 1998]. This condition is also present in developed countries [Johnson-Wimbley and Graham 2011, Killip *et al*. 2007]. The most frequent causes of iron deficiency are heme oglobinopathies, infections, deficiencies of micronutrients (e.g., iron, folate, vitamin B12), cancer and gastrointestinal pathology, such as bowel disease [Killip *et al*. 2007, Johnson-Wimbley and Graham 2011, Saunders *et al.* 2013, Pasricha *et al*. 2021], blood loss due to menstrual periods and gastrointestinal bleeding, but also restricted diets [Gisbert *et al*. 2009, Johnson-Wimbley and Graham 2011].

The Wistar rat (*Rattus norvegicus*) is often used as experimental animal in biomedical research because physiologically it has high similarities with human body [Bogunjoko *et al.* 1983, Alferez *et al.* 2011, Ghasemiet *et al*. 2021, Yeung *et al.* 2021]. We investigated the effect of diet supplemented with dried ostrich meat on blood morphological parameters of rats with induced iron deficiency. To our knowledge this was the first study to show how ostrich meat in the diet affects the hematological blood parameters using animal model of rats with iron deficiency.

In the current study in rats with iron deficiency fed with iron deficient diet all the parameters remained constant from the start till the end of experiment remaining out of the optimal range during the experiment, as was presented in Table 1. On the other hand, in rats with iron deficiency fed with standard diet and ostrich meat, significant differences were observed in RBC, HGB, HCT, MCV and MCH. In iron deficient rats receiving iron deficient diet with ostrich meat significant differences were observed in all morphological subgroups except Fe level. In both of those groups provided with ostrich meat significant increase from the onset of the experiment to its termination was observed for all affected parameters, except for RBC. In healthy male rats of 8-16 weeks old the ranges of the investigated parameters are as following: RBC (7.27-9.65 $10^6/\mu L$), HGB (13.7-17.6 g/dL), HCT (39.6-52.5%), MCV (48.9-57.9 fL), MCH (17.1-20.4 pg) [Giknis and Clifford 2008]. An increase in the value of MCV observed in iron deficient rats in all dietary treatment groups except for those fed only with the standard diet, may indicate an increase in the plasma volume. A similar result was observed in animals previously treated with iron-ferritin isolate [Zielińska-Dawidziak *et al*. 2012, 2014].

In the current study ferritin levels significantly increased during this study in iron-deficient rats fed with the iron deficient diet with ostrich meat (59.60 ng/mL vs. 91.90 ng/mL, $p=0.005$). Ferritin is a major iron-storage protein and is present not only in cells but also in sera, and the serum ferritin level is positively correlated with body iron stores [Watanabe *et al*. 2001]. Iron can be utilized by target cells or stored, bound

Table 1. Blood morphological parameters in none iron-deficient and iron deficient rats depending on the diet fed

Effect		Time of sampling during experiment	RBC(10^{12}/L)		HGB (g/dL)		HCT (%)		MCV (fl)		MCH (pg)		Ferritin(ng/mL)		Fe (µmol/L)	
			mean	STD	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD
None iron-deficient rats	standard diet (SD)	beginning	7.37	0.65	14.8^{A}	1.38	46.95^{A}	3.93	63.5^{Aa}	1.05	20.07^{Aa}	0.38	44.70	16.36	38.72	6.03
		end	7.9^{B}	0.36	15.15	0.97	46.82	2.77	59.25^{Ab}	2.49	19.18b	0.59	45.6^{A}	13.68	34.6^{A}	2.13
	iron deficiency diet (FeD)	beginning	7.3^{Ba}	0.35	14.94^{A}	1.21	45.92^{Aa}	2.91	$63A^{a}$	1.22	20.46^{Aa}	1.39	59.9^{Aa}	17.21	40.68^{A}	3.74
		end	7.09^{Bb}	1.02	12.94^{A}	1.72	39.48^{Ab}	5.10	55.75^{Ab}	1.67	18.33^{Ab}	0.71	26.81^{b}	16.14	36.8^{A}	7.13
	standard diet and ostrich meat (SDO)	beginning	7.85	0.14	14.56	0.30	46.67	0.80	59.95	0.98	18.69	0.28	55.80	13.33	41.30	10.17
		end	7.61	0.66	14.11	1.37	44.85	3.73	59.25	2.55	18.61	0.88	51.10	12.62	37.60	6.34
	iron deficiency diet and ostrich meat (FeDO)	beginning	7.81	0.16	15.09^{a}	0.39	45.72^{a}	1.48	58.07	1.49	19.16	0.33	16.9^{Bb}	25.14	33.90	7.78
		end	7.49	0.22	14.44^{b}	0.80	43.27^{b}	2.47	57.78	2.28	19.29	0.68	45.5^{a}	13.22	39.90	7.41
Iron-deficient rats	standard diet (SD)	beginning	9.01^{a}	0.68	13.32^{B}	0.70	31.96^{Bb}	3.58	35.41^{Bb}	1.24	14.81^{Bb}	0.37	42.6^{a}	3.41	35.04a	2.33
		end	8.36^{Ab}	0.47	15.34	0.72	46.32^{a}	2.04	55.5^{Ba}	2.78	18.4^{a}	1.12	29.5^{Bb}	2.51	27.7^{Bb}	5.10
	iron deficiency diet (FeD)	beginning	9.35^{A}	0.52	10.28^{B}	0.78	27.47^{B}	1.34	29.44^{B}	2.13	10.99^{B}	0.94	42.6^{B}	3.41	35.04^{B}	2.33
		end	9.31^{A}	0.91	10.44^{B}	1.04	29.35^{B}	3.38	31.56^{B}	3.47	11.23^{B}	1.13	42.60	3.41	35.04^{B}	2.33
	standard diet and ostrich meat (SDO)	beginning	9.5^{a}	0.91	13.67^{b}	1.04	34.01^{b}	3.38	35.91^{b}	3.47	14.31^{b}	1.13	37.40	11.64	33.84	5.35
		end	7.98^{b}	0.58	15.49^{a}	0.60	47.32^{a}	2.48	59.38^{a}	2.67	19.49^{a}	1.53	39.90	15.30	34.10	2.40
	iron deficiency diet and ostrich meat (FeDO)	beginning	10.46^{a}	0.20	12.76^{b}	0.19	34.41^{b}	0.95	32.26^{b}	1.31	12.11^{b}	1.20	59.6^{b}	13.99	32.8^{b}	6.30
		end	9.15^{b}	0.66	16.26^{a}	1.19	46.82^{a}	3.22	51.13^{a}	3.23	17.76^{a}	0.71	91.9^{Aa}	20.25	37.9^{a}	6.10

HGB – hemoglobin; RBC – red blood cell count; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; Fe – iron.
Mean values bearing different small letters (a, b) indicate statistically significant differences at ($p \leq 0.05$) between the times of the sampling within rat group and diet fed.
Mean values bearing different capital letters (A, B) indicate statistically significant differences at ($p \leq 0.05$) between rat groups within the sampling time and diet fed.

to ferritin, mainly in the liver. Thus, ferritin is a good indicator of total body iron, being an acute phase protein, it can be increased in inflammatory states. As the rats did not show any signs of compromised health over the course of experiment, we attribute this raise to the presence of ostrich meat in the animals' diet.

Overall, in the iron-deficient rats fed with the standard diet with ostrich meat all blood morphology parameters returned to the optimal levels of the healthy animals. In rats with iron deficiency fed with standard diet and ostrich meat but also iron deficiency diet with ostrich meat such parameters as HCT, MCV, MCH, RBC and ferritin returned at the end of experiment to optimal range (Tab. 1). Similar tendencies regarding blood hematological was obtained by Zhang *et al.* [1988], where the bioavailability of iron in meat occurred to be better than from spinach (*Spinacea oleracea L.*). Also Sun *et al*. [2022] obtained comparable results regarding some hematological parameters like hemoglobin. No significant effect of the iron-deficient diet on iron deficient rats blood morphological parameters was observed between beginning and the end of experiment.

In the non iron-deficient group of rats fed with standard diet MCV and MCH levels decreased during the experiment, while in non iron-deficient group of rats fed with the iron-deficient diet the significant decrease was observed in the level of the following parameters: RBC HCT, MCV, MCH and in the ferritin level. Rats without iron deficiency receiving iron deficient diet with dried ostrich meat, presented optimal iron levels, but lower ferritin levels when comparing the end to the beginning of the experiment (45.50 ng/mL vs.16.90 ng/mL, p=0,022).

In turn, no significant effect of the standard diet with ostrich meat was observed on non iron deficient rats' blood morphological parameters (Tab. 1).

In conclusion, in our study, we showed that dried ostrich meat can be used as a good source of heme iron. However, it is important to consider that to estimate the Fe availability of meat one must not only take account the concentration and type of each Fe-containing compound present, but also their possible synergistic effects, the presence of chelating agents or extent of cooking.

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