

White Stork (*Ciconia ciconia*) Nestlings Affected By Agricultural Practices? Assessment Of Integrated Biomarker Responses.

Dora Bjedov ¹, Mirna Velki ², Lucija Sara Kovačić ², Lidija Begović ², Ivan Lešić ², Luka Jurinović ³, Tibor Mikuska ⁴, Mirta Sudarić Bogojević ², Sandra Ečimović ², and Alma Mikuška ^{2,*}

Table S1. Detailed results of pairwise *post-hoc* statistical analysis of the adopted models, performed with *emmeans* package in R (version 4.2.3.). Fixed effects are variables *biomarker response* and *sampling location*, while random effect is the variable *nest*. *P* value was adjusted by Tukey method. Significant comparisons are bolded.

			estimate	SE	DF	t.ratio	<i>p</i> value
ChE	Plasma	Baranja - Jakuševac	3.20	2.54	49	-1.258	0.4253
		Baranja - Srijem	-4.66	2.00	49	-2.332	0.0607
		Jakuševac - Srijem	-7.86	2.63	49	-2.985	0.0121
CES	Plasma	Baranja - Jakuševac	4.44	4.13	49	1.074	0.5345
		Baranja - Srijem	3.02	3.42	49	0.882	0.6539
		Jakuševac - Srijem	-1.42	4.33	49	-3.27	0.9427
	S9	Baranja - Jakuševac	0.45	0.45	50	0.987	0.5881
		Baranja - Srijem	-0.30	0.35	50	-0.856	0.6702
		Jakuševac - Srijem	-0.75	0.46	50	-1.607	0.252
GST	Plasma	Baranja - Jakuševac	0.51	0.88	48	0.577	0.8992
		Baranja - Srijem	1.02	0.74	48	1.38	0.3594
		Jakuševac - Srijem	0.51	0.93	48	0.553	0.8455
	S9	Baranja - Jakuševac	0.93	1.51	49	0.618	0.8109
		Baranja - Srijem	0.24	1.24	49	0.193	0.9796
		Jakuševac - Srijem	-0.69	1.56	49	-0.446	0.8964
GR	Plasma	Baranja - Jakuševac	10.40	25.80	48	0.404	0.9141
		Baranja - Srijem	53.20	21.40	48	2.484	0.0429
		Jakuševac - Srijem	42.80	26.90	48	1.594	0.258
	S9	Baranja - Jakuševac	-77.10	99.80	50	-0.773	0.7212
		Baranja - Srijem	-182.10	80.60	50	-2.26	0.071
		Jakuševac - Srijem	-104.90	102.00	50	-1.028	0.5627
GSH	Plasma	Baranja - Jakuševac	-189	296	48	-0.638	0.8002
		Baranja - Srijem	399	246	48	1.62	0.247
		Jakuševac - Srijem	588	308	48	1.905	0.1482
	S9	Baranja - Jakuševac	890	827	47	1.076	0.5336
		Baranja - Srijem	859	698	47	1.23	0.4419
		Jakuševac - Srijem	-31	870	47	-0.036	0.9993
ROS	Plasma	Baranja - Jakuševac	-6.66667	3.96	50	-1.682	0.2221

	Baranja - Srijem	-6.65789	3.23	50	-2.059	0.109
	Jakuševac - Srijem	0.00877	4.11	50	0.002	1.000
S9	Baranja - Jakuševac	-12.939	6.02	51	-2.149	0.0901
	Baranja - Srijem	0.544	4.84	51	0.112	0.9931
	Jakuševac - Srijem	13.483	6.20	51	2.176	0.085

ChE – cholinesterase; CES – carboxylesterase; GST – glutathione S-transferase; GR – glutathione reductase; GSH – glutathione; ROS – reactive oxygen species; estimate – estimate of the size effect, i.e. the difference between the estimated marginal means; SE – standard error; DF – degrees of freedom; t.ratio – test statistic to generate the *p* value.

Table S2. Results of molecular sex determination in white stork (*Ciconia ciconia*) nestlings' blood and biomarker response in females and males for each sampling location – Jakuševac, Baranja and Srijem.

		Jakuševac				Baranja				Srijem			
		Females		Males		Females		Males		Females		Males	
<i>n</i>		6		3		12		11		7		12	
		mean	<i>SD</i>	mean	<i>SD</i>	mean	<i>SD</i>	mean	<i>SD</i>	mean	<i>SD</i>	mean	<i>SD</i>
ChE	Plasma	28.38	12.39	28.86	0.65	26.89	4.50	35.11	7.62	29	7.99	35	7.62
(nmol min ⁻¹ mg _{PROT} ⁻¹)	S9	4.79	1.42	4.68	2.02	4.38	1.35	4.55	2.25	4	1.15	5	2.25
CES	Plasma	33.27	21.38	33.42	7.97	36.87	8.85	39.66	12.67	33	8.25	40	12.67
(nmol min ⁻¹ mg _{PROT} ⁻¹)	S9	8.71	2.20	7.77	0.37	8.30	1.34	8.50	1.31	8	0.81	8	1.31
GST	Plasma	13.57	2.99	12.97	2.59	13.04	1.90	12.29	1.54	13	3.10	12	1.54
(nmol min ⁻¹ mg _{PROT} ⁻¹)	S9	13.49	3.40	14.41	4.05	14.64	4.37	13.63	5.44	14	4.46	14	5.44
GR	Plasma	252.30	80.06	293.42	102.37	319.87	118.95	233.74	56.73	230	99.76	234	56.73
(pmol min ⁻¹ mg _{PROT} ⁻¹)	S9	957.08	337.47	802.88	209.49	808.62	289.84	1000.93	230.87	989	195.53	1001	230.87
GSH	Plasma	4451.06	1114.80	3459.78	1220.64	4170.51	1299.04	3586.88	446.30	3388	779.64	3587	446.30
(RFU)	S9	21028.33	2147.23	20814.83	2380.48	22254.23	1360.51	21189.09	2806.66	22040	1115.05	21189	2806.66
ROS	Plasma	120.89	10.69	109.89	20.38	112.49	9.54	120.03	7.49	118	9.20	120	7.49
(RFU)	S9	47.17	10.80	57.22	27.69	39.38	15.78	39.75	17.18	33	17.11	40	17.18

ChE – cholinesterase; CES – carboxylesterase; GST – glutathione S-transferase; GR – glutathione reductase; GSH – glutathione; ROS – reactive oxygen species; RFU – relative fluorescence; *n* – number of nestlings, *SD* – standard deviation.

Table S3. Descriptive statistics of esterase activity and oxidative stress biomarkers from two blood fractions (plasma and S9) analysed in white stork (*Ciconia ciconia*) nestlings from landfill Jakuševac and agricultural areas Baranja and Srijem during breeding seasons 2020^a, 2021^b and 2022^c.

			Jakuševac			Baranja			Srijem		
			2020	2021	2022	2020	2021	2022	2020	2021	2022
ChE (nmol min ⁻¹ mg _{PROT} ⁻¹)	Plasma	<i>n</i>	NA	8	10	18	17	24	25	29	19
		min.	NA	20.39	16.22	7.45	17.03	19.61	11.87	15.62	21.20
		max.	NA	30.57	51.51	31.10	61.98	37.56	36.87	47.55	47.47
		range	NA	10.18	35.29	23.65	44.95	17.95	25.00	31.93	26.26
	S9	<i>n</i>	NA	8	10	16	15	24	23	27	20
		min.	NA	1.28	3.29	1.62	0.45	2.65	2.11	1.04	2.37
		max.	NA	4.47	7.00	5.99	10.40	8.02	7.84	8.54	10.03
		range	NA	3.18	3.70	4.37	9.94	5.36	5.73	7.51	7.66
CES (nmol min ⁻¹ mg _{PROT} ⁻¹)	Plasma	<i>n</i>	NA	8	10	18	17	24	25	30	19
		min.	NA	20.47	10.22	8.04	17.22	17.78	10.67	22.14	19.69
		max.	NA	79.10	62.77	48.22	40.46	53.44	55.44	68.77	67.80
		range	NA	58.63	52.55	40.18	23.24	35.66	44.77	46.63	48.11
	S9	<i>n</i>	NA	7	10	17	11	24	25	24	20
		min.	NA	2.06	6.67	2.53	4.42	6.38	2.75	1.00	6.64
		max.	NA	6.31	12.84	17.72	9.10	10.65	16.03	9.24	11.42
		range	NA	4.25	6.18	15.19	4.68	4.28	13.28	8.24	4.78
GST (nmol min ⁻¹ mg _{PROT} ⁻¹)	Plasma	<i>n</i>	NA	7	10	18	16	24	25	30	17
		min.	NA	6.36	9.87	8.59	4.42	9.39	10.24	3.97	9.30
		max.	NA	8.98	17.22	49.60	10.18	18.77	52.54	12.17	17.49
		range	NA	2.63	7.35	41.01	5.76	9.38	42.30	8.20	8.19
	S9	<i>n</i>	NA	8	10	16	16	24	23	27	19
		min.	NA	18.98	8.30	8.54	4.64	8.60	8.93	14.44	7.06
		max.	NA	54.42	17.90	19.57	78.17	25.22	26.23	109.40	25.52
		range	NA	35.44	9.61	11.03	73.53	16.62	17.30	94.93	18.46
GR (pmol min ⁻¹ mg _{PROT} ⁻¹)	Plasma	<i>n</i>	NA	8	10	16	12	24	24	24	19
		min.	NA	91.13	141.50	167.60	25.58	158.30	107.10	63.75	144.50
		max.	NA	267.70	364.70	972.20	407.70	605.80	573.00	605.70	431.60
		range	NA	176.60	223.20	804.60	382.10	447.50	466.00	542.00	287.10
	S9	<i>n</i>	NA	8	10	16	15	23	24	27	20
		min.	NA	275.20	535.40	558.30	113.00	479.50	371.30	472.60	556.40
		max.	NA	1711.00	1534.00	1439.00	4886.00	1543.00	3671.00	3105.00	1623.00
		range	NA	1436.00	998.20	881.00	4773.00	1064.00	3300.00	2633.00	1067.00
GSH (RFU)	Plasma	<i>n</i>	NA	8	10	17	17	24	24	29	18
		min.	NA	4535	2062	4722	2138	2940	7557	2685	2012
		max.	NA	8741	6306	11030	7626	7426	16397	7803	4571
		range	NA	4206	4244	6308	5487	4486	8840	5118	2558
	S9	<i>n</i>	NA	8	10	17	17	23	25	30	17
		min.	NA	12834	17294	7755	1606	17990	11100	1254	15363
		max.	NA	22434	23564	39418	27547	25407	40886	28833	24685
		range	NA	9600	6270	31662	25941	7418	29787	27579	9323

ROS (RFU)	Plasma	<i>n</i>	NA	14	10	17	23	24	23	36	19
		min.	NA	84.22	90.33	64.67	79.89	92.33	84.33	80.56	97.67
		max.	NA	125.60	137.30	83.67	176.20	137.70	108.00	170.90	133.00
		range	NA	41.34	47.00	19.00	96.33	45.33	23.67	90.33	35.33
	S9	<i>n</i>	NA	8	10	18	16	24	25	30	20
		min.	NA	25.00	26.67	15.58	17.33	21.33	19.25	15.67	21.33
		max.	NA	72.67	80.67	63.58	91.33	72.00	58.92	113.30	71.67
		range	NA	47.67	54.00	48.00	74.00	50.67	39.67	97.67	50.33

NA – not analysed; ChE – cholinesterase; CES – carboxylesterase; GST – glutathione S-transferase; GR – glutathione reductase; GSH – glutathione; ROS – reactive oxygen species; RFU – relative fluorescence; *n* – number of sampled nestlings, min. – minimum value; max. – maximum value; ^a reference #22; ^b reference #23; ^c the present study.