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Article

The Year of the Honey Bee (*Apis mellifera* L.) with Respect to Its Physiology and Immunity: A Search for Biochemical Markers of Longevity

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Supplymentary Materials

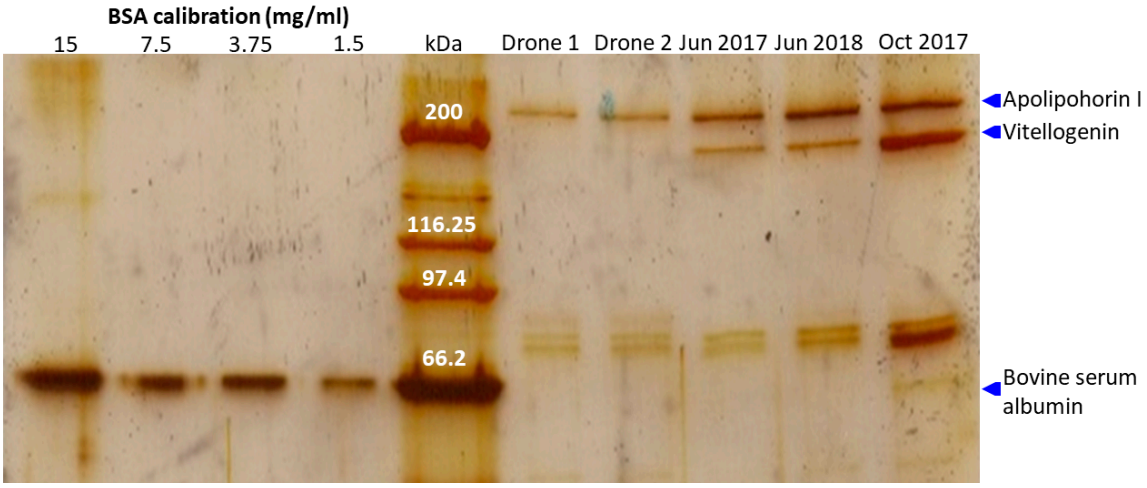

Figure S1. To double-check vitellogenin bands identified at Coomasie stained gels, some gels were stained in parallel by silver according to Kirkeby *et al.* [1]. Gel electrophoretic analysis of vitellogenin concentration in drones (Drone 1, 2) and honey bee workers from summer season (Jun 2017, 2018) and winter season (Oct 2017). Bovine serum albumin was used as calibration to calculate concentration of vitellogenin. Size of proteins used in ladder (SDS-PAGE Molecular Weight Standard, Bio-rad, 161-0317, USA) is indicated in the figure.

Figure S2. Western blot analysis of vitellogenin level in haemolymph of summer/winter population compared to drone haemolymph. For western blot, haemolymph samples were diluted 100×. Diluted samples were subjected to SDS-PAGE and electrotransfered onto Immobilon-P Membrane (Millipore) and immunodetected using primary antibody (Vitellogenin, kindly provided by RNDr. Dalibor Kodrík, CSc., Institute of entomology, Biology Centre CAS, 1:5000) and secondary antibody (Sigma-Aldrich, A0545, 1:5000). The blots were visualized by Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA) in western blot imagining system Fusion SL (Vilber, France).

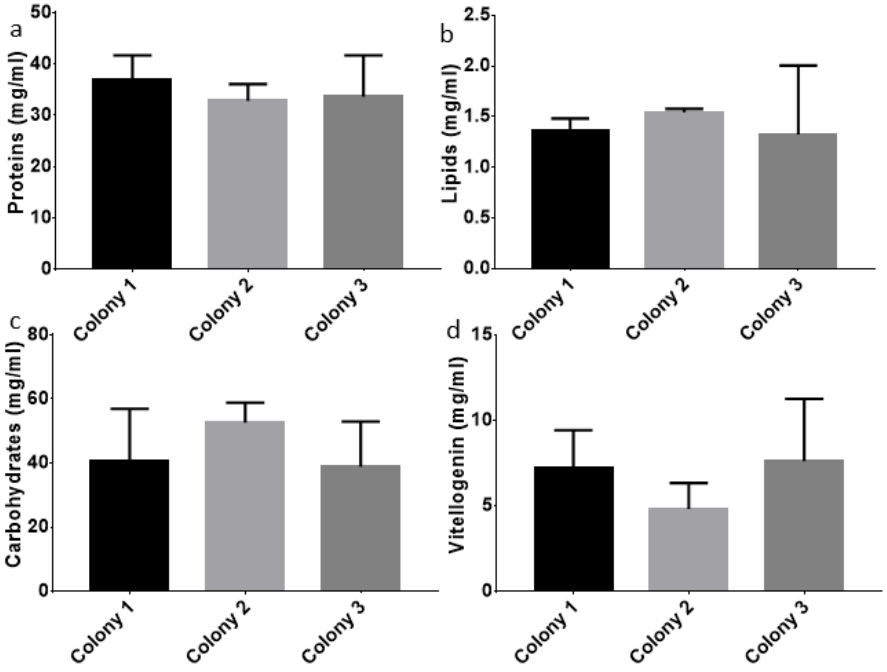

Figure S3. Comparison of physiological parameters: total concentration of proteins (**a**), lipids (**b**), carbohydrates (**c**) and vitellogenin (**d**) among experimental bee hive (Colony 1) and two different bee hives (Colony 2 and 3). The measurement was performed in June 2018. No significant difference was observed at the *p*-value 0.05. Columns indicate mean + SD, $n = 3–6$.

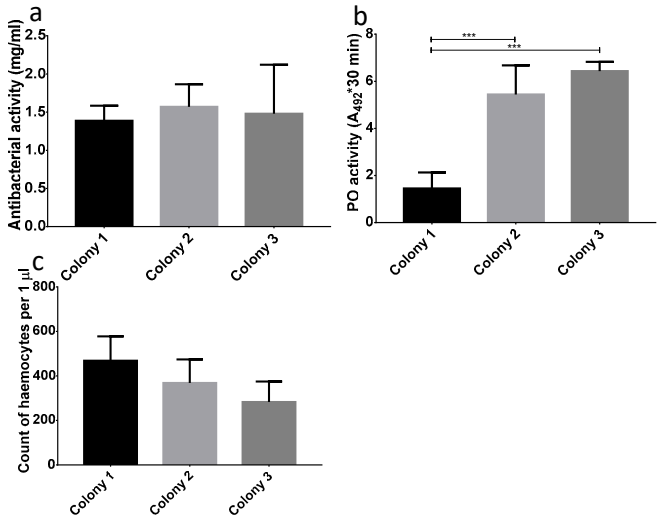

Figure S4. Comparison of immunological parameters: antibacterial activity (**a**), phenoloxidase activity (**b**) and count of haemocytes (**c**) among experimental bee hive (Colony 1) and two different bee hives (Colony 2 and 3). The measurement was performed in June 2018. Columns indicate mean + SD, n = 3–6. Asterisk indicates significant difference *** $p < 0.001$, ns = not significant.

Table S1. Compilation of statistical data for each tested season. The table contain n—sample size, mean, median, SD—standard deviation and SE—standard error for every tested parameter.

	Summer 2017					Winter 2017/2018					Summer 2018				
	n	**Mean**	**Median**	**SD**	**SE**	**n**	**Mean**	**Median**	**SD**	**SE**	**n**	**Mean**	**Median**	**SD**	**SE**
Proteins	12	35.83	40.33	10.7	3.089	27	61.95	62.4	13.77	2.65	19	31.69	32.49	6.297	1.445
Lipids	13	3.513	3.098	1.405	0.389	19	3.916	4.044	0.897	0.206	15	2.512	2.554	0.969	0.25
Carbohydrates	13	56.94	50.21	19.42	5.387	22	64.96	67.64	14.48	3.087	15	59.91	57.39	29.78	7.689
Vitellogenin	13	8.024	8.384	5.705	1.582	19	17.78	17.21	4.648	1.066	15	3.015	2.439	2.651	0.684
Antibacterial activity	13	0.665	0.459	0.435	0.121	19	3.64	3.353	1.557	0.357	15	2.349	2.526	0.795	0.205
PO activity	8	1.88	1.3	1.507	0.533	22	2.38	1.303	2.11	0.449	22	1.183	1.165	0.551	0.118
Haemocytes	18	494.8	412.5	288.5	67.99	24	495.6	431.3	154.1	31.46	16	403.9	393.8	139	34.76

Table S2. *p*-Values of Dunn´s test calculated by GraphPad software. The table contain *p*-values for comparison of every season.

	Summer 2017 × Winter 2017/2018	**Summer 2017 × Summer 2018**	**Summer 2018 × Winter 2017/2018**
Proteins	<0.001	0.94	<0.001
Lipids	0.42	0.20	0.001
Carbohydrates	0.65	>0.99	>0.99
Vitellogenin	0.002	0.27	<0.001
Antibacterial activity	<0.001	0.003	0.12
PO activity	>0.99	>0.99	0.30
Haemocytes	>0.99	>0.99	0.31

Reference

1. Kirkeby, S.; Moe, D.; Bòg-Hansen, T.C. The silver staining procedure of sodium dodecyl sulfate-gels may be accelerated by shortening fixation time. *Electrophoresis* **1993**, *14*, 51–55.