

Journal: Apidologie

Antibiotic treatment impairs protein digestion in the honeybee, *Apis mellifera*

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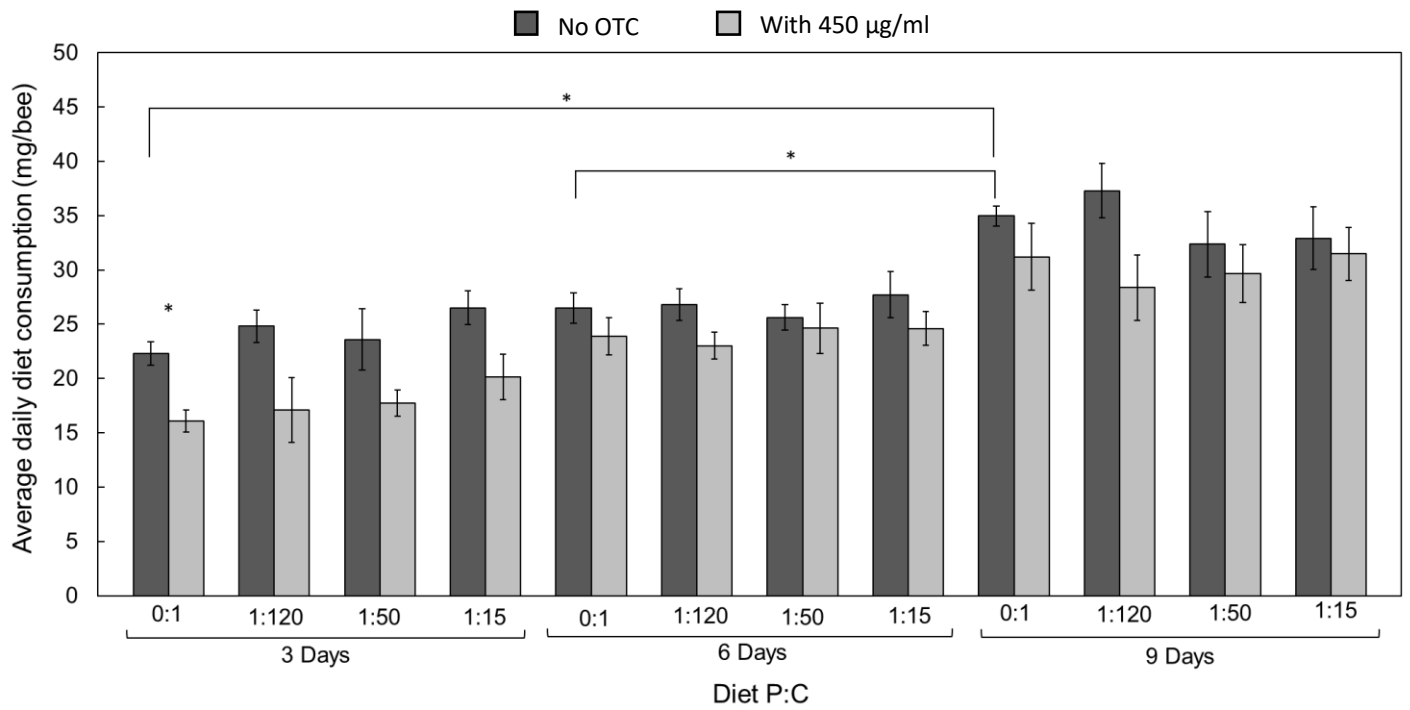
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Supplementary Figures S1 and S2

Supplementary Table SI and SII



Supplementary Figure S1. Hoarding cage containing newly emerged worker bees. Newly emerged workers were collected from the frames within 24 h of emergence and placed in clear PVC hoarding cages (8.5 x 8.5 x 4 cm; 50 bees per cage), each with 20 holes for ventilation in the lid. Each cage contained a piece of wax foundation (7 x 3 cm) for the bees to aggregate on. Plastic feeding vials (2 ml Eppendorf tubes) with feeding holes were inserted horizontally into the cages, one with water and one with experimental diet, provided fresh daily.



Supplementary Figure S2. Average daily diet consumption. Indicated is the average diet consumed per day across varying levels of dietary protein in the presence and absence of 450 µg/ml oxytetracycline (OTC) in bees fed for 3, 6 and 9 days. Data are presented as the mean ± SE (N = 6; 50 bees per cage). Significant differences are indicated with an asterisk (*) (Welch ANOVA, $p < 0.05$).

Supplementary Table SI. The levels of individual free amino acids excreted by honeybees after feeding for 3, 6 and 9 days on diets with varying P:C ratios (no oxytetracycline). (50 bees per cage).

	Diet (P:C)	N	Mean	Std. Error	Minimum	Maximum
Total Free Amino Acids μg per bee	Day 3 1:15	3	11.15	0.81	9.78	12.58
	Day 3 1:50	3	10.85	1.84	7.19	13.00
	Day 3 1:120	3	7.42	1.38	5.15	9.90
	Day 6 1:15	3	9.76	2.27	6.91	14.24
	Day 6 1:50	3	9.07	2.01	5.53	12.51
	Day 6 1:120	3	6.34	0.58	5.23	7.17
	Day 3 0:1	2	6.63	1.05	5.58	7.68
	Day 6 0:1	3	8.34	0.99	6.84	10.20
His ng per bee	Day 3 1:15	3	114.13	82.59	14.80	278.10
	Day 3 1:50	3	7.57	4.25	0.10	14.80
	Day 3 1:120	3	14.53	12.26	0.00	38.90
	Day 6 1:15	3	159.93	97.07	0.00	335.20
	Day 6 1:50	3	6.73	5.30	0.00	17.20
	Day 6 1:120	3	421.03	228.33	125.10	870.20
	Day 3 0:1	2	10.45	2.25	8.20	12.70
	Day 6 0:1	3	0.67	0.67	0.00	2.00
Ser ng per bee	Day 3 1:15	3	363.17	29.87	309.40	412.60
	Day 3 1:50	3	319.57	32.33	266.10	377.80
	Day 3 1:120	3	252.00	98.17	145.40	448.10
	Day 6 1:15	3	326.93	58.72	240.50	439.00
	Day 6 1:50	3	240.93	61.03	174.10	362.80
	Day 6 1:120	3	188.67	16.29	159.80	216.20
	Day 3 0:1	2	213.85	38.05	175.80	251.90
	Day 6 0:1	3	251.30	55.31	142.80	324.20
Arg ng per bee	Day 3 1:15	3	126.00	57.89	54.40	240.60
	Day 3 1:50	3	100.93	33.31	34.90	141.60
	Day 3 1:120	3	121.03	16.01	101.70	152.80
	Day 6 1:15	3	54.77	15.36	30.00	82.90
	Day 6 1:50	3	74.20	30.96	24.30	130.90
	Day 6 1:120	3	136.20	16.90	110.20	167.90
	Day 3 0:1	2	132.55	42.55	90.00	175.10
	Day 6 0:1	3	97.20	9.21	81.10	113.00
Lys ng per bee	Day 3 1:15	3	229.07	24.01	194.00	275.00
	Day 3 1:50	3	234.13	53.32	139.40	323.90
	Day 3 1:120	3	135.87	55.00	67.10	244.60
	Day 6 1:15	3	189.87	57.10	109.20	300.20
	Day 6 1:50	3	146.40	28.26	115.10	202.80
	Day 6 1:120	3	89.33	15.10	73.20	119.50
	Day 3 0:1	2	128.40	23.00	105.40	151.40
	Day 6 0:1	3	166.40	50.35	75.00	248.70

Asp ng per bee	Day 3 1:15	3	285.10	65.84	157.80	377.90
	Day 3 1:50	3	126.13	2.63	122.70	131.30
	Day 3 1:120	3	149.43	22.35	106.20	180.90
	Day 6 1:15	3	116.60	24.44	70.70	154.10
	Day 6 1:50	3	163.20	36.71	121.70	236.40
	Day 6 1:120	3	87.30	5.13	81.20	97.50
	Day 3 0:1	2	127.25	26.05	101.20	153.30
	Day 6 0:1	3	115.93	40.12	42.40	180.50
Glu ng per bee	Day 3 1:15	3	1147.33	160.72	847.90	1398.30
	Day 3 1:50	3	851.17	64.56	763.20	977.00
	Day 3 1:120	3	719.03	147.73	534.30	1011.10
	Day 6 1:15	3	950.00	131.52	788.20	1210.50
	Day 6 1:50	3	795.50	234.72	495.10	1258.10
	Day 6 1:120	3	623.43	82.86	467.10	749.20
	Day 3 0:1	2	728.35	0.45	727.90	728.80
	Day 6 0:1	3	734.67	172.14	394.70	951.70
Thr ng per bee	Day 3 1:15	3	300.53	23.90	254.50	334.70
	Day 3 1:50	3	335.10	70.43	194.30	409.20
	Day 3 1:120	3	201.57	72.52	127.70	346.60
	Day 6 1:15	3	297.07	85.92	177.10	463.60
	Day 6 1:50	3	263.83	51.55	163.10	333.20
	Day 6 1:120	3	124.50	6.43	115.60	137.00
	Day 3 0:1	2	166.55	43.35	123.20	209.90
	Day 6 0:1	3	207.50	51.06	109.00	280.10
Ala ng per bee	Day 3 1:15	3	864.77	131.99	643.30	1099.90
	Day 3 1:50	3	1006.77	148.09	762.80	1274.20
	Day 3 1:120	3	746.17	171.23	529.10	1084.10
	Day 6 1:15	3	769.07	150.60	571.30	1064.70
	Day 6 1:50	3	677.23	140.86	465.40	944.00
	Day 6 1:120	3	644.17	86.58	535.70	815.30
	Day 3 0:1	2	581.80	0.20	581.60	582.00
	Day 6 0:1	3	882.00	149.68	590.50	1086.80
Pro ng per bee	Day 3 1:15	3	761.30	90.01	582.90	871.40
	Day 3 1:50	3	863.00	301.84	315.80	1357.40
	Day 3 1:120	3	533.33	118.16	342.70	749.60
	Day 6 1:15	3	751.30	110.73	561.90	945.40
	Day 6 1:50	3	515.43	43.37	453.50	599.00
	Day 6 1:120	3	461.73	74.68	353.80	605.10
	Day 3 0:1	2	590.00	144.00	446.00	734.00
	Day 6 0:1	3	583.30	110.13	397.80	778.90
Cys ng per bee	Day 3 1:15	3	23.07	13.95	0.00	48.20
	Day 3 1:50	3	29.10	12.22	14.00	53.30
	Day 3 1:120	3	24.23	8.08	10.90	38.80
	Day 6 1:15	3	41.13	18.68	6.50	70.60
	Day 6 1:50	3	17.77	6.59	10.30	30.90

	Day 6 1:120	3	16.50	8.53	0.00	28.50
	Day 3 0:1	2	25.55	14.55	11.00	40.10
	Day 6 0:1	3	18.77	5.37	10.60	28.90
Lys ng per bee	Day 3 1:15	3	679.80	103.22	536.40	880.10
	Day 3 1:50	3	579.07	109.46	446.30	796.20
	Day 3 1:120	3	470.33	104.52	298.30	659.20
	Day 6 1:15	3	526.43	40.29	446.80	576.90
	Day 6 1:50	3	634.20	170.64	358.70	946.40
	Day 6 1:120	3	344.33	77.44	253.50	498.40
	Day 3 0:1	2	413.80	62.50	351.30	476.30
	Day 6 0:1	3	510.17	63.47	387.80	600.60
Tyr ng per bee	Day 3 1:15	3	2657.50	449.27	1765.50	3197.20
	Day 3 1:50	3	1936.90	266.96	1538.00	2443.70
	Day 3 1:120	3	1560.00	371.65	825.10	2023.90
	Day 6 1:15	3	1488.77	639.07	621.90	2735.60
	Day 6 1:50	3	1537.70	202.29	1138.20	1792.80
	Day 6 1:120	3	1517.67	567.01	518.30	2481.50
	Day 3 0:1	2	1352.95	881.55	471.40	2234.50
	Day 6 0:1	3	2339.60	229.79	1940.70	2736.70
Met ng per bee	Day 3 1:15	3	24.03	21.29	0.00	66.50
	Day 3 1:50	3	6.03	3.26	0.00	11.20
	Day 3 1:120	3	3.80	2.53	0.00	8.60
	Day 6 1:15	3	16.70	14.65	0.00	45.90
	Day 6 1:50	3	4.83	2.42	0.00	7.30
	Day 6 1:120	3	1.03	1.03	0.00	3.10
	Day 3 0:1	2	9.20	3.00	6.20	12.20
	Day 6 0:1	3	3.00	1.50	0.00	4.60
Val ng per bee	Day 3 1:15	3	443.97	84.16	290.60	580.70
	Day 3 1:50	3	444.53	153.95	283.10	752.30
	Day 3 1:120	3	242.70	112.78	84.40	461.00
	Day 6 1:15	3	444.97	159.67	231.20	757.30
	Day 6 1:50	3	326.77	74.07	188.20	441.40
	Day 6 1:120	3	133.87	3.55	126.80	138.00
	Day 3 0:1	2	186.20	103.30	82.90	289.50
	Day 6 0:1	3	293.33	105.09	102.20	464.60
	Total	23	320.12	40.60	82.90	757.30
Ile ng per bee	Day 3 1:15	3	418.70	83.53	253.90	524.80
	Day 3 1:50	3	387.83	127.72	255.00	643.20
	Day 3 1:120	3	239.23	106.84	103.90	450.10
	Day 6 1:15	3	351.40	132.55	181.10	612.50
	Day 6 1:50	3	277.90	72.08	134.30	360.60
	Day 6 1:120	3	124.93	3.87	117.20	128.80
	Day 3 0:1	2	185.45	111.45	74.00	296.90
	Day 6 0:1	3	251.57	96.88	76.50	411.00
Leu ng per bee	Day 3 1:15	3	911.87	225.40	537.20	1316.30

	Day 3 1:50	3	938.23	359.38	553.90	1656.40
	Day 3 1:120	3	484.50	221.28	175.30	913.30
	Day 6 1:15	3	927.27	452.76	384.10	1826.30
	Day 6 1:50	3	615.40	166.82	336.20	913.20
	Day 6 1:120	3	259.00	25.98	227.90	310.60
	Day 3 0:1	2	383.70	219.60	164.10	603.30
	Day 6 0:1	3	591.97	215.53	225.10	971.40
Phe ng per bee	Day 3 1:15	3	677.37	123.10	445.00	864.00
	Day 3 1:50	3	770.73	223.70	460.40	1205.00
	Day 3 1:120	3	402.00	184.48	184.90	768.90
	Day 6 1:15	3	800.77	372.09	329.60	1535.20
	Day 6 1:50	3	456.47	128.94	235.00	681.60
	Day 6 1:120	3	231.70	28.57	191.30	286.90
	Day 3 0:1	2	327.20	126.50	200.70	453.70
Day 6 0:1	3	495.33	149.45	248.20	764.50	
Gln ng per bee	Day 3 1:15	3	34.13	4.45	25.50	40.30
	Day 3 1:50	3	20.00	4.94	10.90	27.90
	Day 3 1:120	3	22.23	8.54	13.10	39.30
	Day 6 1:15	3	29.97	8.14	14.00	40.70
	Day 6 1:50	3	12.80	2.20	8.40	15.10
	Day 6 1:120	3	10.83	2.38	7.70	15.50
	Day 3 0:1	2	20.10	7.90	12.20	28.00
Day 6 0:1	3	9.53	3.96	2.90	16.60	
Trp ng per bee	Day 3 1:15	3	124.23	105.61	14.80	335.40
	Day 3 1:50	3	258.40	184.06	57.80	626.00
	Day 3 1:120	3	94.37	69.36	9.90	231.90
	Day 6 1:15	3	70.50	48.41	17.90	167.20
	Day 6 1:50	3	109.43	80.24	27.60	269.90
	Day 6 1:120	3	70.83	54.76	13.10	180.30
	Day 3 0:1	2	88.60	82.90	5.70	171.50
Day 6 0:1	3	104.43	69.49	28.50	243.20	
OHPro ng per bee	Day 3 1:15	3	856.37	294.57	456.20	1430.90
	Day 3 1:50	3	1459.43	539.53	450.40	2295.10
	Day 3 1:120	3	760.07	189.05	440.90	1095.20
	Day 6 1:15	3	1302.30	124.31	1136.70	1545.70
	Day 6 1:50	3	668.70	166.36	380.80	957.10
	Day 6 1:120	3	736.63	42.84	692.90	822.30
	Day 3 0:1	2	776.50	560.60	215.90	1337.10
Day 6 0:1	3	566.50	201.80	189.40	879.60	
GABA ng per bee	Day 3 1:15	3	50.73	25.50	0.00	80.60
	Day 3 1:50	3	68.80	19.49	30.60	94.60
	Day 3 1:120	3	59.63	15.83	28.80	81.30
	Day 6 1:15	3	41.40	6.75	34.30	54.90
	Day 6 1:50	3	45.37	16.44	12.50	62.30
	Day 6 1:120	3	39.67	7.61	24.50	48.40

	Day 3 0:1	2	37.45	37.45	0.00	74.90
	Day 6 0:1	3	34.10	14.42	18.50	62.90
Orn ng per bee	Day 3 1:15	3	2657.50	449.27	1765.50	3197.20
	Day 3 1:50	3	1936.90	266.96	1538.00	2443.70
	Day 3 1:120	3	1560.00	371.65	825.10	2023.90
	Day 6 1:15	3	1488.77	639.07	621.90	2735.60
	Day 6 1:50	3	1537.70	202.29	1138.20	1792.80
	Day 6 1:120	3	1517.67	567.01	518.30	2481.50
	Day 3 0:1	2	1352.95	881.55	471.40	2234.50
	Day 6 0:1	3	2339.60	229.79	1940.70	2736.70

Supplementary Table SII. Digestive efficiency of bees fed varying protein diets in the presence or absence of oxytetracycline. Data presented as the mean \pm SE (N=6; 50 bees per cage). OTC: oxytetracycline (450 μ g/ml).

3 Days		
Diet P:C	Digestion Efficiency (%)	% Protein excreted
1:15	96.34 \pm 0.59	3.66 \pm 0.59
1:50	84.22 \pm 3.96	15.78 \pm 3.96
1:120	74.80 \pm 5.67	25.20 \pm 5.67
1:15 + OTC	94.44 \pm 1.96	5.56 \pm 1.96
1:50 + OTC	79.41 \pm 8.11	20.59 \pm 8.11
1:120 + OTC	40.15 \pm 9.71	59.85 \pm 9.71
6 Days		
Diet P:C	Digestion Efficiency (%)	% Protein excreted
1:15	98.62 \pm 0.22	1.38 \pm 0.22
1:50	94.12 \pm 1.20	5.88 \pm 1.20
1:120	85.62 \pm 2.55	14.38 \pm 2.55
1:15 + OTC	95.72 \pm 0.46	4.28 \pm 0.46
1:50 + OTC	86.52 \pm 1.71	13.48 \pm 1.71
1:120 + OTC	61.52 \pm 4.51	38.48 \pm 4.51