

Supplementary Materials:

Supplementary information for: Sustainable Agriculture: Nutritional Benefits of Wheat–Soybean and Maize–Sunflower Associations for Hibernation and Reproduction of Endangered Common Hamsters

Supplementary Methods

Table S1. Macronutrient and micronutrient composition of the crops selected for the experiment. This table is based on available data before the experiment was conducted (AFZ et al., 2011; USDA SR-21, 2016). Data are given for seeds of each crop except fodder radish, as data were not available for these seeds. Avg: average.

Analysis	Unit	Food Item				
		<i>Wheat</i>	<i>Maize</i>	<i>Sunflower</i>	<i>Soybean</i>	<i>Fodder Radish</i>
Main analysis	Unit	Avg	Avg	Avg	Avg	Avg
<u>Dry matter</u>	% as fed	87.0	23.4	92.8	88.8	/
<u>Crude protein</u>	% DM	12.6	9.4	16.6	39.6	/
<u>Lipids</u>	% DM	3.6	4.4	47.9	21.3	/
<u>Total sugars</u>	% DM	3.2	2.1	2.7	8.7	/
<u>Starch</u>	% DM	69.1	73.4	1.3	6.4	/
<u>Gross energy</u>	MJ/kg DM	18.2	18.7	28.7	23.6	/
Minerals	Unit	Avg	Avg	Avg	Avg	Avg
<u>Calcium</u>	g/kg DM	0.7	0.5	2.6	3.2	/
<u>Magnesium</u>	g/kg DM	1.2	1.2	3	2.4	/
<u>Manganese</u>	mg/kg DM	40	5	19	29	/
<u>Zinc</u>	mg/kg DM	31	21	50	43	/
<u>Copper</u>	mg/kg DM	6	5	18	19	/
<u>Iron</u>	mg/kg DM	78	37	52	121	/
Amino acids	Unit	Avg	Avg	Avg	Avg	Avg
Leucine	% protein	6.5	12	6.0	7.5	/
Lysine	% protein	2.9	3.1	3.9	6.2	/
Tryptophan	% protein	1.2	0.7	1.6	1.3	/
Fatty acids	Unit	Avg	Avg	Avg	Avg	Avg
Palmitic	% lipids	25	11	6	10	/
Palmitoleic	% lipids	1	Traces	Traces	Traces	/
Stearic	% lipids	1	2	5	4	/
Oleic	% lipids	12	28	18	20.7	/
Linoleic	% lipids	55	56	69	55	/
Linolenic	% lipids	5.4	1	0.3	7.3	/
Vitamins	Unit	Avg	Avg	Avg	Avg	Avg
B3	mg/100 g	1.8	<1	8.3	0.4	/

Supplementary Results

Table S2. Proportions (%) of indexes 1, 2 and 3 recorded for each sex on each diet throughout winter.

<i>Diet</i>	%_1	%_2	%_3	<i>Sex</i>	%_1	%_2	%_3
<i>Mrad</i>	43.3	6.3	50.3	F	32.9	4.3	62.9
				M	53.8	8.4	37.8
<i>Msoy</i>	58.3	9.6	32.1	F	48.2	7.9	43.9
				M	68.3	11.4	20.3
<i>Msunf</i>	48.2	4.5	47.3	F	43.9	30.3	53.1
				M	52.5	5.9	41.6
<i>Wrad</i>	55.0	8.4	36.6	F	54.6	6.7	38.7
				M	55.5	10.0	34.5
<i>Wsoy</i>	47.0	6.1	46.9	F	38.5	6.3	55.2
				M	55.5	5.9	38.7
<i>Wsunf</i>	57.8	7.4	34.9	F	30.9	5.0	34.1
				M	54.6	9.7	35.7

Note: The three diets in bold are those on which individuals were significantly more active than on the three others (see Results section and Figure 1A in the manuscript and summary Figure S1 below). A score of 1 represents hamsters in deep torpor, and a score of 2 indicates individuals in shallow torpor or in a deep sleep. Finally, a score of 3 is attributed to hamsters that were either active or in a light sleep.

Table S3. Model selection looking at the effect of diet (base and complement), sex and body mass on activity index. Models with Delta AICc < 4 and null and full models are presented.

Full Model: Score ~ (Base+Comp+Sex+Body mass)^2+(1 Piece)+(1 Ind)+(1 Date)						
Model #	Fixed Factors in the Model	df	LogLik	AICc	Delta AICc	Weight
28	Base * Complement + Sex	12	-2619,504	5263,1	0	0,084
10	Base + Sex	8	-2623,81	5263,7	0,56	0,063
92	Base * Complement + Base * Sex	14	-2617,979	5264,1	0,98	0,051
32	Base * Complement + Sex + Body mass	13	-2619,176	5264,5	1,36	0,042
74	Base * Sex	10	-2622,436	5264,9	1,84	0,033
348	(Base + Complement + Sex)^2	16	-2616,396	5265	1,86	0,033
192	(Base + Complement + Body mass)^2 + Sex	17	-2615,405	5265	1,89	0,032
14	Base + Sex + Body mass	9	-2623,502	5265,1	1,96	0,031
448	(Base + Complement + Body mass)^2 + Complement * Sex	20	-2612,46	5265,2	2,08	0,03
Null	(Null)	1	-2631,09	5272,2	9,09	0,001
Full	(Full)	22	-2611,65	5267,31	4,21	0,009

Table S4. Hamsters' average body mass (g) according to their diet, sex and periods. Three periods are represented: OH = onset of hibernation, PH = post-hibernation and PR = post-reproduction. Means±SEM are shown in grams.

PERIOD	DIET	M-SOY	M-SUNF	M-RAD	W-SOY	W-SUNF	W-RAD
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	OH	252.9 ± 14.2	253.1 ± 14.2	252.6 ± 14.2	253.6 ± 14.2	253.9 ± 14.2	254.1 ± 14.2
	PH	194.1 ± 11.9	231.3 ± 11.9	216.9 ± 11.9	234.6 ± 11.9	212.9 ± 11.9	188.4 ± 11.9
	PR	224.3 ± 12.5	256.1 ± 13.6	229.7 ± 13.2	257.3 ± 13.0	240.2 ± 12.5	203.2 ± 13.2
PERIOD	SEX	Males			Females		
	OH	271.7 ± 8.2			235.0 ± 8.2		
	PH	230.3 ± 6.9			195.8 ± 6.9		
	PR	257.0 ± 7.1			213.3 ± 7.9		

Table S5. Output of the model looking at the effect of the diet and the body mass on females' parturition rate. The full model is presented. Considering the limited number of fixed effects considered, we did not perform model selection. P-values from the summary and ANOVA are provided.

Model: Parturition ~ (Diet*Body Mass)							
Fixed Effects	LR Chisq	df	Pr(>Chisq)	Estimate	Std.Error	z value	Pr(< z)
Diet	6.9132	5	0.2272				
Body mass	0.7771	1	0.3780	2.93	4.45	0.657	0.511
Diet:Body mass	7.8081	5	0.1671				

Table S6. Model selection for the effect of the diet, the body mass and the period (birth or weaning) on litter size.

Complete model: Litter size ~ (Diet*Period+Body mass after hibernation)+(1 Mother)							
Model #	Fixed Effects	df	LogLik	AICc	Delta AICc	Weight	Model Outputs
1	Body mass+Period	4	-61.85	132.91	0.00	0.30	Body mass: estimate 0.32±0.17, X ² = 2.97, p = 0.085 Period: weaning estimate -1.13±0.30, X ² = 15.49, p < 0.001
2	Period	3	-63.21	133.12	0.21	0.27	Period: weaning estimate -1.13±0.29, X ² = 16.41, p < 0.001
3	Period+Diet	8	-56.23	133.43	0.52	0.23	Period: weaning estimate -1.13±0.29, X ² = 18.39, p < 0.001 Diet: X ² = 19.39, p = 0.002
4	Body mass+ Period+Diet	9	-55.28	134.98	2.08	0.11	Body mass: estimate 0.21±0.16, X ² = 2.22, p = 0.136 Period: weaning estimate -1.13 ± 0.29, X ² = 19.43, p < 0.001 Diet: X ² = 18.76, p = 0.002
5	Period+Diet+ Period:Diet	13	-47.21	135.58	2.68	0.08	Period: X ² = 33.24, p < 0.001 Diet: X ² = 24.70, p < 0.001 Period:Diet: X ² = 15.56, p = 0.008
6	Body mass+Period+ Diet+Period:Diet	14	-46.25	138.77	5.86	0.02	
7	Diet	7	-66.47	150.67	17.76	0.00	
8	Body mass	3	-72.09	150.88	17.97	0.00	
9	(Null)	2	-73.45	151.23	18.33	0.00	
10	Body mass+Diet	8	-65.52	152.00	19.09	0.00	

Note: Outputs of models with Delta AICc<4 are provided. Since models 1–3 have similar AICc values and similar weights, and considering that (i) we specifically investigated the effect of the diet in our study and that (ii) we found an effect of the diet on Δ body mass during winter (and thus, some of the differences in body mass are explained by the diet; see §3.3), model 3 is discussed in the article (see §3.5) and written in bold in the table. Multiple comparisons between diets are presented for this model in Table S7. Significant variables in each model and corresponding p-values are represented in bold in the model outputs column.

Table S7. Multiple comparisons of model 3 from Table S6 on diet effect on litter size.

Model: Litter size ~ (Diet+Period)+(1 Mother)						
Contrast	Estimate	SE	df	t.ratio	P-Values	Adjusted P-Values (Tukey)
Wrad–Wsoy	–1.504	0.806	13	–1.866	0.0848	0.4616
Wrad–Wsunf	0.000	0.883	13	0.000	1.0000	1.0000
Wrad–Mrad	–0.629	0.814	13	–0.772	0.4538	0.9676
Wrad–Msoy	–0.262	0.821	13	–0.319	0.7545	0.9994
Wrad–Msunf	–0.916	0.802	13	–1.143	0.2738	0.8552
Wsoy–Wsunf	1.504	0.510	13	2.950	0.0113	0.0935
Wsoy–Mrad	0.875	0.378	13	2.316	0.0376	0.2549
Wsoy–Msoy	1.242	0.394	13	3.155	0.0076	0.0661
Wsoy–Msunf	0.588	0.351	13	1.673	0.1182	0.5701
Wsunf–Mrad	–0.629	0.522	13	–1.203	0.2503	0.8278
Wsunf–Msoy	–0.262	0.534	13	–0.492	0.6312	0.9956
Wsunf–Msunf	–0.916	0.503	13	–1.820	0.0918	0.4866
Mrad–Msoy	0.366	0.410	13	0.894	0.3877	0.9414
Mrad–Msunf	–0.288	0.369	13	–0.779	0.4500	0.9664
Msoy–Msunf	–0.654	0.385	13	–1.697	0.1134	0.5560

Note: This model is the one discussed in the manuscript. Significant differences are presented in bold. Multiple comparisons were not possible with the Wrad group, as only one female gave birth, and none of her pups survived.

Supplementary figure



Figure S1. Signs of cartilage atrophy in the ears of common hamsters on the Mrad diet after hibernation. Animals were under anesthesia when the pictures were taken.