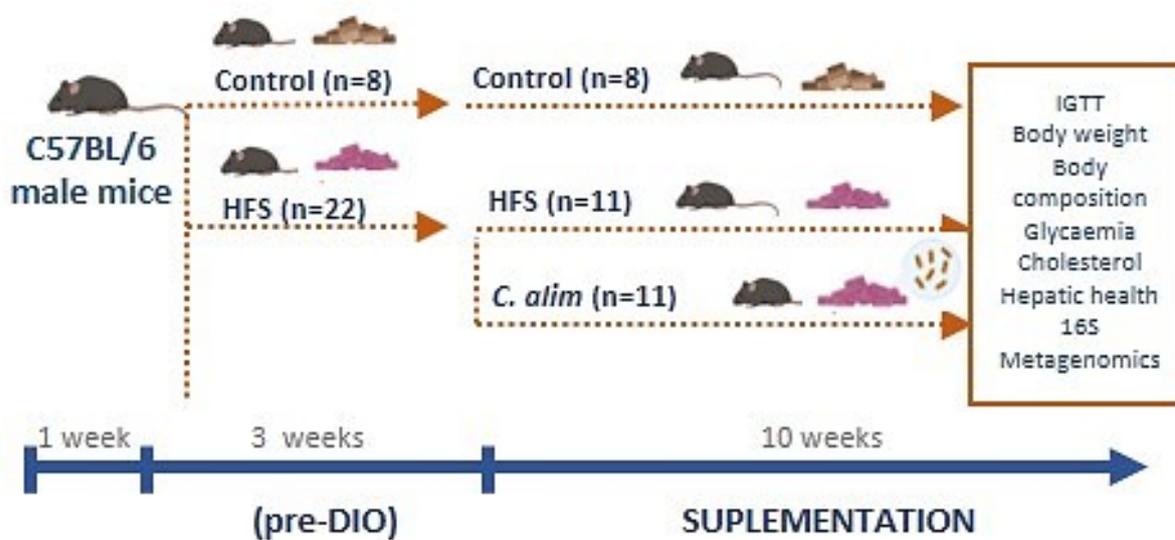
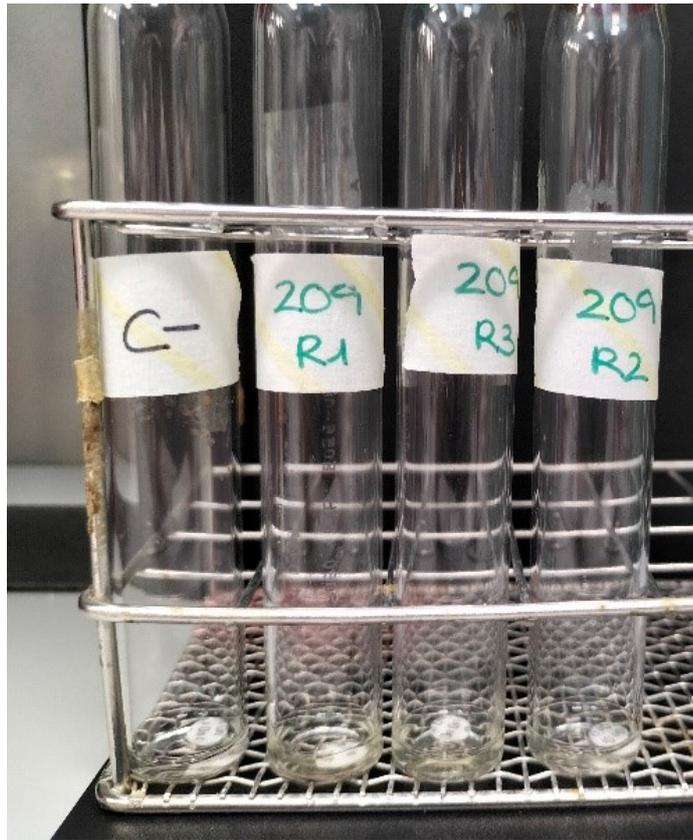


## SUPPLEMENTARY INFORMATION

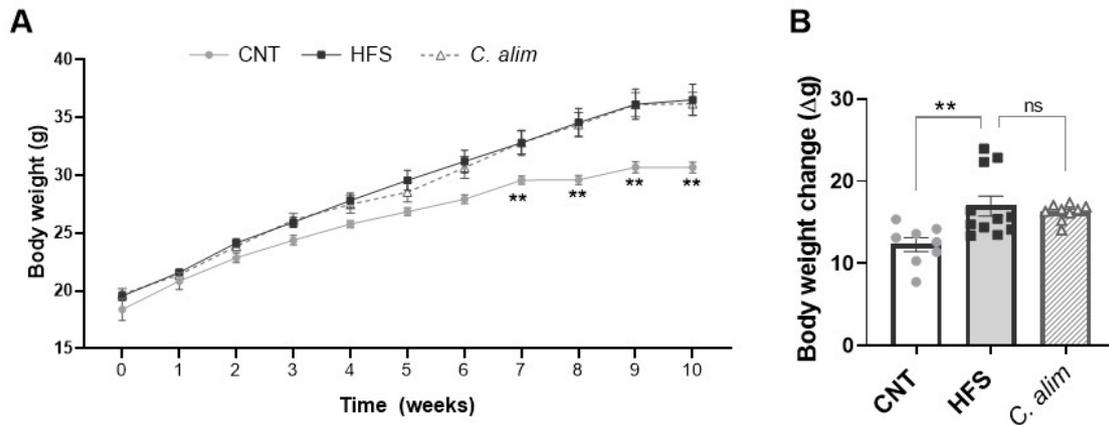
Supplementary figures:



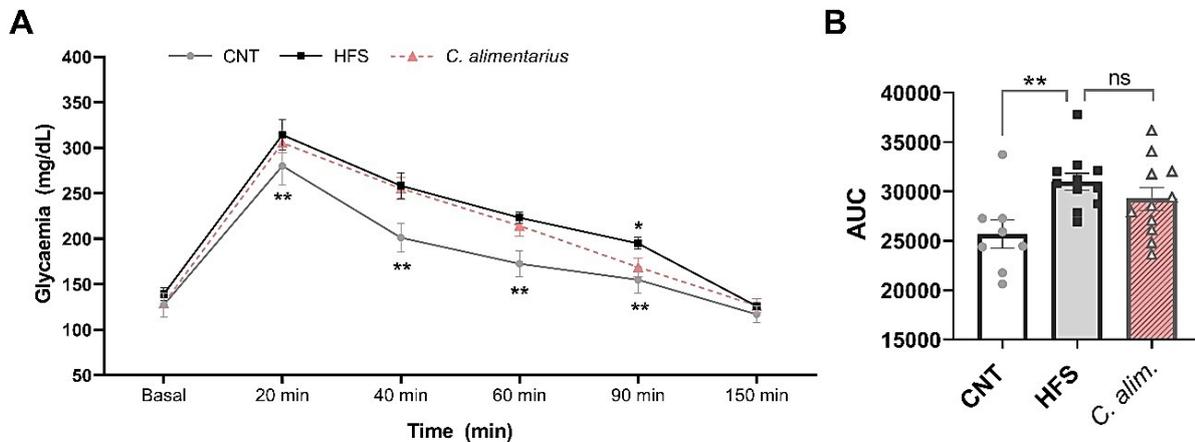
**Figure S1:** Schematic representation of the experimental design used for the functional evaluation of the effect of supplementation with *C. alimentarius* CNTA 209 in a mouse model with diet-induced obesity.



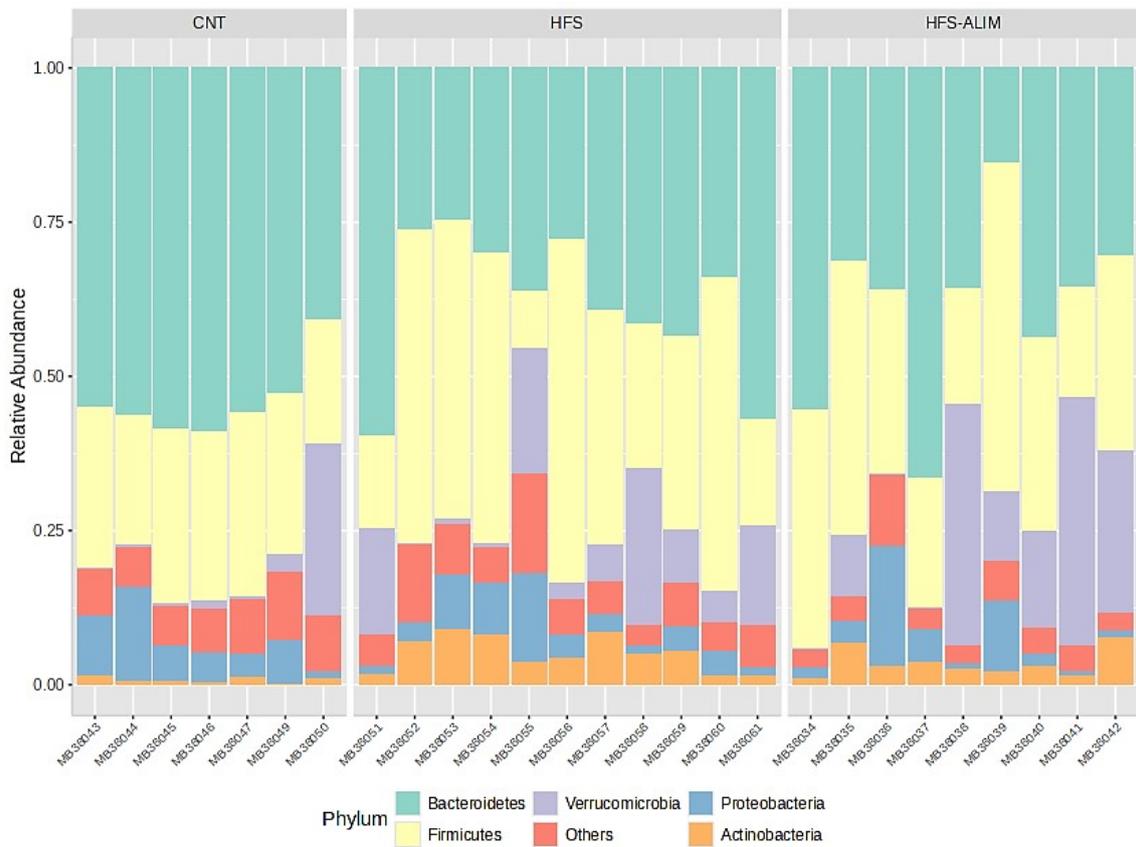
**Figure S2:** Semiquantitative *in vitro* test demonstrating the production of  $\beta$ -galactosidase by *C. alimentarius* CNTA 209. Three replicates are shown. C- ,negative control.



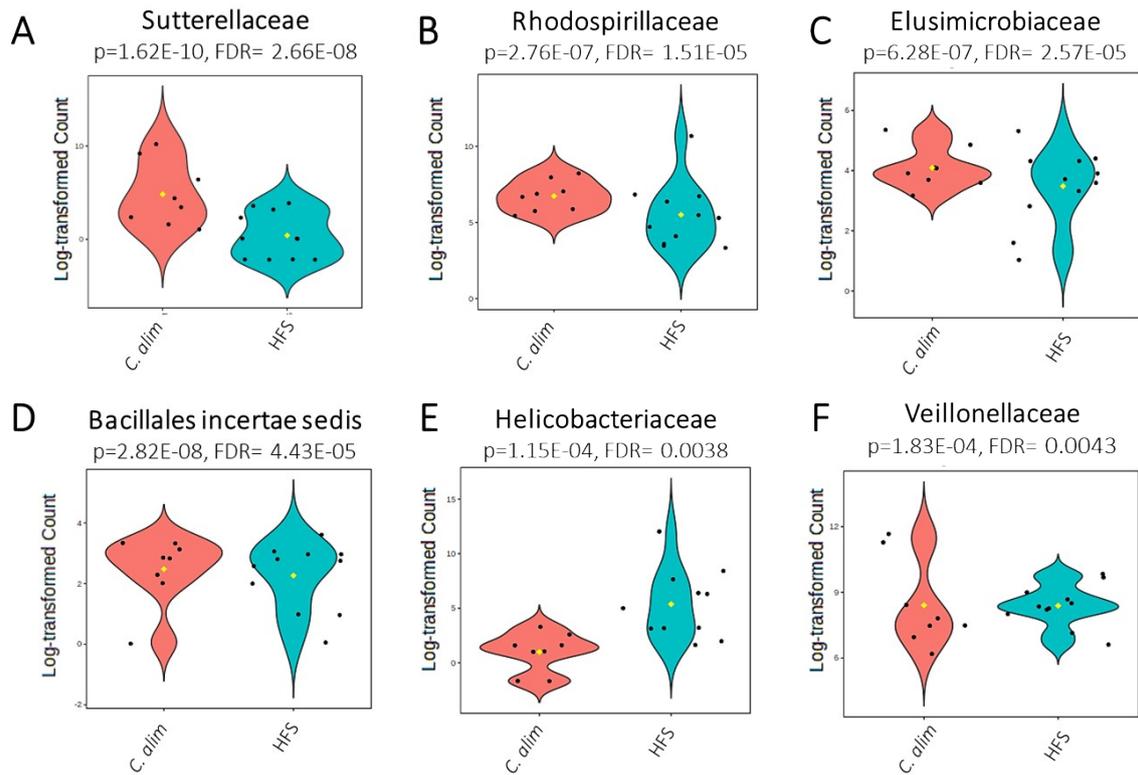
**Figure S3.** A) Body weight record determined weekly for each experimental group, throughout the 10 weeks of the study. Mean  $\pm$  SEM. Statistical differences were evaluated by the one-way ANOVA test followed by Duncan test as multiple comparisons when Anova  $p < 0.05$ . \*\* $p < 0.01$  vs HFS group. B) Body weight change along the study. Mean  $\pm$  SEM. Statistical differences were evaluated by the one-way ANOVA test followed by Duncan test as multiple comparisons when Anova  $p < 0.05$ . CNT: control diet group; HFS: high-fat-sucrose diet group; *C. alim*: *C. alimentarius* CNTA 209 group.



**Figure S4:** A) Effects of *C. alimentarius* 209 on blood glucose levels after the IPGTT performed during week 9 of supplementation. B) Area under the curve of the GTT. Results are expressed as the mean  $\pm$  SEM. Statistical differences were analysed by the one-way ANOVA followed by Duncan test as multiple comparisons when Anova  $p < 0.05$ . \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , ns, not significant comparing with the HFS-group. CNT: control diet group; HFS: high-fat-sucrose diet group; *C. alim*: *C. alimentarius* CNTA 209 group.



**Figure S5:** Relative tax abundance at Phylum level in the different samples of CNT, HFS and HFS-C. alim groups. Only the five more abundant phyla are represented.



**Figure S6:** Representation of the normalized data of those families with statistically significant differences between the *C. alim* and HFS groups. A) Sutterellaceae. B) Rhodospirillaceae. C) Elusimicrobiaceae. D) Bacillales incertidae sedis. E) Helicobacteriaceae. F) Veillonellaceae. Metagenomic statistical analyses were calculated using Deseq2, considering a minimum p value  $<0.01$ , and  $FDR < 0.005$ . HFS: high-fat-sucrose diet group; *C. alim*: *C. alimentarius* CNTA 209 group.

## Supplementary tables:

**Table S1:** Growth parameters of *C. alimentarius* CNTA 209.

Parameters	<i>C. alimentarius</i> CNTA 209
Initial bacteria count (A) (Log CFU/mL)	6.22
Maximum growth rate (B) (hours <sup>-1</sup> )	0.25
Difference between the initial bacteria count and the maximum	2.54
Time required by microorganism to achieve the maximum growth	6.24
Lag time (LPD) (hours)	2.24
R value	1
RMSE value	0.05

RMSE: Root mean square error.

**Table S2:** Diet intake (g per day) per mouse, and total intake of all HFD-fed groups. Mean  $\pm$  SEM. Control-diet mice were fed *ad libitum*.

	HFS	<i>C. alim</i>	Anova
Diet intake (g / day / mouse)	2.27 $\pm$ 0.01	2.33 $\pm$ 0.29	ns
Total intake (kcal)	672.42 $\pm$ 2.52	690.45 $\pm$ 2.15	ns
Food Efficiency ( $\Delta$ body weight / total kcal)	2.52 $\pm$ 0.17	2.39 $\pm$ 0.03	ns

ns: not significant.

**Table S3:** Liver, spleen and kidney's weight of the different groups of the study. Mean  $\pm$  SEM.

	CNT	HFS	<i>C. alim</i>	Anova
Liver (g)	1.011 $\pm$ 0.054	1.063 $\pm$ 0.044	1.062 $\pm$ 0.042	ns
Spleen (g)	0.089 $\pm$ 0.006	0.085 $\pm$ 0.007	0.082 $\pm$ 0.006	ns
Kidney (g)	0.165 $\pm$ 0.007	0.157 $\pm$ 0.004	0.161 $\pm$ 0.005	ns

**Table S4:** For each sex and treatment, weekly food consumption data are presented, expressed as grams of food/animal/day. These data are obtained using the formula: food consumption = (Given (g) - Remaining (g))/(number of animals \* days). T corresponds to the group treated with *C. alimentarius* and C to the control group.

		Administered dose (%)									
Group		Acclimatation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
MALE ♂	T	23.5	22.1	24.1	29.7	29.3	26.3	25.9	25.9	25.8	25.8
	C	23.5	21.9	24.1	29.8	30.0	26.7	25.9	25.9	25.8	25.9
FEMALE ♀	T	15.2	14.6	16.0	18.5	17.1	15.4	15.5	15.6	15.6	15.7
	C	14.8	14.5	17.1	18.0	17.5	15.7	15.8	15.3	15.9	15.5

**Table S5:** Data on % intake are presented, sorted by test group and sex, as a measure of the actual dose administered. T corresponds to the group treated with *C. alimentarius*, and C to the control group.

		Administered dose (%)									
Group		Acclimatation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
MALE ♂	T	100.0%	100.0%	99.5%	99.1%	97.6%	100.0%	99.8%	99.5%	99.3%	99.1%
	C	100.0%	95.2%	99.6%	99.2%	100.0%	100.0%	99.8%	99.6%	99.2%	99.5%
FEMALE ♀	T	89.6%	92.9%	105.7%	90.5%	100.0%	100.0%	98.7%	95.8%	99.6%	96.8%
	C	87.0%	96.6%	135.5%	93.4%	100.0%	97.9%	98.7%	95.8%	99.6%	96.8%

**Table S6:** Weight record (in grams) of the male (M) and female (F) rats of the toxicological assay. The table shows, ordered by sex and test group, the descriptive statistics of centralization and dispersion (mean and standard deviation). Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group.

Group	Weight record (g)										
	Arrival	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
T M♂	272.8	309.6	337.2	351.5	381.4	411.8	422.8	436.7	441.5	455.6	462.5
	6.5	9.2	10.9	12.4	12.8	15.1	10.6	20.2	21.2	24.6	25.1
C M♂	256.0	295.0	334.7	350.5	392.1	430.2	442.2	471.3	453.9	462.6	473.4
	6.6	8.3	9.1	7.8	15.0	25.4	29.3	39.5	30.9	28.0	27.0
T F♀	156.0	176.1	187.2	197.7	207.7	215.4	220.1	222.9	226.1	229.1	231.6
	3.8	3.8	6.5	6.3	11.1	10.1	10.4	9.1	9.0	9.7	10.0
C F♀	157.4	180.1	186.2	197.7	213.3	222.8	229.4	232.4	235.4	235.8	222.6
	3.9	9.5	11.0	11.2	13.1	18.4	17.7	15.0	15.5	15.3	11.6

**Table S7:** Weight gain (in grams) of the male (M) and female (F) rats of the toxicological assay. The table shows, ordered by sex and test group, the descriptive statistics of centralization and dispersion (mean and standard deviation). Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group.

Group	Weight gain ( $\Delta g$ )											
	Beginning - Arrival	Week 1 - Beginning	Week 2 - 1	Week 3 - 2	Week 4 - 3	Week 5 - 4	Week 6 - 5	Week 7 - 6	Week 8 - 7	Week 9 - 8	Sacrifice - Beginning	Sacrifice - Arrival
T M♂	36.8	27.6	14.3	29.9	30.4	11.0	13.9	4.8	14.0	6.9	152.9	189.6
	3.9	5.9	2.6	4.1	6.5	5.6	10.7	2.5	4.7	2.7	17.3	19.9
C M♂	39.0	39.8	15.7	41.6	38.2	12.0	29.1	-17.5	8.7	10.8	178.4	217.4
	5.8	5.4	8.7	12.3	27.1	5.2	40.1	46.5	7.0	6.2	24.4	23.5
Group	Weight gain ( $\Delta g$ )											
	Beginning - Arrival	Week 1 - Beginning	Week 2 - 1	Week 3 - 2	Week 4 - 3	Week 5 - 4	Week 6 - 5	Week 7 - 6	Week 8 - 7	Week 9 - 8	Sacrifice - Beginning	Sacrifice - Arrival
T F♀	20.1	11.1	10.5	10.0	7.7	4.7	2.9	3.1	3.0	2.5	55.5	75.6
	5.9	3.9	0.6	5.1	2.3	3.2	1.7	2.6	0.8	1.7	7.8	11.8
C F♀	22.8	6.0	11.6	15.5	9.6	6.5	3.0	3.0	0.5	-13.3	42.4	65.2
	9.5	3.0	2.7	8.4	6.1	4.8	2.9	2.9	1.3	6.7	13.0	11.9

**Table S8:** Hematological Parameters. For each sex, ordered by study group, the descriptive statistics for centralization and dispersion (mean and standard deviation) Statistical analyses (Mann Whitney U) were carried out to compare the Treated (T, *C. alimentarius*) versus the Control (C) group.

MALE ♂	Hematological Parameters								
	WBC (x10 <sup>3</sup> cell /mL)	RBC (x10 <sup>6</sup> cél/mL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	Platelets (x10 <sup>3</sup> cell/mL)	Reticulocytes (%)
T M♂	4.67	8.43	15.1	43.2	51.3	17.9	34.9	736	2.93
	1.00	0.31	0.4	1.0	0.7	0.2	0.3	82	0.45
C M♂	5.55	8.81	15.3	44.2	50.2	17.4	34.6	847	3.30
	1.34	0.52	0.6	1.7	2.7	0.9	0.2	81	0.19
FEMALE ♀	Hematological Parameters								
	WBC (x10 <sup>3</sup> cell /mL)	RBC (x10 <sup>6</sup> cell /mL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	Plaquetas (x10 <sup>3</sup> cell /mL)	Reticulocitos (%)
T F♀	2.93	7.62	14.3	41.1	54.0	18.8	34.7	641	3.01
	0.87	0.22	0.5	1.2	1.1	0.5	0.3	93	0.64
C F♀	3.24	7.91	14.7	42.7	53.9	18.6	34.5	735	2.72
	0.99	0.22	0.4	1.3	1.5	0.4	0.3	31	0.41

WBC: White Blood Cells; RBC: Red Blood Cells; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

**Table S9:** Absolute differential cell count. For each sex, ordered by study group, the descriptive statistics for centralization and dispersion (mean and standard deviation) Statistical analyses (Mann Whitney U) were carried out to compare the Treated (T, *C. alimentarius*) versus the Control (C) group.

MALE ♂	Absolute differential count (x10 <sup>3</sup> cell/μL)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
TM	0.79	3.52	0.09	0.10	0.18
	0.23	0.73	0.03	0.05	0.06
CM	1.47	3.59	0.12	0.10	0.27
	0.81	0.59	0.03	0.04	0.17
FEMALE ♀	Absolute differential count (x10 <sup>3</sup> cell/μL)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
TF	0.34	2.46	0.04	0.04	0.06
	0.15	0.83	0.02	0.01	0.03
CF	0.42	2.63	0.04	0.03	0.12
	0.12	0.98	0.02	0.02	0.05

**Table S10:** Coagulation parameters. For each sex, ordered by study group, the descriptive statistics for centralization and dispersion (mean and standard deviation) Statistical analyses (Mann Whitney U) were carried out to compare the Treated (T, *C. alimentarius*) versus the Control (C) group.

Coagulation parameters			
MALE ♂	Fibrinogen (mg/dL)	Thromboplastin Time (TP) (s)	Activated Partial Thromboplastin Time (APTT)
TM	195.1	17.0	19.5
	15.4	0.2	4.4
CM	213.7	17.7	24.1
	19.4	1.6	4.9
FEMALE ♀	Fibrinogen (mg/dL)	Thromboplastin Time (TP) (s)	Activated Partial Thromboplastin Time (APTT)
TF	126.8	14.7	19.8
	20.5	1.8	5.9
CF	131.5	15.7	23.1
	7.8	1.2	5.4

**Table S11:** Biochemical parameters after the intervention. It collects for each sex, ordered by study group, the descriptive statistics of centralization and dispersion (mean and standard deviation). Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group. (\*) significant (p<0,05).

MALE (♂)		Biochemical parameters												
		Albumin (g/dL)	Urea (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirrubine (mg/dL)	Cholesterol (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)	CPK (g/dL)	Triglycerides (mg/dL)	LDH (mg/dL)
Final	T	3,9	31	68	21	54	0,04	81	0,34	134	5,7	447	56	481
		0,3	3	10	3	13	0,01 (*)	9	0,07	15	0,3	89	12	122
	C	4,0	39	64	19	58	0,07	88	0,33	146	5,6	366	73	374
		0,4	8	6	3	9	0,02	17	0,07	27	0,4	58	20	99
FEMALE (♀)		Biochemical parameters												
		Albumin (g/dL)	Urea (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirrubine (mg/dL)	Cholesterol (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)	CPK (g/dL)	Triglycerides (mg/dL)	LDH (mg/dL)
Final	T	4,4	40	77	20	28	0,06	71	0,43	106	5,5	452	39	458
		0,2	8	21	6	9	0,02	14	0,09	14	0,5	129 (*)	7	96 (*)
	C	4,5	44	95	21	23	0,07	70	0,44	93	5,7	675	53	790
		0,4	11	30	14	6	0,02	6	0,09	29	0,9	132	14	246

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alanine Transaminase; CPK: Creatine phosphokinase; LDH: Lactate dehydrogenase.

**Table S12:** Urine biochemistry of individualized rats. The table collects the individual data of the urine biochemistry analyses separated by sex and by group. Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group. (\*) significant (p<0,05).

Animal ID		Density (mg/dL)	pH	Leucocytes (cell/ $\mu$ L)	Nitrites	Proteins (mg/dL)	Glucose (mg/dL)	Ketone bodies (mg/dL)	Urobilinogen (mg/dL)	Bilirrubin (mg/dL)	Erythrocyt es (cell/ $\mu$ L)	Color
MALE ♂	TM1	1.010	7.0	negative	positive	25.00	normal	negative	normal	negative	10	pale amber.
	TM2	1.010	7.0	negative	positive	negative	normal	negative	normal	negative	10	pale amber
	TM3	1.005	7.0	negative	negative	negative	normal	negative	normal	negative	10	pale amber
	TM4	1.015	7.0	negative	positive	25.00	normal	negative	normal	negative	negative	pale amber
	TM5	1.015	6.5	negative	positive	25.00	normal	negative	normal	negative	10	amber
	CM1	1.015	7.0	negative	negative	75.00	normal	negative	normal	negative	10	pale amber
	CM2	1.015	7.0	25.0	positive	25.00	normal	5.00	normal	negative	10	pale amber
	CM3	1.020	6.5	25.0	positive	25.00	normal	negative	normal	negative	negative	amber
	CM4	1.020	6.0	25.0	negative	25.00	normal	5.00	normal	negative	negative	amber
	CM5	1.010	7.0	negative	positive	25.00	normal	negative	normal	negative	negative	pale amber
FEMALE ♀	TF1	1.005	7.0	negative	negative	negative	normal	negative	normal	negative	negative	pale amber
	TF2	1.015	6.5	negative	negative	25.00	normal	negative	normal	negative	negative	pale amber
	TF3	1.010	7.0	negative	positive	25.00	normal	negative	normal	negative	10	pale amber
	TF4	1.020	6.0	negative	negative	25.00	normal	negative	normal	negative	negative	amber
	TF5	1.020	6.0	negative	negative	negative	normal	negative	normal	negative	negative	amber
	CF1	1.015	6.0	25.0	negative	negative	normal	negative	normal	negative	10	pale amber
	CF2	1.030	5.0	25.0	positive	75.00	normal	5.00	normal	1.0	negative	brown
	CF3	1.020	6.0	negative	negative	25.00	normal	negative	normal	negative	negative	amber
	CF4	1.025	6.0	negative	positive	75.00	normal	5.00	normal	1.0	10	brown
	CF5	1.020	6.5	negative	negative	25.00	normal	negative	normal	negative	10	pale amber

**Table S13:** Absolute and relative (% vs total body weight) weight of the organs of male rats. Mean  $\pm$  standard deviation. Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group.

ID Group	Absolute organ weight (g)						
	Spleen	Heart	Liver	Thymus	Kidneys	Testes	Adrenals
TM	0.686	1.141	12.639	0.443	2.565	4.009	0.091
	0.079	0.115	1.769	0.093	0.206	0.374	0.023
CM	0.745	1.252	12.083	0.602	2.700	4.244	0.103
	0.087	0.131	1.788	0.205	0.277	0.476	0.026
ID Group	Relative organ weight (%)						
	Spleen	Heart	Liver	Thymus	Kidneys	Testes	Adrenals
TM	0.157	0.260	2.872	0.101	0.587	0.934	0.021
	0.018	0.021	0.215	0.020	0.068	0.139	0.006
CM	0.164	0.275	2.662	0.134	0.594	0.934	0.023
	0.020	0.033	0.438	0.044	0.084	0.139	0.005

**Table S14:** Absolute and relative (% vs total body weight) weight of the organs of female rats. Mean  $\pm$  standard deviation. Statistical analyses (Mann Whitney U) were carried out to compare the Treated (*C. alimentarius*) versus the Control (C) group.

ID Group	Absolute organ weight (g)						
	Spleen	Heart	Liver	Thymus	Kidneys	Testes	Adrenals
TF	0.450	0.680	6.041	0.397	1.343	0.102	0.088
	0.060	0.090	0.457	0.071	0.129	0.023	0.010
CF	0.443	0.839	6.187	0.366	1.382	0.096	0.094
	0.093	0.214	0.762	0.108	0.131	0.009	0.025
ID Group	Relative organ weight (%)						
	Spleen	Heart	Liver	Thymus	Kidneys	Testes	Adrenals
TF	0.211	0.319	2.841	0.186	0.634	0.048	0.042
	0.020	0.031	0.184	0.027	0.078	0.012	0.004
CF	0.213	0.398	2.940	0.174	0.657	0.046	0.045
	0.049	0.079	0.262	0.046	0.050	0.003	0.014