Effect of microplastics on rumen metabolism

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Microplastics problem

A survey revealed that milk in cartons is contaminated by microplastics

Which could be the source of contamination?

Packaging?

Processing?

Transfer soil - plant - animal?

Microplastic source in animal

Environmental pollution

Plastic degradation

Microplastic production

The microplastics are particles with a lenght variable from 10 µm to 1 µm and a diameter less than 10 µm, containing bisphenols or phthalates as additive

Where they can be found?

Crop and pasture

for soil (i.e., mulching, mechanical processing ecc..)

Water

(i.e., groundwater pollution)

Processing chain

(i.e, grinding, blowing, crushing ecc..)

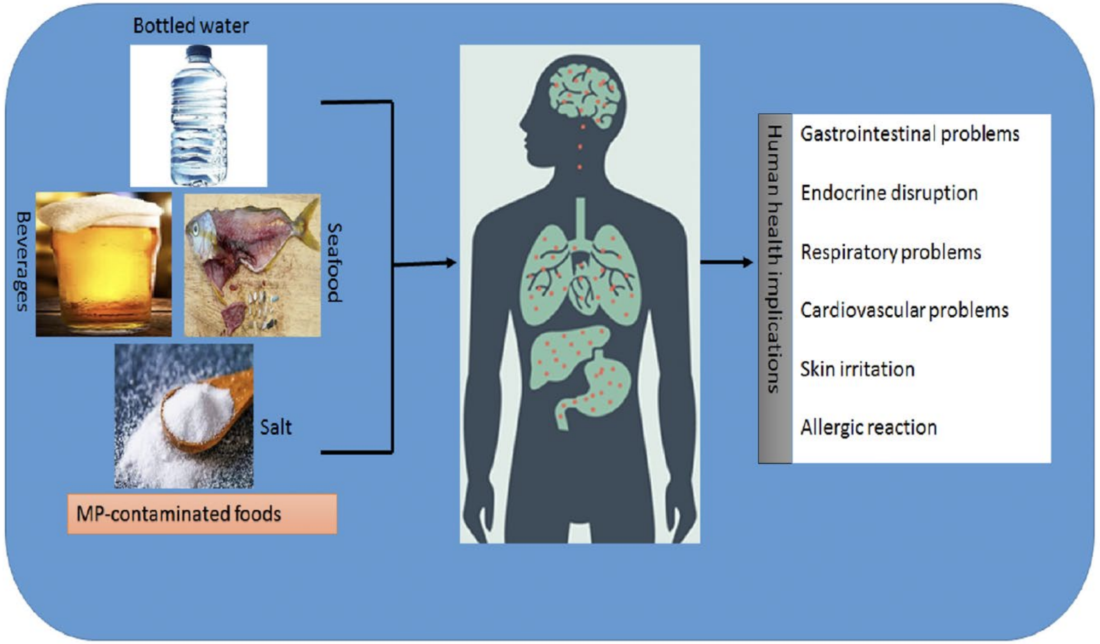
Thus

Ingredients of animal diet polluting by microplastics

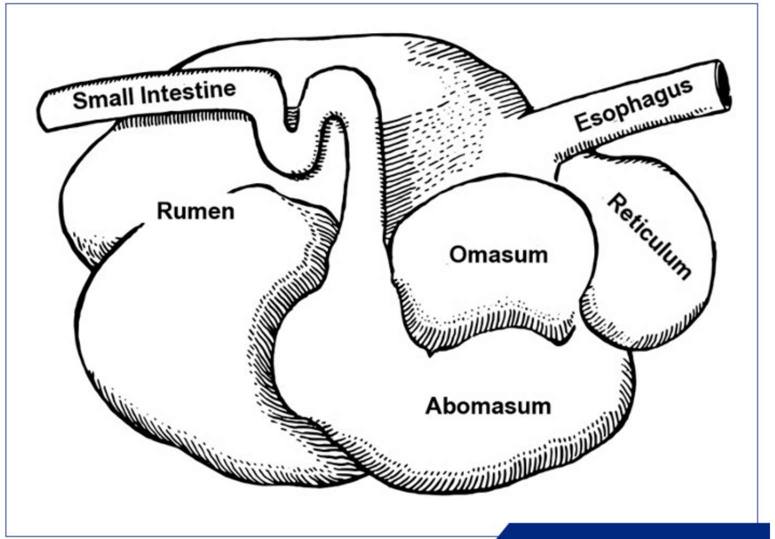
Microplastics problem

If microplastics interfere with animal metabolism, the contaminants can reach the end product of the animal production chain. The pollutants in the food make it dangerous for human health provoking dysmetabolism.

Bisphenols and phthalates are harmful to human health



Ruminant efficient



- Rumination (diet stratification, and double digestion)
- Fiber fermentation (cellulose and hemicellulose)
- Fat lipolysis and biohydrogenation
- Microbial protein production
- Nutrient absorption (ammonia and volatile fatty acid)

Ratio of rumen microbial community is affected by diet

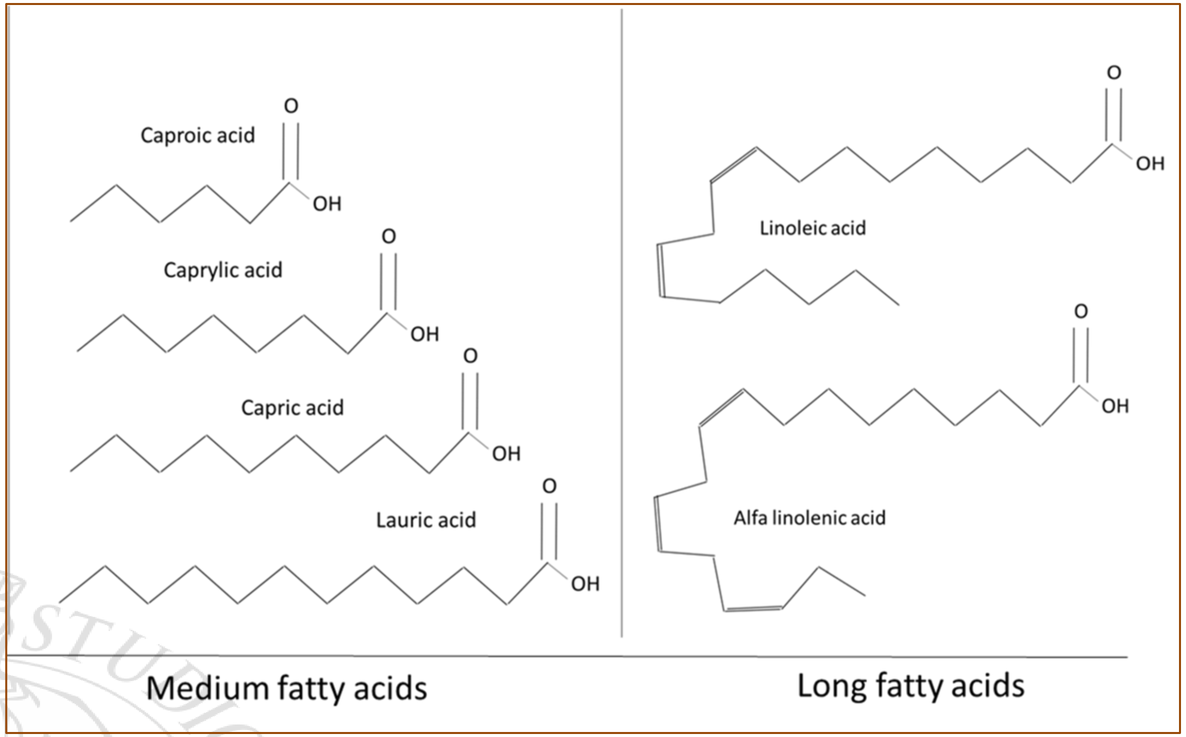
Microorganisms are involved in different metabolism pathways

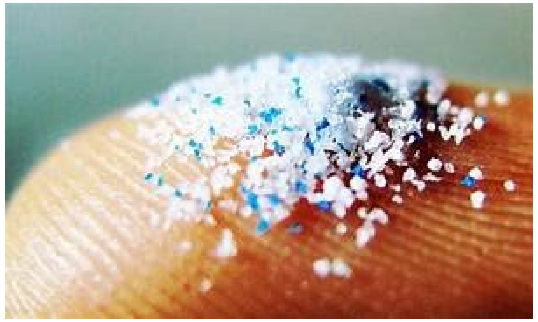
What are the effect of MPs on rumen microbial community???

Synergic Actors: Bacteria, Archea, Fungi, Protozoa

Scope

Preliminary study on the effect of several bisphenols and phthalates as additives for microplastic from plastic degradation on rumen microbiota biomarker through an *in vitro* trial





Materials and Methods

Experimental design.	*Chemical analysis.*	*Microbiota analysis.*
0,01 g of control feed per tube 4 ml of inoculum (1/4 LR + 3/4 saliva) 12 replicates per microplastic sample 1 sampling times (72 h)	Fatty acid profile characterization and residuals of microplastics determination after fermentation.	Rumen microbial community characterization by Metagenomic approach. Work in progress

Additives for microplastics *in vitro* fermented

- Bisphenol A
- Bisphenol S
- Bisphenol AF
- Bisphenol C
- Bisphenol F
- Monomethylphthalate
- Diethylphthalate
- Monobuthylphthalate
- Dimethylphthalate
- Monobenzylphthalate
- Dioctylphthalate

Concentration of 0.83 mg/mL

Materials and Methods

FATTY ACID

Fatty acids were extracted using one-pot methylation following the Kramer et al., 1997 protocol.

The fatty acid profile was determined by gas Chromatography using a GC-FID.

The C9:0 and C19:0 methyl esters were used as internal standard for fatty acid quantification.

Statistical analysis of fatty acid

$$y_{ij} = \mu + D_i + e_{ij}$$

MICROBIOTA

The DNA was extracted using Fast DNA Spin for soil kit (MP Biomedicals, Solon, OH)

The V3-V4 region of the 16S rRNA gene was PCR-amplified with Pro341F and Pro805R primers (Takahashi et al. 2014).

Sequencing in progress

Results and discussion

Fatty acid from C18:0 carbon chain biohydrogenation process

Fatty acid	BIS_A	BIS_AF	BIS_C	BIS_F	BIS_S	CON	DI_ET_PHT	DIM_ET_PHT	DIOCTYL_PHT	MON_BEN_PHT	MON_BUT_PHT	MON_MET_PHT	SEM	p value
C18	14.597d	17.039cd	16.857cd	21.375bcd	29.047abcd	40.493a	34.515abc	40.32a	26.058abcd	23.466abcd	38.995ab	31.488abcd	4.388	<0.0001
C18:1 t4	0.015bc	0c	0c	0.014bc	0.079ab	0.117a	0c	0c	0c	0c	0c	0.057abc	0.017	<0.0001
C18:1 t5	0.008c	0.056bc	0.071abc	0.115ab	0.075abc	0.091abc	0.111ab	0.096abc	0.042bc	0.081abc	0.084abc	0.153a	0.023	0.0012
C18:1 t6-8	0.056d	0.158cd	0.158cd	0.251bcd	0.502ab	0.664a	0.254bcd	0.361bc	0.285bcd	0.378bc	0.372bc	0.654a	0.061	<0.0001
C18:1 t9	0.161b	0.180b	0.491ab	0.696a	0.812a	0.795a	0.734a	0.756a	0.830a	0.693a	0.663a	0.865a	0.096	<0.0001
C18:1 t10	0.131d	0.219cd	0.217cd	0.290bcd	0.447ab	0.450ab	0.340abc	0.327abcd	0.288bcd	0.351abc	0.328abcd	0.503a	0.046	<0.0001
C18:1 t11	0.987cd	0.850d	1.283bcd	2.751a	2.086ab	1.714bcd	1.506bcd	1.533bcd	1.599bcd	1.976abc	1.591bcd	1.953abc	0.228	<0.0001
C18:1 t12	0.157d	0.214cd	0.198cd	0.472a	0.382abc	0.327abcd	0.371abc	0.293abcd	0.247bcd	0.351abcd	0.316abcd	0.409ab	0.048	<0.0001
C18:1 c9	26.429ab	26.570a	25.792ab	22.267ab	19.594b	11.017c	10.280c	8.435c	11.279c	10.019c	9.273c	9.331c	1.632	<0.0001
C18:1 c7	2.926a	0.150b	0.146b	0.250b	0.171b	0.183b	0.281b	0.272b	0.148b	0.319b	0.393b	0.297b	0.395	0.0003
C18:1 c11	0.262	0.503	0.436	0.635	0.995	0.326	0.522	0.427	0.570	0.609	0.598	0.471	0.163	0.1220
C18:1 c12	4.086a	1.843bcd	3.258ab	1.108cd	2.146bcd	2.156bcd	1.591cd	1.067d	2.507bc	2.028bcd	2.075bcd	1.946bcd	0.343	<0.0001
C18:1 c13+t1	0.065ab	0b	0.117ab	0.069ab	0.284a	0.103ab	0b	0b	0b	0b	0b	0.039b	0.051	0.0008
C18:1 c14+c1	0.235	0.243	0.308	0.252	0.407	0.434	0.424	0.383	0.211	0.346	0.386	0.471	0.076	0.0684
C18:1 c15	0.325bc	0.189c	0.438bc	0.313bc	0.273bc	0.378bc	0.722b	0.701b	0.624bc	0.675b	0.617bc	1.309a	0.112	<0.0001
C18:2 c9c12	9.328a	8.635ab	8.670ab	7.587abc	4.488bcd	2.581d	5.468abcd	4.204cd	8.671ab	6.419abcd	5.503abcd	4.005cd	1.015	<0.0001
C18:3 c9t12t15	0.004b	0.080ab	0.045ab	0b	0.091ab	0.090ab	0.201a	0b	0b	0b	0b	0.115ab	0.040	0.0006
C18:3 c9c12c15	2.995a	2.439b	1.869c	1.484cd	1.212de	0.838ef	0.824ef	0.621f	0.672f	0.638f	0.771ef	0.810ef	0.118	<0.0001
C18:2 c9t11	0.196bc	0.514a	0.775a	0.503ab	0.120c	0.086c	0.121c	0.015c	0c	0.069c	0.071c	0.106c	0.068	<0.0001
C18:3 c9t11c15	0.070ab	0c	0.070ab	0c	0.048abc	0.094a	0c	0.017bc	0c	0c	0c	0c	0.016	<0.0001

higher value

lower value

median value

Bisphenols affect differently microbial fatty acid metabolism compared to phthalates.

Results and discussion

Iso and anteiso form of fatty acids as indicators of cellulolytic and amylolytic metabolism

Fatty acid	BIS_A	BIS_AF	BIS_C	BIS_F	BIS_S	CON	DI_ET_PHT	DIM_ET_PHT	DIOCTYL_PHT	MON_BEN_PHT	MON_BUT_PHT	MON_MET_PHT	SEM	p value
C5 iso	0.532bcd	0.512d	0.536bcd	0.562bcd	0.659bcd	0.587bcd	1.503ab	1.443abc	1.94a	1.695a	1.016abcd	1.06abcd	0.228	<0.0001
C13 iso	0.170	0.249	0.225	0.238	0.145	0.206	0.249	0.149	0.109	0.185	0.214	0.259	0.044	0.1089
C13	1.737a	0.681abc	0.154c	0.493bc	1.286ab	0.601abc	0.340bc	0.735abc	0.633abc	1.03abc	0.200c	0.366bc	0.259	0.0004
C15 iso	0.242	0.299	0.322	0.259	0.213	0.242	0.343	0.272	0.157	0.281	0.301	0.371	0.056	0.1769
C15 ante	0.033b	0.077ab	0.046b	0.108ab	0.04b	0.067ab	0.187a	0.098ab	0.044b	0.045b	0.058ab	0.124ab	0.033	0.0091
C15	0.883bc	0.773c	0.829bc	1.154abc	0.884bc	1.238abc	1.188abc	1.366abc	1.169abc	1.723a	1.304abc	1.533ab	0.182	0.0008
C17 ante	0.545ab	0.486b	0.584ab	1.064a	0.438b	0.686ab	0.758ab	0.850ab	0.610ab	1.067a	0.771ab	0.960ab	0.134	0.0010
C17	0.471d	0.590d	0.716bcd	0.658cd	0.597cd	1.051abc	0.960abcd	0.892abcd	1.076abc	1.319a	1.138ab	1.151ab	0.115	<0.0001
C18 iso	0.022	0.061	0.022	0	0.085	0.06	0.017	0.032	0	0.039	0	0.038	0.027	0.2509
C20 ante	0.823bc	1.758a	1.025b	0.669bc	0.313c	0.404c	0.549bc	0.381c	0.373c	0.417c	0.307c	0.643bc	0.126	<0.0001

higher value

lower value

Bisphenols affect microbial fatty acid metabolism similarly to phthalates.

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Results and discussion

C10:0
C12:0
C13:0
C14:0
C15:0
C16:0
C17:0
C18:0
C20:0
C21:0
C22:0
C23:0

Treatment							Mean
CON	A						68.0
DIM_ET_PHT	A						67.4
MON_BUT_PHT	A	B					66.2
MON_MET_PHT	A	B	C				62.6
DI_ET_PHT	A	B	C				62.0
MON_BEN_PHT	A	B	C				58.9
BIS_S		B	C	D			56.1
DIOCTYL_PHT			C	D	E		55.1
BIS_F				D	E	F	47.6
BIS_AF					E	F	44.5
BIS_C						F	44.0
BIS_A						F	43.0
p value							< 0.0001

Significant differences of **SATURATED** mean value among treatments

Results and discussion

Significant differences of **UNSATURATED** mean value among treatments

C12:1
C14:1
C16:1
C17:1
C18:1 t6-8
C18:1 t9
C18:1 t10
C18:1 t11
C18:1 t12
C18:1 c9
C18:1 c7
C18:1 c11
C18:1 c12
C18:1
c14+c1
C18:1 c15
C20:1
C22:1

TREATMENT			MEAN
BIS_A	A		37.2
BIS_C	A		35.5
BIS_AF	A		32.7
BIS_F	A		31.5
BIS_S	A		29.2
MON_MET_PHT		B	20.7
CON		B	20.5
DIOCTYL_PHT		B	20.2
MON_BEN_PHT		B	20.0
DI_ET_PHT		B	18.9
MON_BUT_PHT		B	18.4
DIM_ET_PHT		B	16.1
p value			<0.0001

Bisphenols affect differently microbial fatty acid metabolism compared to phthalates selectively.

Conclusion

Bisphenols and phtalates act differently on rumen metabolism.

Bisphenols interfere with rumen biohydrogenation process, favouring the accumulation of oleic acid and linolenic acid concentrations.

The effect on the cellulolytic and amylolytic metabolisms seem to be similar for both kinds of microplastics.

Data on rumen microbiota and on residual microplastic post-fermentation should evidence if the microbial community ratio is altered by different microplastics and if microorganisms are able to metabolize microplastics. More research is needed.

In the future, we will study how the feed is concentrated in microplastics and how they may interfere with milk or meat quality profile.

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Thank you for your attention