DEFATTED INSECT MEALS: impact on in vitro ruminal fermentation and lipid biohydrogenation

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1. Introduction

Renna et al. (2023), Anim. Front. 13(4): 102-111

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Table 1. Diets containing insect-derived products compared to control diets: impact on nutrient digestibility and ruminal fermentation parameters

Ruminant species	Insect species	Insect form	Inclusion level, %	IVOMD	IVDMD	Intake	IDNDN	Total VFA	Ac:Pr	pH	NH_3-N	Reference
Bovine	HI1	larvae meal	20	↓	↓					↑	↓	Jayanegara et al. (2017a)
	HI2	larvae meal	20	↓	↓					↑	↓	
	HI1	larvae meal	40	↓	↓					↑	↓	
	HI2	larvae meal	40	↓	↓			↓		↑	↓	
Bovine	HI	larvae oil	5									Jayanegara et al. (2020)
	OS	larvae oil	5	↓								
	TM	larvae oil	5	↓	↓							
	ZM	larvae oil	5	↓	↓							
	GB	nymph oil	5	↓	↓							
Bovine	HI	larvae oil	1		↓							Jayanegara et al. (2021a)
			2	↓	↓						↑	
			3	↓	↓						↑	
			4	↓	↓						↑	
			5	↓	↓						↑	
Bovine	ACD	adult meal	10							↑	↑	Ahmed et al. (2021)
	BP	adult meal	10							↑	↑	
	GB	adult meal	10							↑		
	BM	pupae meal	10							↑	↑	
Bovine (beef)[#]	HI	larvae meal	36									Fukuda et al. (2022)
Bovine	GB	adult meal[*]	4-16		↑					↑		Phesatcha et al. (2022)
Bovine	HI	larvae meal	20	↓	↓			↓		↓		Mulianda et al. (2020)
		larvae meal, chemical defatting	20					↑			↑	
		larvae meal, mechanical defatting	20		↓			↓			↓	
Bovine (crossbred cattle)[#,†]	BM	defatted pupae meal	1.4									Rashmi et al. (2022)
			2.7					↓			↓	
			4.1								↓	
Ovine	HI	larvae oil	2									Hervás et al. (2022)
	ACD	adult oil	2									
	BM	pupae oil	2									
Ovine	ALD	larvae meal	100	↓				↓	↑	↑	↓	Renna et al. (2022b)
	HI	larvae meal	100	↓				↓	↑	↑	↓	
	MD	larvae meal	100	↓				↓	↑	↑	↓	
	TM	larvae meal	100	↓				↓		↑	↓	
	BL	subadult meal	100	↓				↓	↑	↑	↓	
	ACD	adult meal	100	↓				↓	↑	↑	↓	
	GB	adult meal	100	↓				↓		↑	↓	
	GS	adult meal	100	↓				↓	↑	↑	↓	
Ovine	TM	larvae meal	100				↑					Toral et al. (2022)
	ZM	larvae meal	100									
	ALD	larvae meal	100									
	ACD	adult meal	100									
Caprine (post-weaning)[#]	GB	adult meal	15									Astuti et al. (2019)
			30									

High dietary lipids in ruminant nutrition:

- reduction of feed intake
- impaired rumen fermentation
- milk fat depression

Palmquist and Jenkins (2017), J. Dairy Sci. 100(12): 10061

In ruminant nutrition, the use of defatted meals could be more appropriate than the use of full-fat meals

AIM

to investigate, in vitro, the effects of the residual ether extract content of defatted HI and TM meals on rumen fermentation characteristics and lipid biohydrogenation

2. Materials and Methods

Insect meals

5 defatted *H. illucens* meals

- 19.7 g EE/kg DM **(HI19.7)**
- 12.8 g EE/kg DM **(HI12.8)**
- 9.2 g EE/kg DM **(HI9.2)**
- 6.6 g EE/kg DM **(HI6.6)**
- 4.7 g EE/kg DM **(HI4.7)**

2 defatted *T. molitor* meals

- 8.1 g EE/kg DM **(TM8.1)**
- 5.7 g EE/kg DM **(TM5.7)**

2 full-fat *H. illucens* and *T. molitor* meals

Renna et al. (2022). J. Anim. Sci. Biotechnol. 13: 138

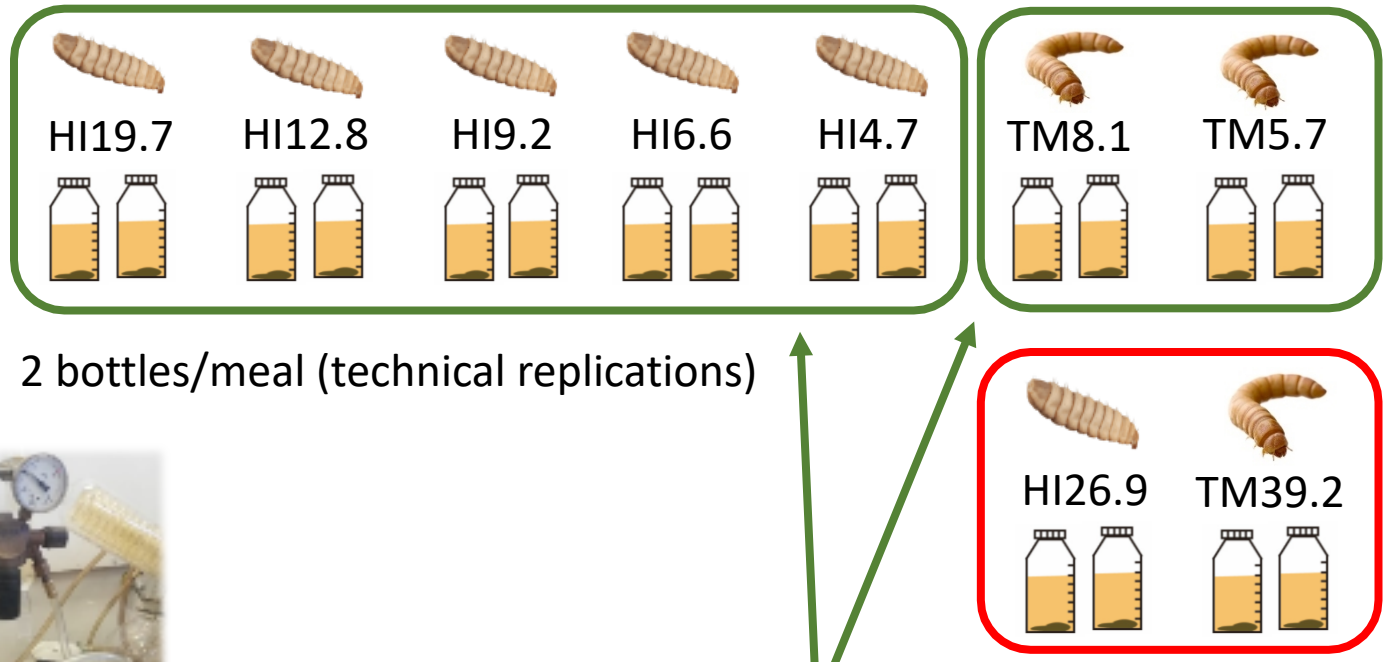
- 26.9 g EE/kg DM **(HI26.9)**
- 39.2 g EE/kg DM **(TM39.2)**

2. Materials and Methods

- 4 cannulated castrated male Texel sheep
- BW: 64.6 ± 7.10 kg
- fed permanent grassland hay and concentrate

In vitro fermentation

- 4 independent incubation runs of 24 h (statistical replications)
- In each incubation: all the meal samples, rumen fluid from one sheep
- 2 bottles/meal (technical replications)



Collection of the supernatant fraction

Preparation of FA samples for high-resolution GC

2. Materials and Methods

Laboratory analyses – insect meals

- DM, ash, CP, EE, ADF, ADL (AOAC methods, 2000; 2003)
- Different K_p according to insect species and meal type (Janssen et al.., 2017)
- NDF (Mertens, 2002)
- NDIN and ADIN (Licitra et al., 1996)
- Chitin (Finke, 2007)
- FA composition (Renna et al., 2020)

Laboratory analyses – rumen content

- VFA (Jouany, 1982)
- NH_3 (Weatherburn, 1967)
- CH_4, CO_2 and H_2 (Macheboeuf et al., 2008)
- FA composition (Alves et al., 2013)

Statistical analysis

- GLM ANOVA procedure in SPSS (v. 27.0)
- Normality and homogeneity of variances
- Model: insect species (2 levels; fixed effect), incubation run (4 levels, random effect), residual EE content (covariate), insect species × EE (fixed effect)

3. Results

Chemical composition of the insect meals (g/100 g DM)

	HI 26.9	HI 19.7	HI 12.8	HI 9.2	HI 7.0	HI 4.7		TM 39.2	TM 8.1	TM 5.7
DM[1]	95.3	95.0	93.4	93.6	94.4	94.0		94.0	93.4	92.5
OM	91.1	93.3	89.8	88.2	87.8	91.8		96.1	94.7	94.8
CP	35.1	41.2	50.9	51.6	50.37	61.3		33.9	56.5	63.8
EE	26.9	19.7	12.8	9.2	7.0	4.7		39.2	8.1	5.7
NDF	28.2	31.5	25.7	26.8	29.8	25.4		22.5	22.5	24.8
ADF	7.9	8.3	8.8	9.1	14.2	10.1		8.2	8.3	9.1
ADL	1.5	0.4	0.8	1.1	1.9	1.2		2.0	1.6	0.6
NDIN	3.1	4.0	3.9	4.1	3.8	3.2		3.5	2.4	4.2
ADIN	2.6	3.2	3.1	3.1	3.0	2.6		3.2	2.0	3.3
Chitin	5.2	5.5	6.8	7.0	7.4	8.4		5.1	6.3	7.8

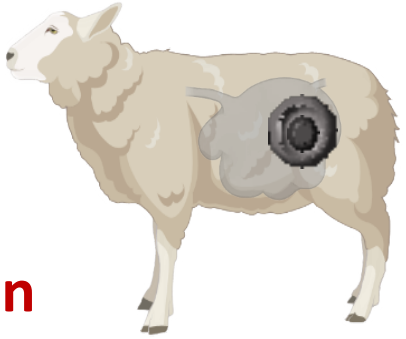
[1] g/100 g

3. Results

Fatty acid composition of the insect meals (g/100 g FA)

	HI 26.9	HI 19.7	HI 12.8	HI 9.2	HI 7.0	HI 4.7	TM 39.2	TM 8.1	TM 5.7
C12:0	26.8	36.3	35.9	43.89	32.8	34.4	0.30	0.39	0.33
C14:0	6.08	8.37	10.2	10.1	8.34	8.50	3.10	2.33	2.86
C16:0	20.0	16.6	19.2	16.5	19.2	17.3	17.2	19.4	16.8
C16:1 *cis*9	1.70	3.07	2.69	3.57	3.01	4.67	1.63	0.92	1.40
C18:0	2.97	3.12	4.51	3.24	4.26	3.37	2.90	5.91	4.61
C18:1 *cis*9	10.8	12.8	18.3	13.6	21.0	16.6	43.2	33.9	38.7
C18:2 n-6	24.5	16.4	5.82	5.48	6.96	10.4	28.0	31.8	30.6
C18:3 n-3	2.01	1.18	0.28	0.47	0.45	1.12	1.42	1.40	1.32
Σ SFA	56.8	65.6	71.7	75.6	66.7	65.9	24.1	29.6	26.3
Σ MUFA	14.0	16.6	21.8	18.1	25.0	22.2	45.7	36.5	41.1
Σ PUFA	28.3	17.6	6.10	5.95	7.46	11.6	29.5	33.2	32.0

3. Results

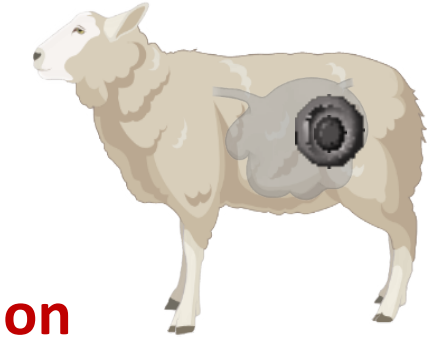


In vitro rumen fermentation parameters after 24 h incubation

										P-value			
	Insect Species	Intercept	Intercept SEM	Species Coefficient	Species SEM	EE Coefficient	EE SEM	EE × Species Coefficient	EE × Species SEM	Intercept	Species	EE	EE × Species
Total gas, mmol/g DM		3.02	0.089	-	-	-0.038	0.0049	-	-	0.000	0.91	0.000	0.27
CH_4, µmol/g DM		743	26.7	-	-	-12.9	1.46	-	-	0.000	0.60	0.000	0.13
CH_4, mmol/mmol total gas	HI TM	0.253	0.0041	-	-	-0.002	0.0002	-0.0006 +0.0006	0.0002	0.000	0.14	0.000	0.017
Total VFA, mmol/g DM		5.83	0.105	-	-	-0.063	0.0057	-	-	0.000	0.60	0.000	0.12
Acetate, g/100 g VFA	HI TM	62.3	0.30	+1.66 -1.66	0.016	-0.18	0.016	-0.045 +0.045	0.0162	0.000	0.000	0.000	0.009
Propionate, g/100 g VFA		15.4	0.22	-	-	+0.122	0.0119	-	-	0.000	0.98	0.000	0.52
Butyrate, g/100 g VFA	HI TM	7.6	0.20	-	-	+0.088	0.0109	+0.055 -0.055	0.0109	0.000	0.37	0.000	0.000
Acetate:Propionate ratio		4.0	0.06	-	-	-0.033	0.0031	-	-	0.000	0.09	0.000	0.49
IVOMD, g/100 g	HI TM	45.9	0.97	+2.41 -2.41	0.966	-0.57	0.053	-0.209 +0.209	0.053	0.000	0.18	0.000	0.000
NH_3-N, mmol/g DM	HI TM	3.94	0.072	-0.23 +0.23	0.072	-0.036	0.0039	-	-	0.000	0.004	0.000	0.96

Abbreviations: VFA, volatile fatty acids; IVOMD, in vitro organic matter disappearance.

3. Results



Fatty acids of rumen digesta (g/100 g FA) after 24 h incubation

										P-value			
	Insect Species	Intercept	Intercept SEM	Species Coefficient	Species SEM	EE Coefficient	EE SEM	EE × Species Coefficient	EE × Species SEM	Intercept	Species	EE	EE × Species
C12:0	HI TM	11.7	1.37	+10.9 -10.9	1.37	-	-	-	-	0.000	0.000	0.574	0.517
C18:0	HI TM	10.76	0.566	-2.53 +2.53	0.566	-0.09	0.031	-	-	0.000	0.000	0.005	0.176
C18:2 n-6	HI TM	10.78	0.764	-7.31 +7.31	0.764	0.12	0.042	-	-	0.000	0.000	0.007	0.062
Σ SFA	HI TM	58.52	1.39	+10.01 -10.01	1.392	-0.18	0.076	-	-	0.000	0.000	0.027	0.107
Σ BCFA		11.46	0.514	-	-	-0.19	0.028	-	-	0.000	0.081	0.000	0.495
Σ MUFA	HI TM	17.73	0.784	-	-	0.14	0.043	-0.23 +0.23	0.043	0.000	0.071	0.002	0.000
Σ PUFA	HI TM	13.11	0.794	-7.42 +7.42	0.794	0.12	0.043	-	-	0.000	0.000	0.013	0.082

Abbreviations: SFA, saturated fatty acids; BCFA, branhced-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

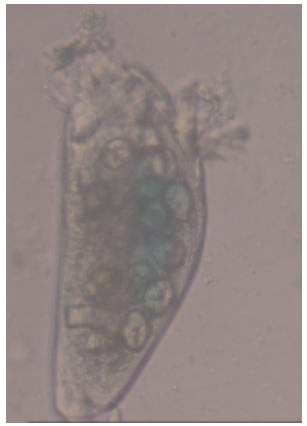
4. Discussion and Conclusions

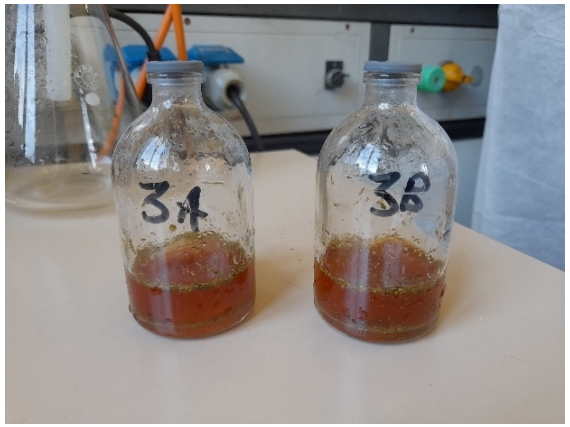
1. **Defatting of insect meals increases the overall ruminal fermentation process**
   - high dietary fat inhibits rumen microorganisms and decreases CHO digestibility
   - good for the animal (VFA main energy currency), bad for the environment (GHG emissions)
   - specific effect on methanogenesis slightly more pronounced at increasing EE with HI than with TM

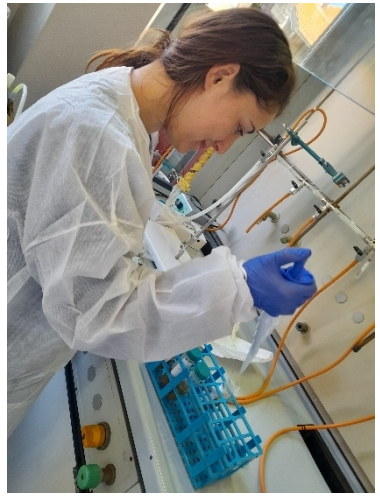
2. **When compared to conventional plant meals, lower IVOMD for the defatted insect meals**
   - chitin content increases with defatting and it is poorly degraded inside the rumen (use of chitinases?)
   - still higher fat in defatted meals than in conventional plant meals

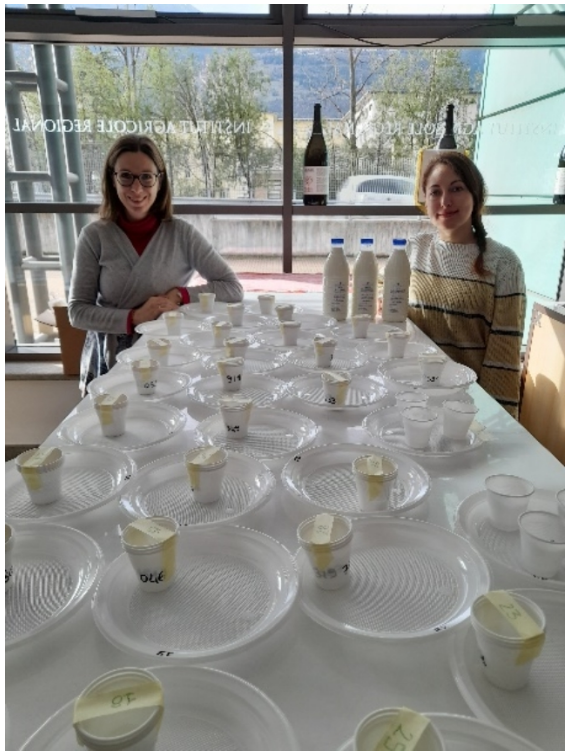
3. **Defatting increases dietary UFA biohydrogenation and synthesis of FA of microbial origin**
   - high dietary fat inhibits rumen microorganisms
   - pay attention to high hypercholesterolemic SFA and low PUFA with HI

5. Further investigations are ongoing!









TIME TO MOVE TO *IN VIVO* TRIALS

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