

EAAP 2019

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Galactomannan fenugreek extract as proposed alternative to antibiotics in young rabbits nutrition

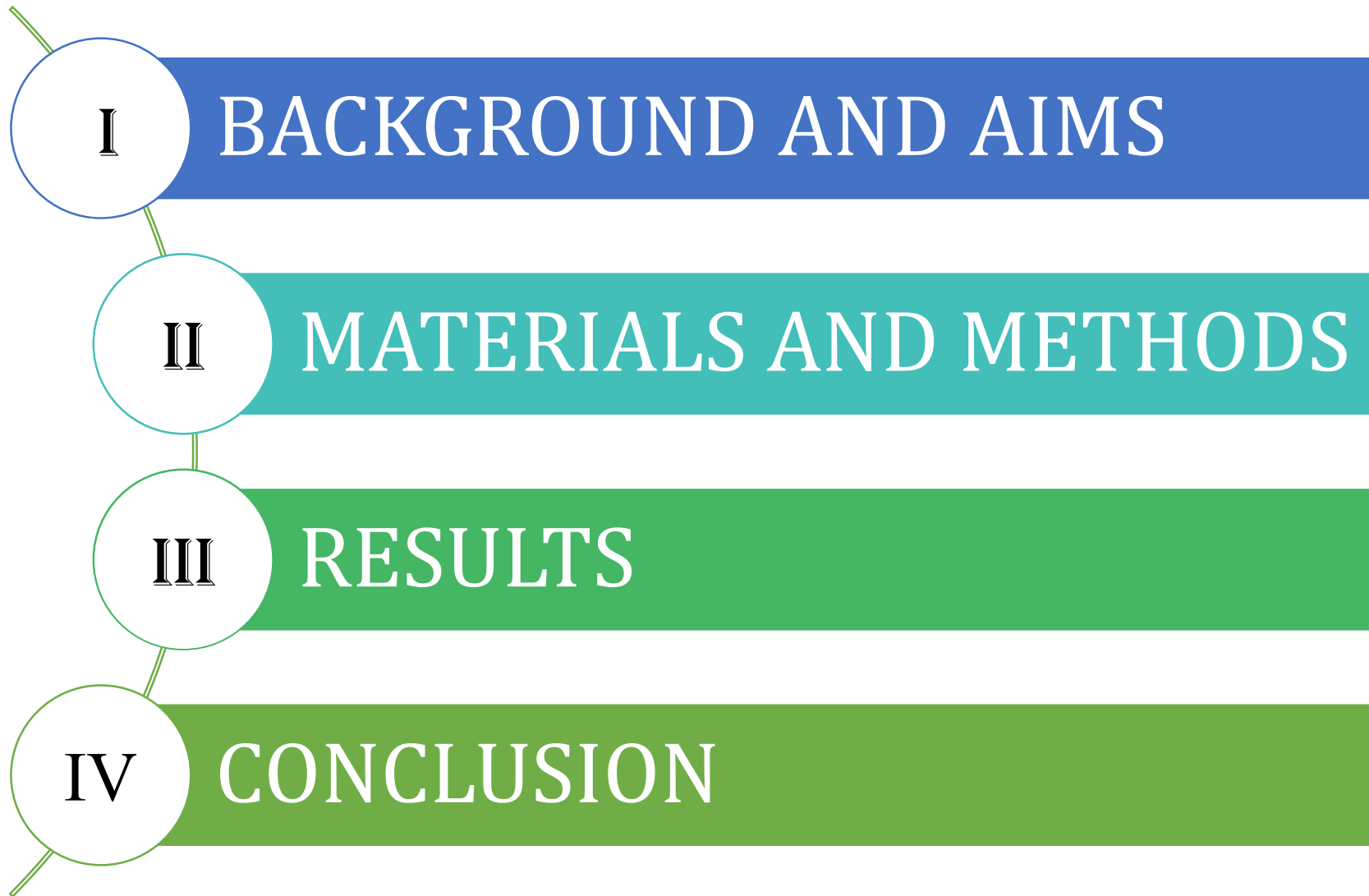
Zemzmi Jihed

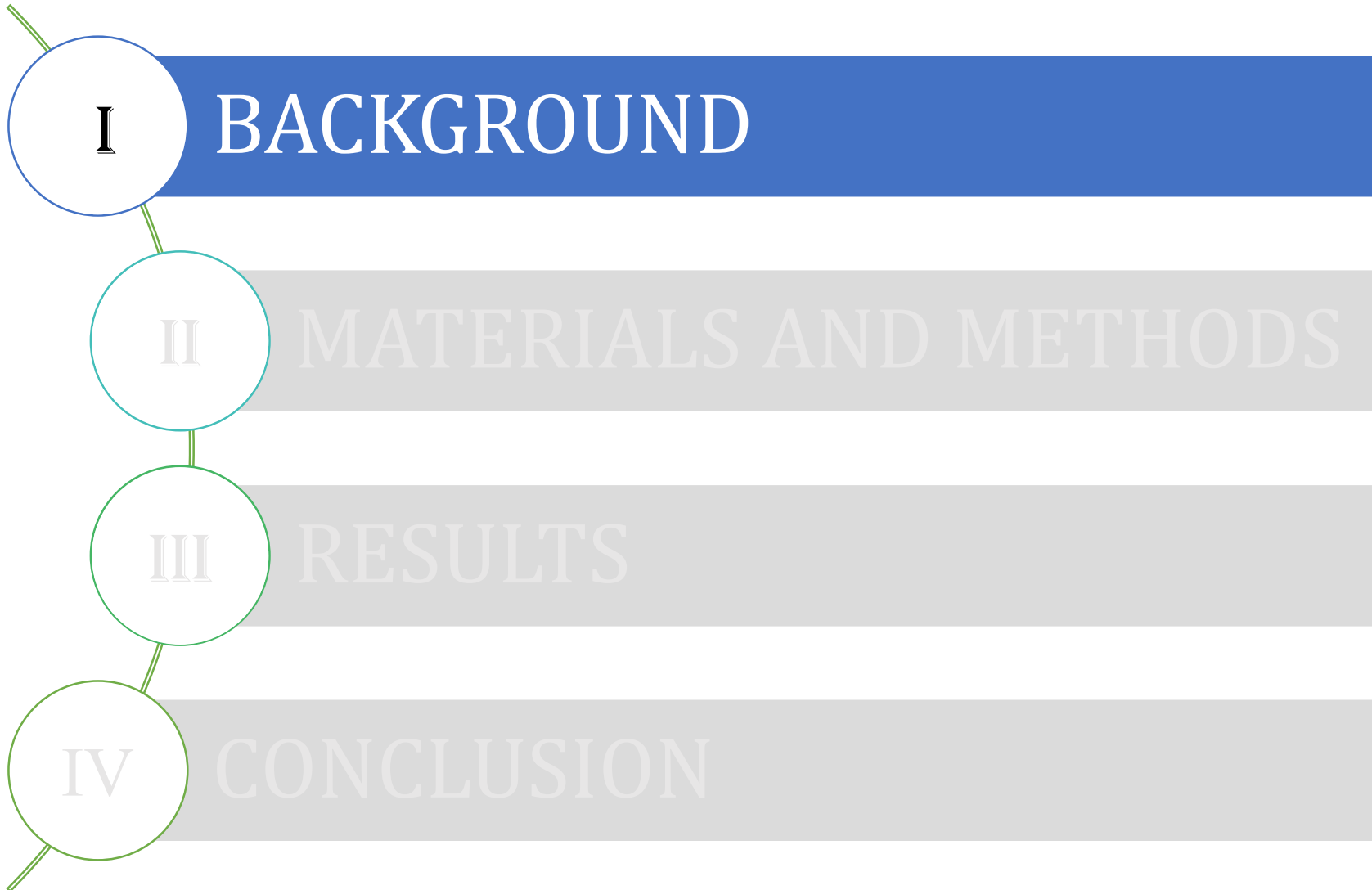
Rodenas L, Blas E, Martínez-Paredes E, López-Lujan MC, Moya J, Najjar T, Pascual JJ



ICTA







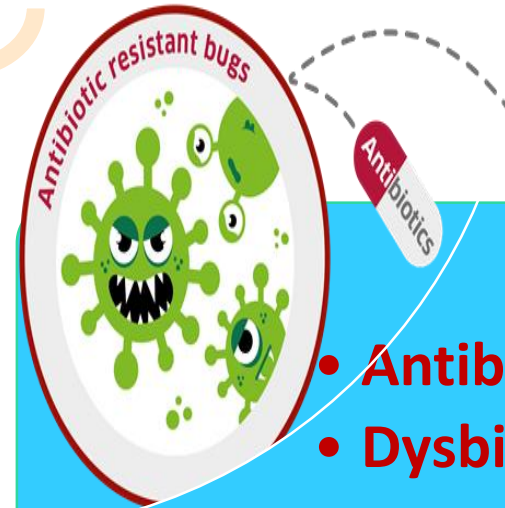
- High incidence of digestive diseases



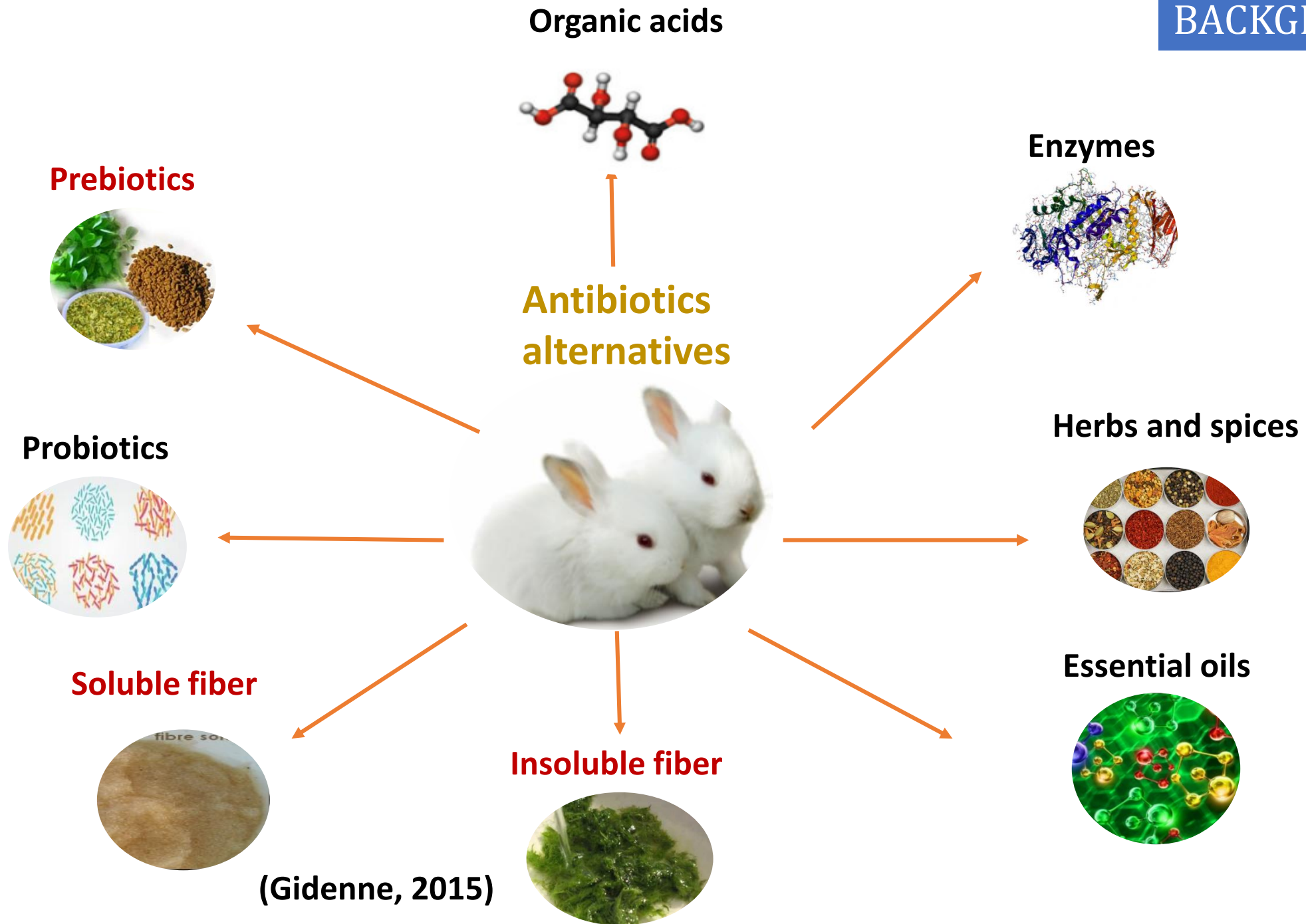
- High dependence on antimicrobials



- Natural alternatives



- Antibioresistance
- Dysbiose



Prebiotics

Fenugreek galactomannan



Antibiotics alternatives



Soluble fiber

Beet pulp



Insoluble fiber

Grape seeds



Prebiotics ???
Galactomannan



Antibiotics alternatives



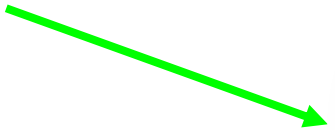
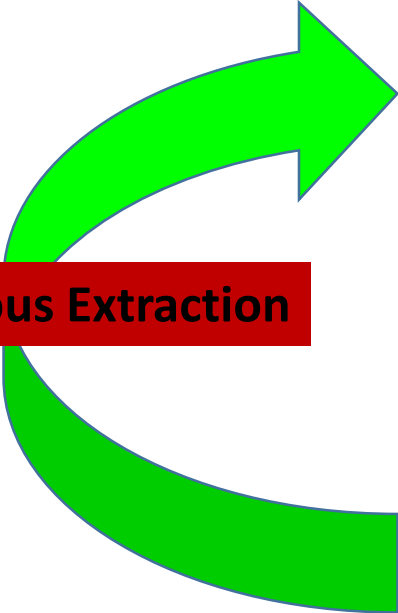
Fenugreek Plant
(*Trigonella foenum graecum*)



Fenugreek Seeds



Aqueous Extraction



Has Fenugreek galactomannan really a prebiotic effect ?

**Not digestible by
gastrointestinal enzymes**

**Highly fermentable by
intestinal bacteria**

**Selectively stimulates the
development and/or activity
of intestinal bacteria**

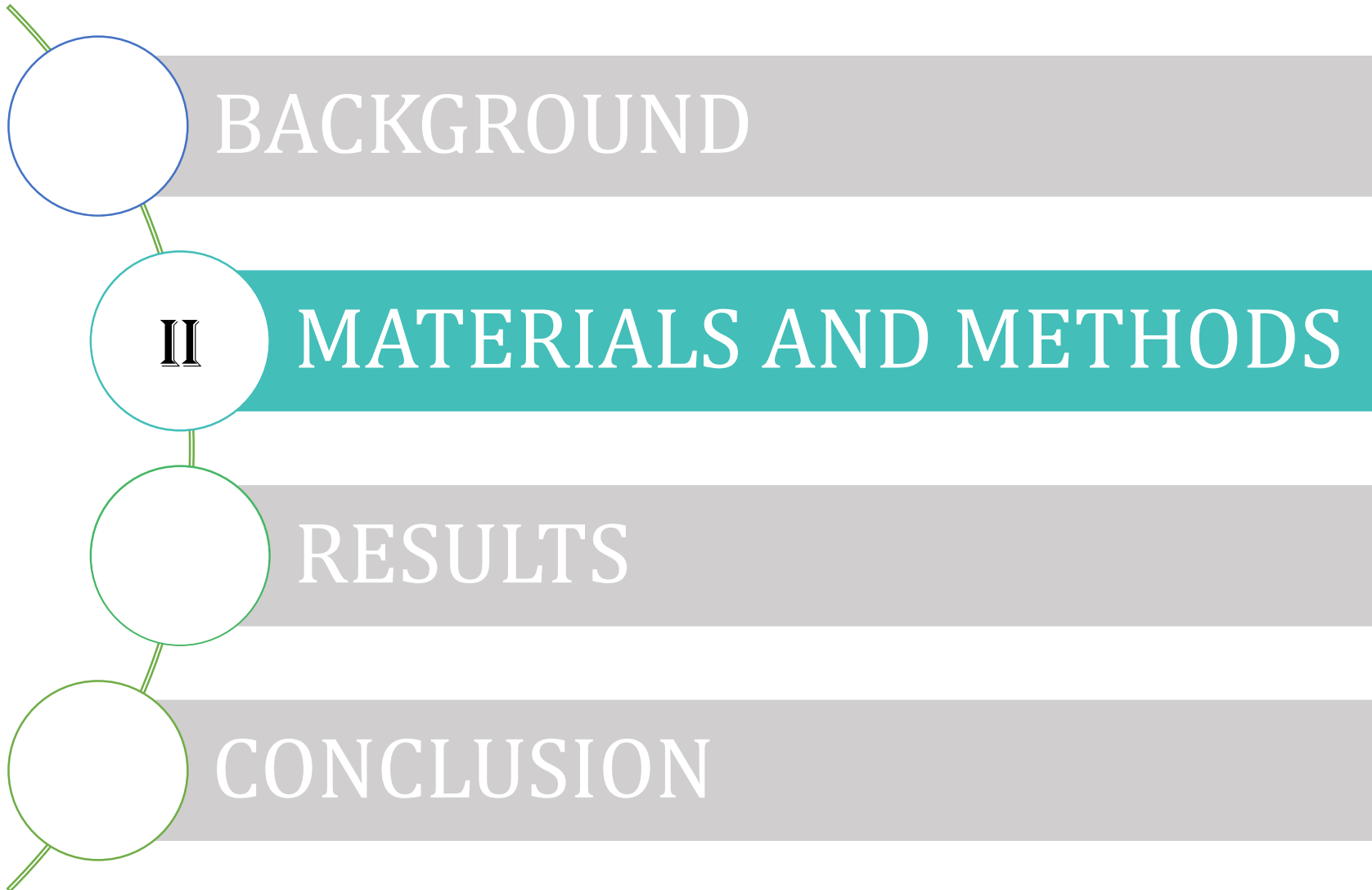


Characterization of fenugreek galactomannan (Chemical composition, purity)

***In vitro* evaluation of the prebiotic effect of fenugreek galactomannan in diets rich in soluble fiber or insoluble fiber (No digestibility and high fermentability)**

The evaluation of the *in vivo* inclusion of 1% of Fenugreek galactomannan in young rabbit's diet with different levels of soluble fiber on health status, faecal digestibility and caecal activity





BACKGROUND

II

MATERIALS AND METHODS

RESULTS

CONCLUSION

Characterization of fenugreek galactomannan

Materials and methods

Fenugreek galactomannan



Chemical characterization:
DM, OM, CP, NDF,
ADF, ADL,



Sugar monomers
Chromatography

Pepsin & Pancreatin digestion



Fenugreek galactomannan



Indigestible residue



Chemical characterization:
DM, OM, CP, NDF,
ADF, ADL,



Sugar monomers
Chromatography

Fenugreek galactomannan:
GM

Rabbit feed rich in **BEET
PULP** (10 %): SF

Rabbit feed rich in **GRAPE
SEEDS** (10 %): IF

+ 0, 0.5, 1, 1.5 and 2% of GM

Pepsin and pancreatin digestion

I

Indigestibles residues

II

Fermentation in rabbit's caecal inoculum: Pressure, pH, VFA, N-NH₃

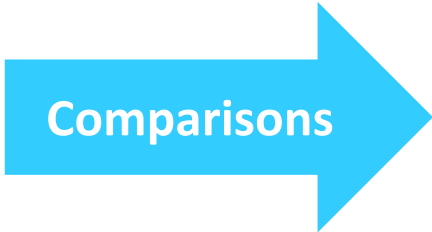


GLM of SAS (2009)



Fixed factors Diets and analysis repetitions

Orthogonal contrasts



GM level (0%GM vs 100% GM)

Lineal effect of GM



Lineal GM_{SF}

Lineal GM_{IF}

SF: 10% Beet pulp diet

IF: 10% Grape sedes diet

GM: Fenugreek galactomannan

In vivo 1% fenugreek galactomannan inclusion: effects on health status, faecal digestibility and caecal activity

Materials and methods

Animals

Group 1 (25 gazapos)

Group 2 (25 gazapos)

Group 3 (25 gazapos)

Group 4 (25 gazapos)

Diets without antibiotics

High soluble fiber diet
H

High soluble fiber diet
+1% GM
HGM

Low soluble fiber diet
L

Low soluble fiber diet
+ 1% GM
LGM

GM: Fenugreek galactomannan

Duration of the trail: weaning, 28 days to 63 day of age

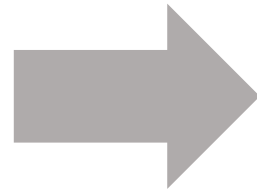
Mortality and morbidity: daily

Faecal digestibility: 49 to 53 days of age (Perez et al., 1995)

Caecal parameters: pH, VFA, N-NH₃ at 63 days of age



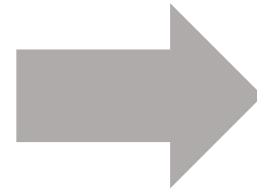
GLM of SAS (2009)



Fecal digestibility

Caecal activities

GENMOD of SAS (2009)



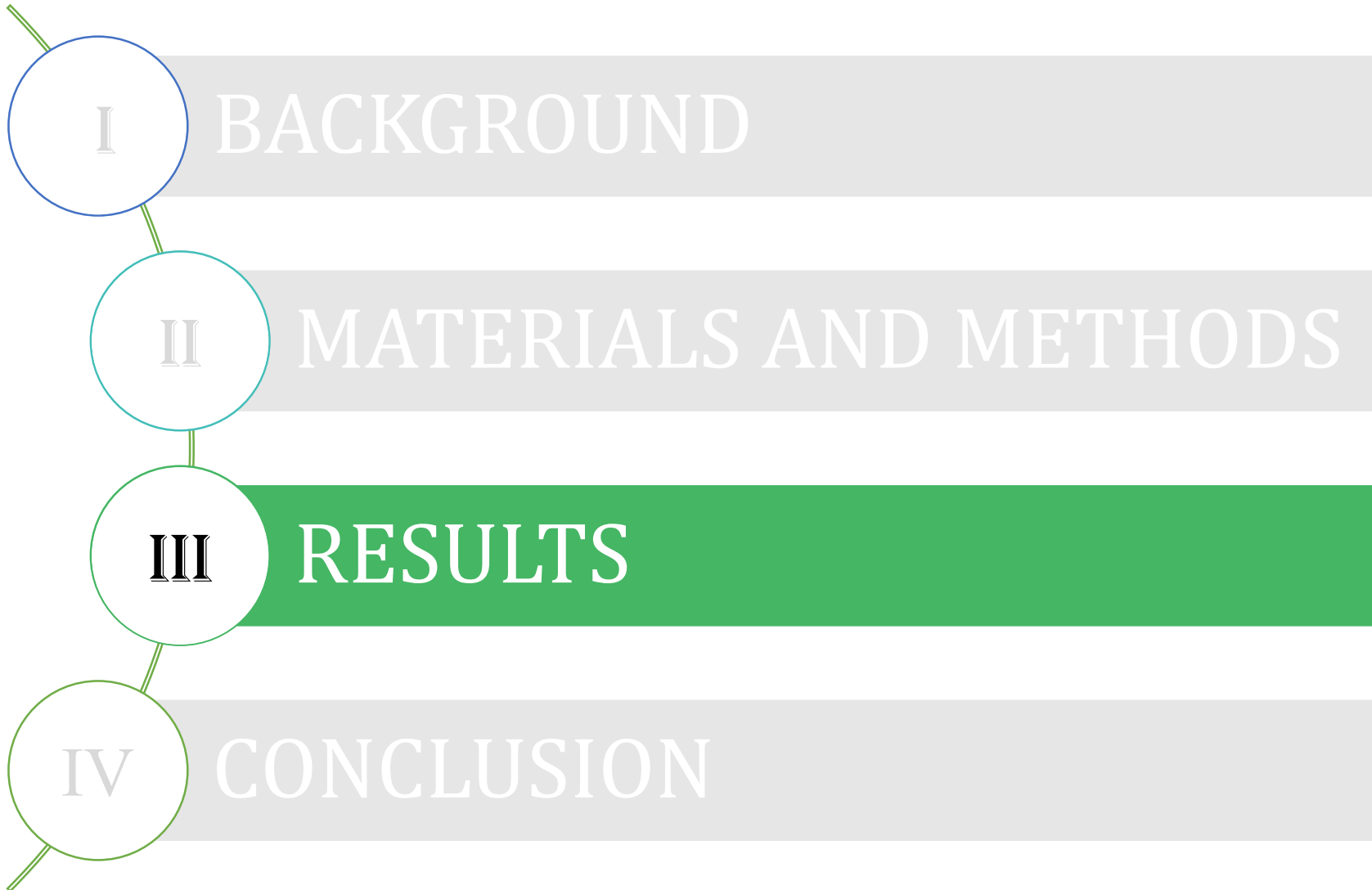
Mortality

Morbidity

Health Risk index

Logistic regression
Binomial distributions

Fixed effects: Fenugreek GM level, Fiber level and their interaction



RESULTS

Characterization

Characterization and no-digestibility of fenugreek GM

	Fenugreek GM	Indigestible residue GM pepsine and pancreatin
Humidity (%)	8.7	7.8
Crude protein (%) DM	22.3	16.4
Mannose (%)	48.5	45.4
Fructose (%)	<0.1	<0.1
Glucose (%)	<0.1	<0.1
Lactose monohydrate(%)	<0.1	<0.1
Maltriose (%)	<0.1	<0.1
Galactose %	51.5	54.6
Galactose/Mannose Ratio	1.1	1.2
Purity (%)	68.8	52.5

84% of fenugreek galactomannan resist to digestion with pepsine pancreatin → the first condition of the prebiotic effect is validated

RESULTS

IN VITRO

Fermentability of fenugreek galactomannan after digestion

	pH	Pression(mbar)	N-NH₃ mg/ml	tVFA mmol/ ml	(%)No-fermentable residue
Indigestible residue of fenugreek GM	5.7 ^a	2.0 ^c	27.0 ^a	46.1 ^c	1.6 ^a
Indigestible residue of soluble fiber diet	6.2 ^a	1.5 ^{bt}	89.4 ^b	21.3 ^b	58.9 ^b
Indigestible residue of insoluble fiber diet	6.3 ^c	1.3 ^a	102.4 ^c	16.3 ^a	66.5 ^c
P-value indigestible residues	***	***	***	***	***
P-value Reptition	***	***	***	***	***

Level of signification:

* p<0.05

** p<0.01

*** p<0.001

Fenugreek GM is totaly fermentable by rabbit's caecal bacteria → the second condition of the prebiotic effect is validated

RESULTS
IN VITRO

Effect of fenugreek GM on fermentation parameters

	0% fenugreek GM- 100% fenugreek GM	Lineal fenugreek GM Soluble Fiber	Lineal fenugreek GM Insoluble Fiber
tVFA	-27,32±3,31***	-0.72±2.44	1.79±2.44
Acetic acid	4.27±3.72	-1.84±2.74	0.12±2.74
Propionic acid	-3.08±0.87*	0.85±0.64	0.71±0.64
Butyric acid	-2.12±2.35	0.19±1.73	-0.92±1.73
Isobutyric acid	-0.15±0.06	0.06±0.04	0.01±0.04
Valeric acid	0.26±0.10*	0.20±0.07**	0.03±0.07
Isovaleric acid	-0.49±0.09***	0.08±0.06	0.04±0.06
Caproic acid	1.23±0.39**	0.42±0.28	-0.00±0.28
N-NH ₃	69.58±6.29***	12.50±5.41*	2.20±5.41

Level of
signification:
* p<0.05
** p<0.01
*** p<0.001

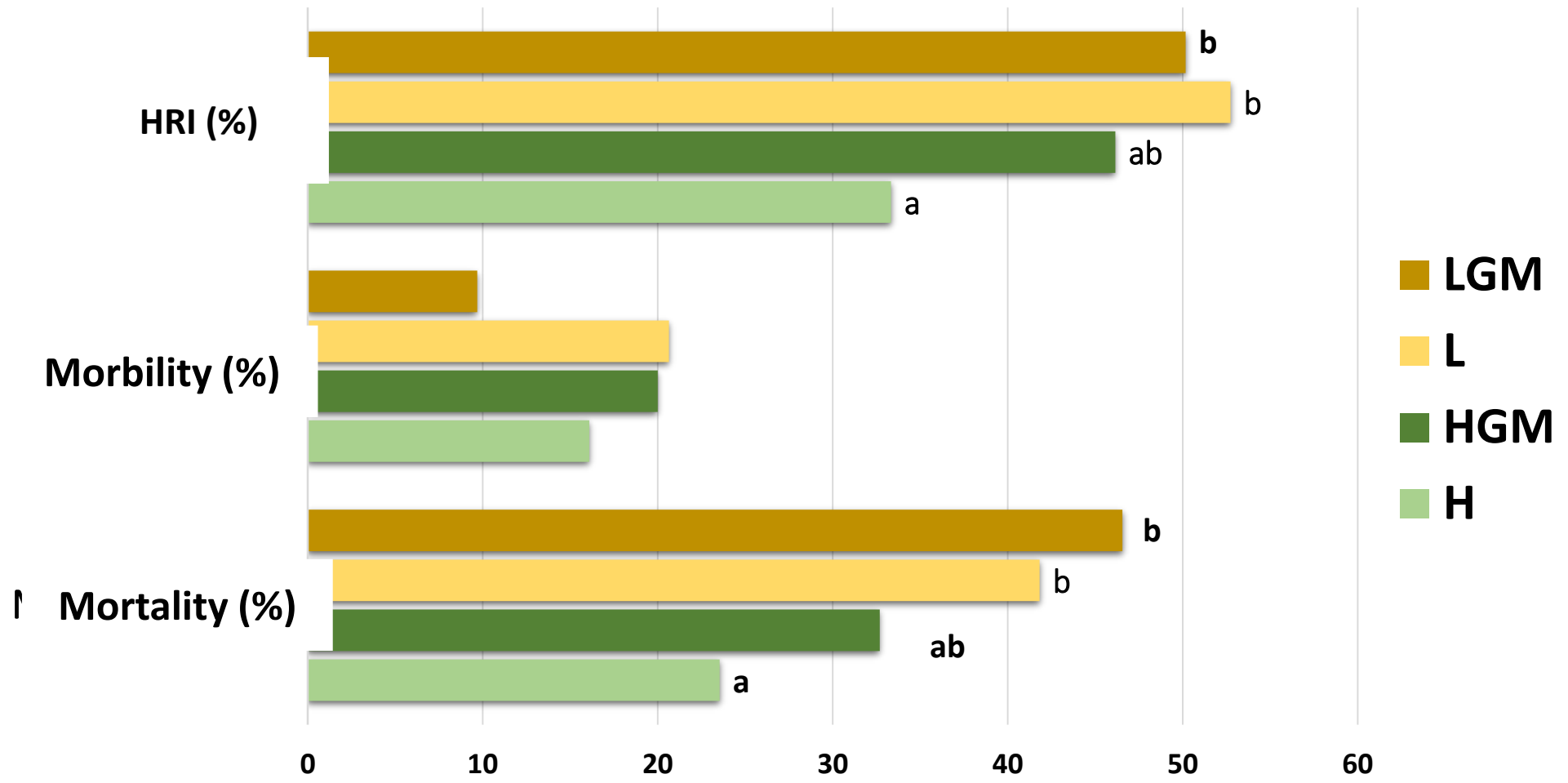
Effect of fenugreek GM on in vitro fermentation parameters

	0% fenugreek GM - 100% fenugreek GM	Lineal fenugreek GM_{Soluble} Fiber	Lineal fenugreek GM Insoluble Fiber
tVFA	-27,32±3,31***	-0.72±2.44	1.79±2.44
Valeric acid	0.26±0.10*	0.20±0.07**	0.03±0.07
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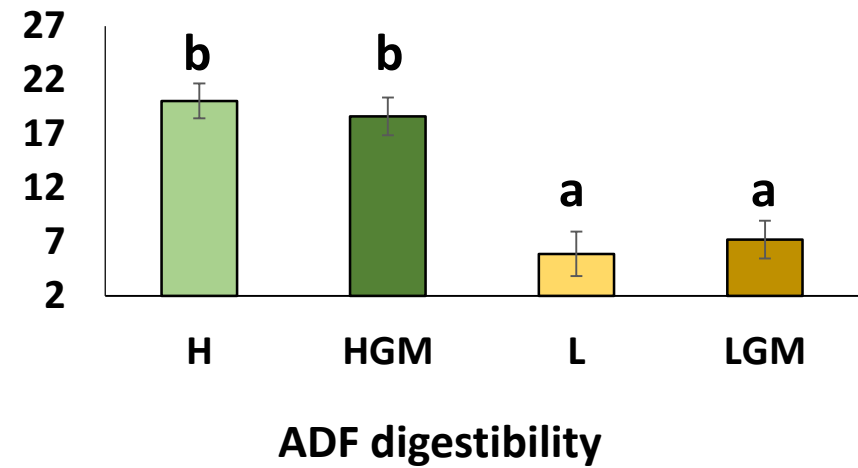
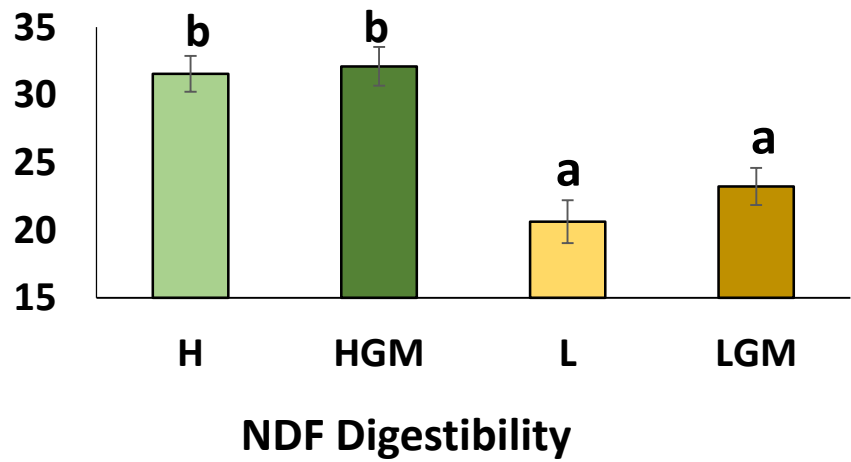
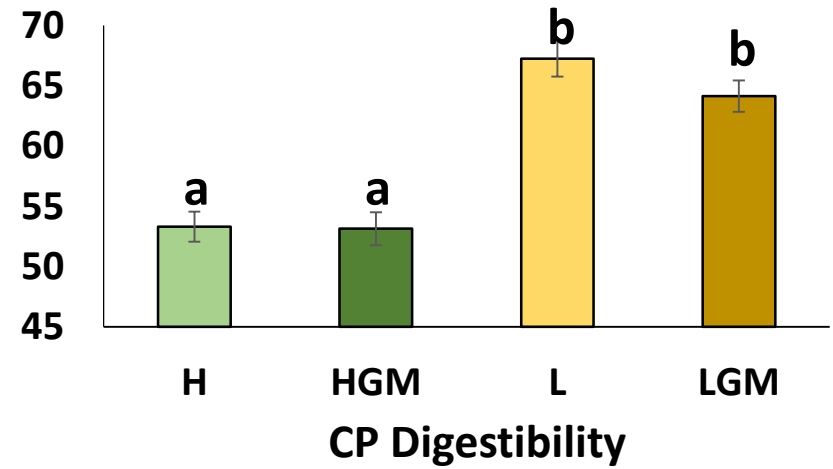
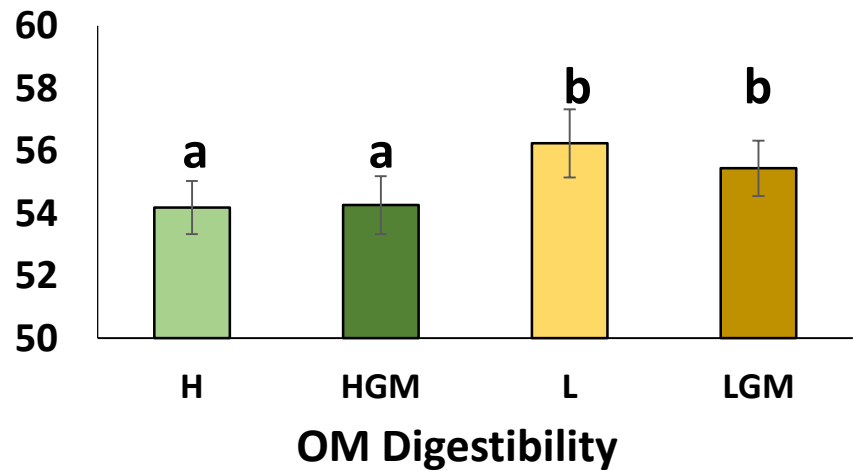
The increasing levels of fenugreek GM affects significantly medium chain fatty acids and N-NH₃ → possible effect on caecal microbiota

Mortality, morbidity and health risk index (HRI)



High soluble fiber diets decrease the risk of digestive disorders

Apparent faecal digestibility (%DM)



LGM increases (P>0.05) NDF digestibility with 2.6 and ADF digestibility with 1.3

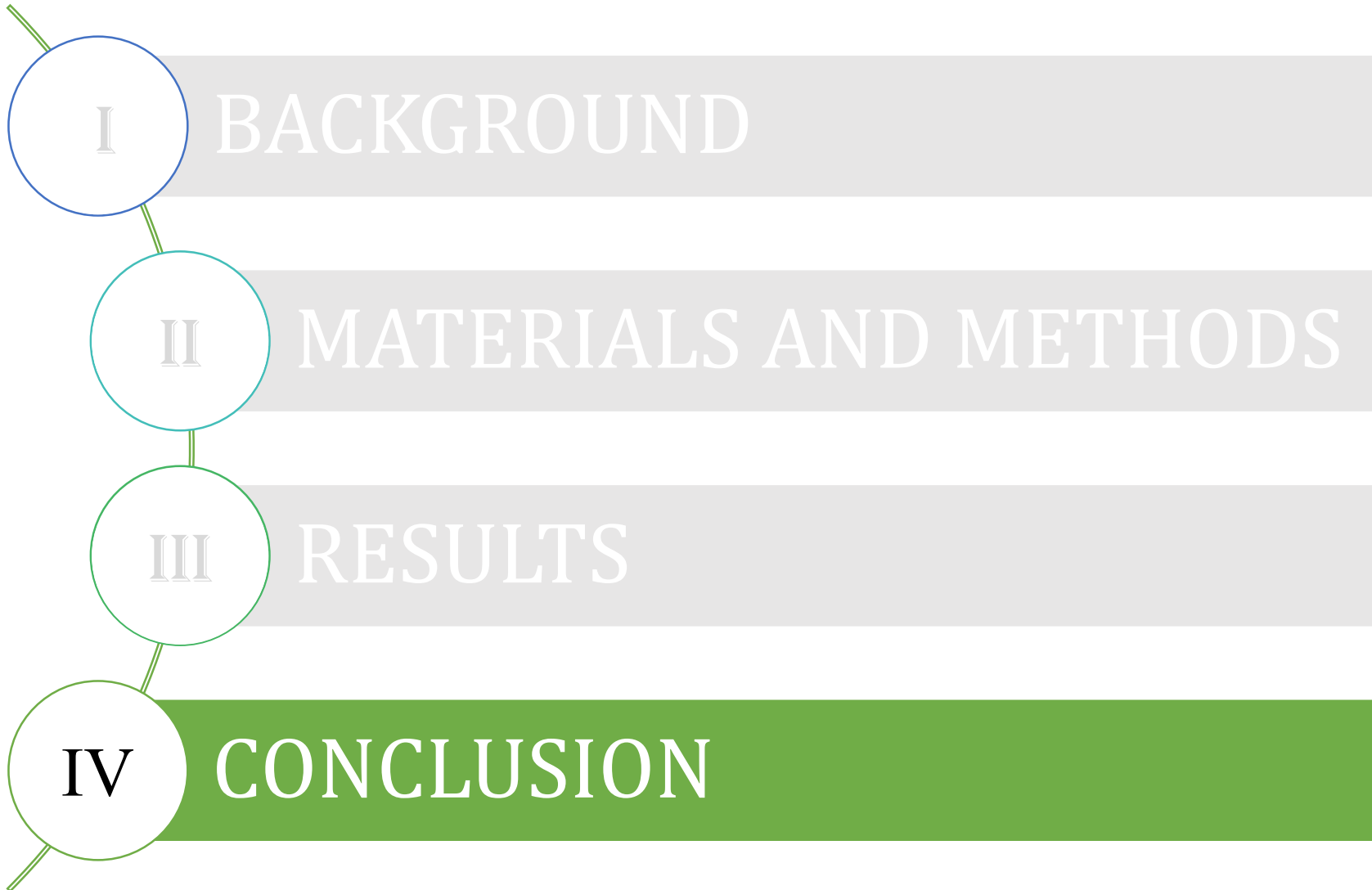
Rabbit's caecal activity parameters

	Pelleted Feed				P-value	
	H	HGM	L	LGM	Fibra	GM
pH	5.87±0.03	5.87±0.03	5.89±0.03	5.88±0.03	0.420	0.843
tVFA	53.78±1.68	53.86±1.74	44.64±1.81	44.02±1.81	<0.001	0.878
Acetic,%	81.22±0.01	81.7±0.01	77.59±0.01	76.19±0.01	<0.001	0.568
Propionic,%	4.14±0.00	4.17±0.00	3.86±0.00	3.87±0.00	0.061	0.896
Butiric,%	13.11±0.01	12.9±0.01	16.7±0.01	17.76±0.01	<0.001	0.526
Caproic, %	0.39±0.00	0.37±0.00	0.83±0.00	1.15±0.00	<0.001	0.010
N-NH₃,mg/L	35.18±8.47	35.75±8.83	92.45±8.64	105.21±8.83	<0.001	0.445

Caproic acid decreases coliform bacteria in the caecum and feces of animals infected with enteropathogenic strain of *E.Coli O103* (**Skrivanova et al., 2008**)

According to **Stewart et al. (1993)** y **Williams et al. (2000)**, short chain fatty acids are the main products of carbohydrates fermentation and for that they need a big amount of N-NH₃ as a source for microbial activity which could explain the low concentration of N-NH₃ in high soluble fibers diets **H** and **HGM**

→possible changes in caecal microbiota



Our Fenugreek galactomannan:

- ❑ Not digestible by stomach and hindgut enzymes
- ❑ Fully fermented by caecal bacteria
- ❑ Enhances fiber fractions digestion
- ❑ Affects medium chain fatty acids concentration in the caecum which suggests a different microbial activity in the presence of GM

➔ Future results of caecal microbiota composition

Thanks For Your Attention!



ICTA

