

**PHYTOBIOTIC USE  
ON TURKEY FARMS:  
FROM IN VITRO TESTS  
ON HISTOMONAS AND EIMERIA  
TO FIELD RESULTS**

**Dr Jean-Marie WATIER**

**Turkey Science and Production Conference**  
Chester, 20th March 2025



**FORKEY<sup>LS</sup>**



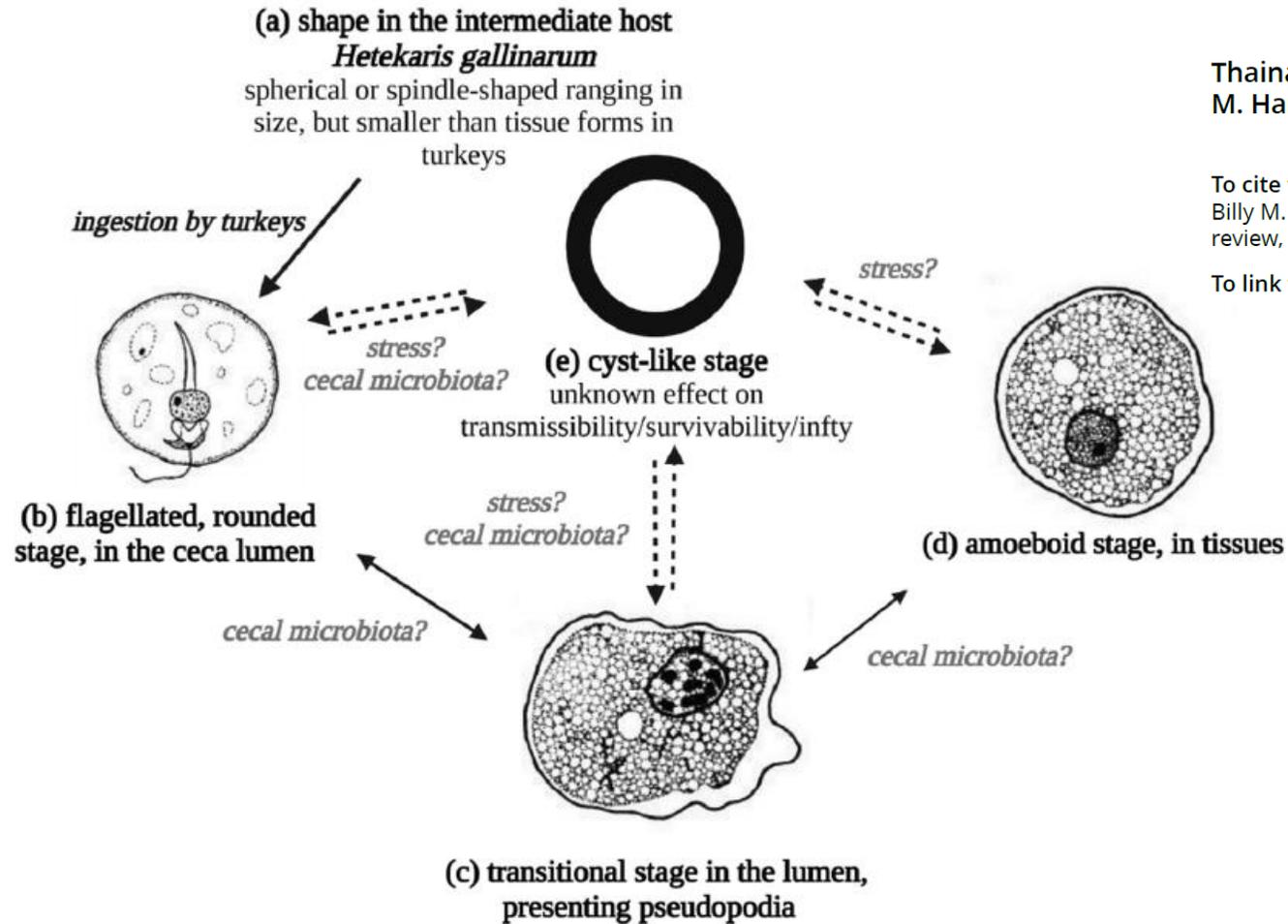
916 T. LANDIM DE BARROS ET AL.

## Uncontroversial facts and new perspectives on poultry histomonosis: a review

Thaina Landim de Barros, Christine N. Vuong, Guillermo Tellez-Isaias & Billy M. Hargis

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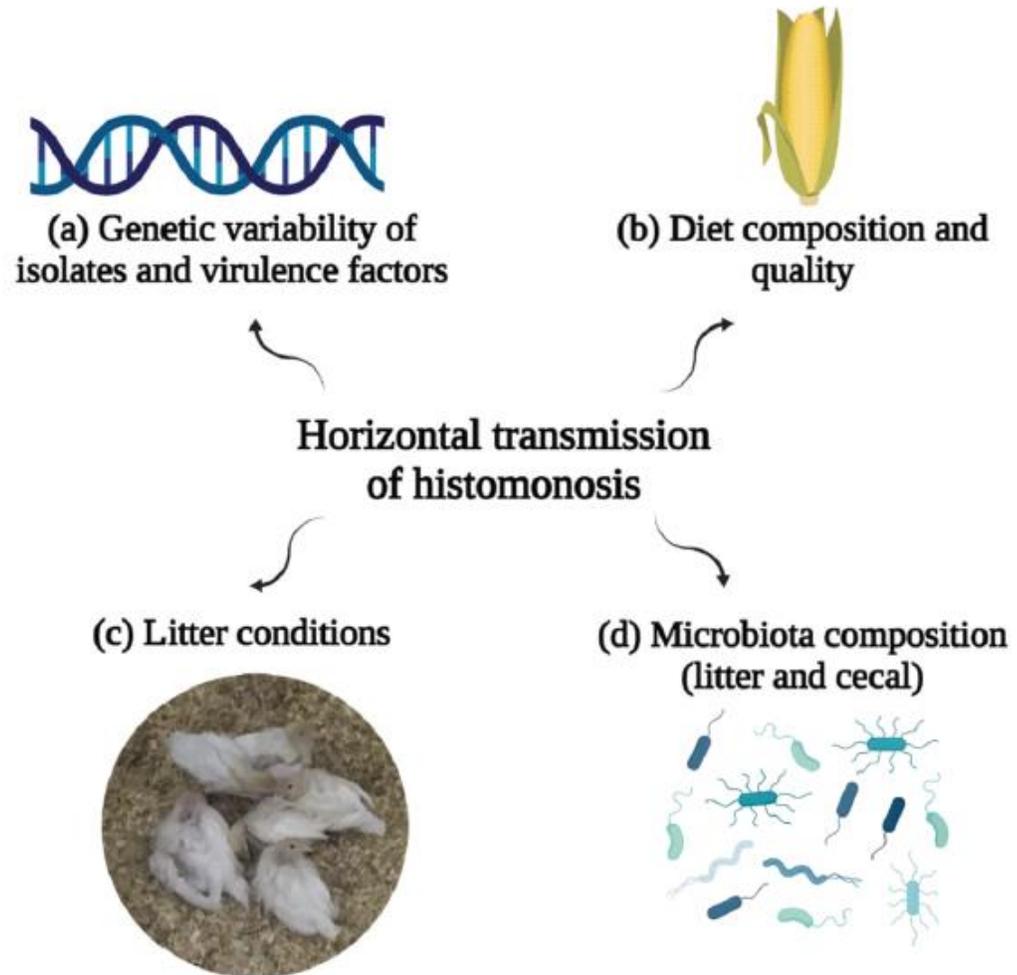
To link to this article: <https://doi.org/10.1080/00439339.2022.2119915>



• shape of *H. meleagridis* in the environment (feces/litter)?

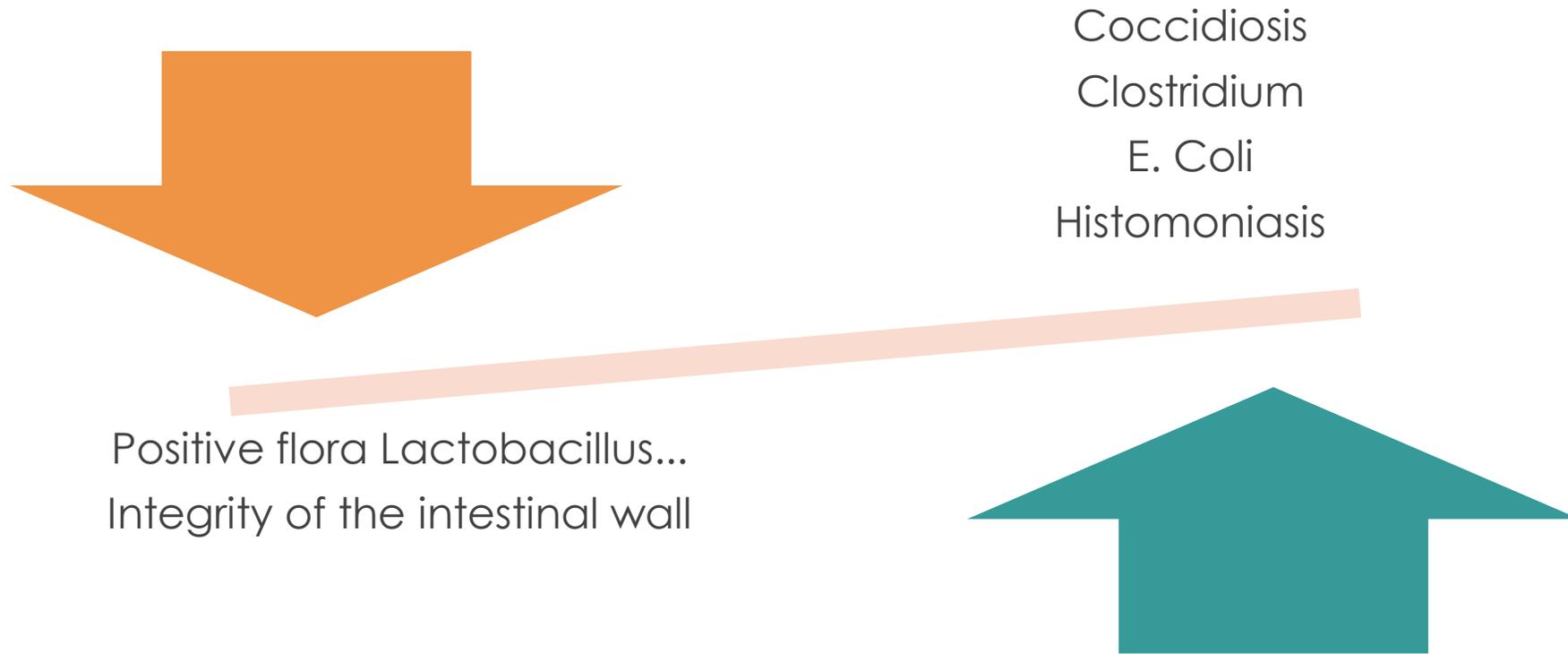
# HISTOMONOSIS HORIZONTAL TRANSMISSION

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**H. meleagridis feeds on E. coli for growth and multiplication**

# THE MAIN CHALLENGES TO TURKEY GUT HEALTH TO PREVENT HISTOMONOSIS



## OBJECTIVE

**Design a solution** with selected active ingredients:

- ✓ **Which act on** parasites and on “negative” bacteria
- ✓ **In order to:** favoring the balance of the microbiota and the gut integrity.



## Benefits of botanical active ingredients

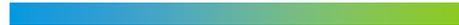
Measured  
in vitro  
activities

### A wide range of activities :

- Antiparasitic (*Histomonas, Eimeria*)
- Antibacterial (*Clostridium...*)
- Intestinal integrity



# **IN VITRO SELECTION OF ACTIVE SUBSTANCES WITH ACTION ON HISTOMONAS**

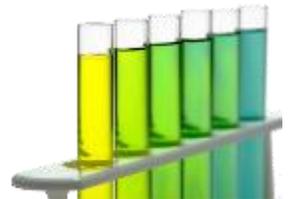


# STUDY OF THE EFFECT OF BOTANICAL ACTIVES ON THE GROWTH AND VIABILITY OF HISTOMONAS MELEAGRIDIS CULTURED IN VITRO.

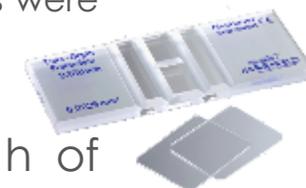


– VIENNA UNIVERSITY- 2019

- In vitro test of active ingredients on *Histomonas meleagridis*
- Validating the effect of IDENA solution: FORKEY LS
- Produced in association with the Clinic of Poultry and Fish Medicine of the University of Veterinary Medicine Vienna.



- H. meleagridis / Turkey / Austria / 2922 / C6 **culture supplemented with Escherichia coli** for use in tests.
- Viable H. meleagridis count by a Neubauer cell counting chamber (bright line haemocytometer) after **24, 48 and 72 h incubation with different compounds selected by Idena.**
  - The Minimum Lethal Concentration (MLC) was determined as the concentration at which no live or motile organisms were detected after 24 h of incubation.
- Live protozoa were counted after 48 and 72 h to confirm the results obtained after 24 h of incubation.
- In addition, **100% eradication was confirmed by inoculation of the respective cell suspension into fresh culture medium** without the addition of a plant substance.



IDENA's plant  
extracts



Antihistomonas  
Activity

# RESULTS



Mixtures	Bioactive conc.(ppm)	x 10 <sup>3</sup> histomonas				Mann- Whitney U	Regrowth
		0H	24H	48H	72H		
1	100	100	169	223	343		
	500	100	149	226	284		
2	100	100	36	16	8	p< 0,05	
	500	100	3	0	0	p< 0,05	
3	10	100	151	175	241		
	50	100	96	117	138		
4- FORKEY LS AB	100 (Incl. 10 EO)	100	44	132	379		
	500 (Incl. 50 EO) Equiv. 2,5 kg/ton AL437AB	100	0	0	0	p< 0,05	<b>NO</b>
5- FORKEY LS	100 (Incl. 10 EO)	100	32	103	241		
	500 (Incl. 50 EO) Equiv. 1 kg/ton AL841	100	0	0	0	p< 0,05	<b>NO</b>
6	10	100	89	167	236		
	50	100	0	0	0	p< 0,05	<b>NO</b>
7	10	100	80	191	151		
	50	100	12	34	67	p< 0,05	
8	100 (Incl. 10 EO)	100	94	97	99		
	500 (Incl. 50 EO)	100	0	0	0	p< 0,05	
9	10	100	128	198	339		
	50	100	102	367	574		
10	60 (Incl. 10 ppm EO)	100	9	55	147		
	300 (Incl. 50 ppm EO)	100	0,6	0	0	p< 0,05	
<b>DMZ</b>	<b>0,4</b>	<b>100</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>p&lt; 0,05</b>	
Negative control		100	154	163	301		

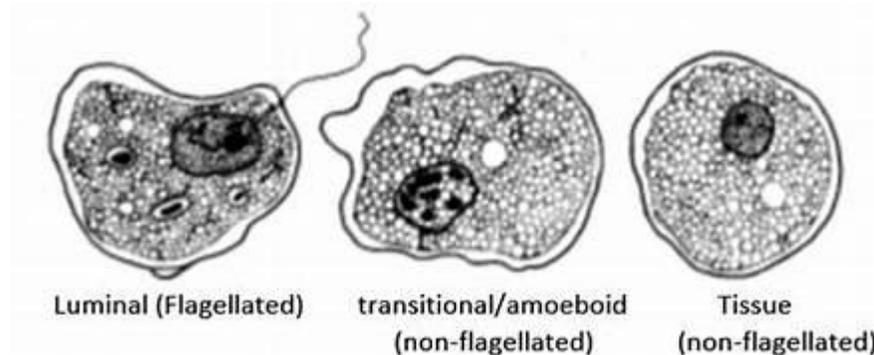
Table 1 : List of substances with the applied concentrations and mean count of *H. meleagridis* before and after 24, 48 and 72 H of incubation

**No regrowth of the parasite in a further incubation in fresh medium**

- => **significant reduction of live *H. meleagridis* cells**, without re-growing in a further culture with fresh medium,

=> FORKEY (concentration of 500 ppm) in vitro (=1 kg/ton in feed of FORKEY) **significantly reduced *H. meleagridis*** and prevented redevelopment after 48 and 72 hours..

- **FORKEY is a good alternative to limit the parasitic risk associated with *Histomonas meleagridis***





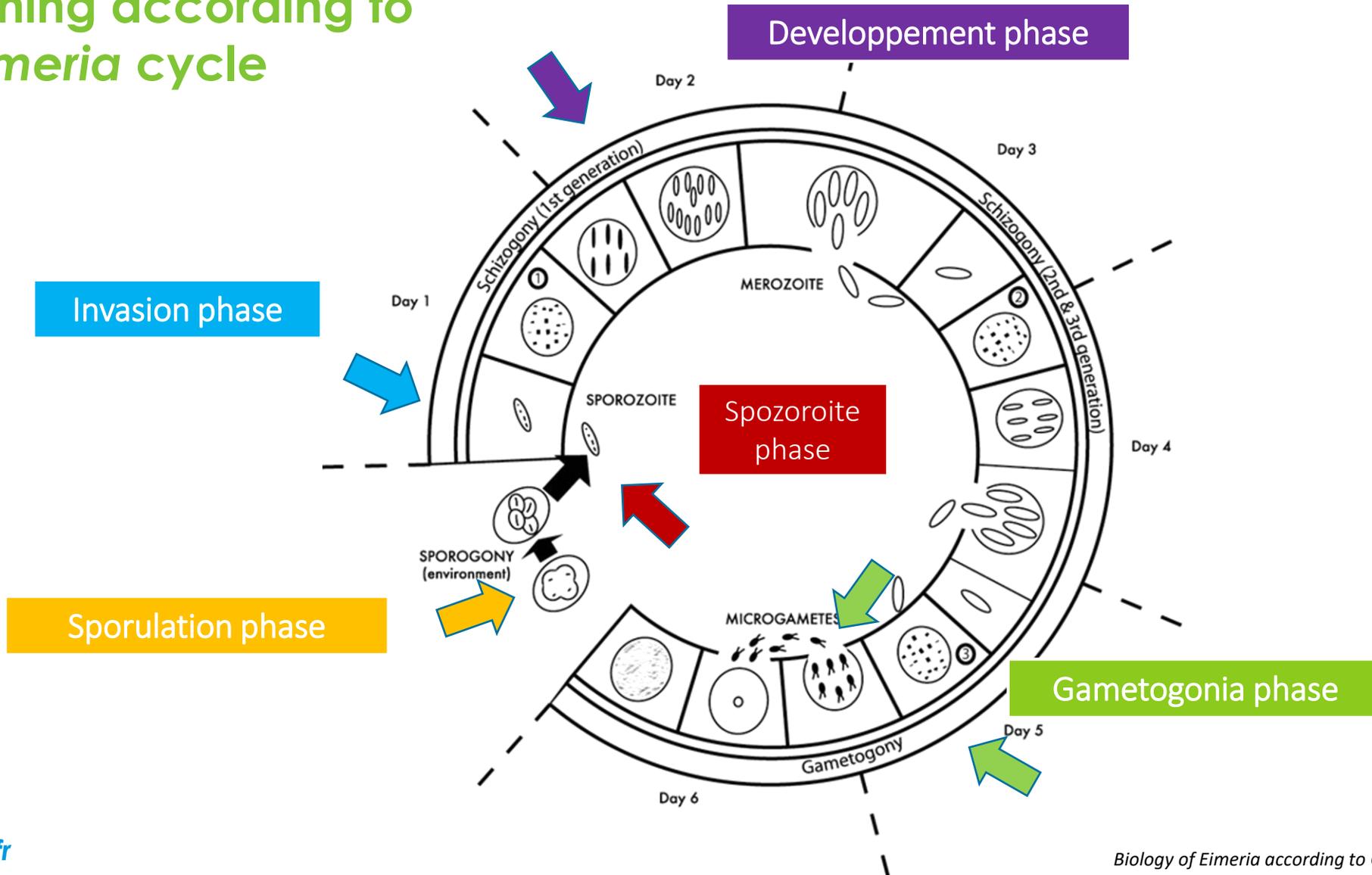
## IN VITRO trials on EIMERIA



INRAE

# IN VITRO TEST ON EIMERIA

## Screening according to the *Eimeria* cycle

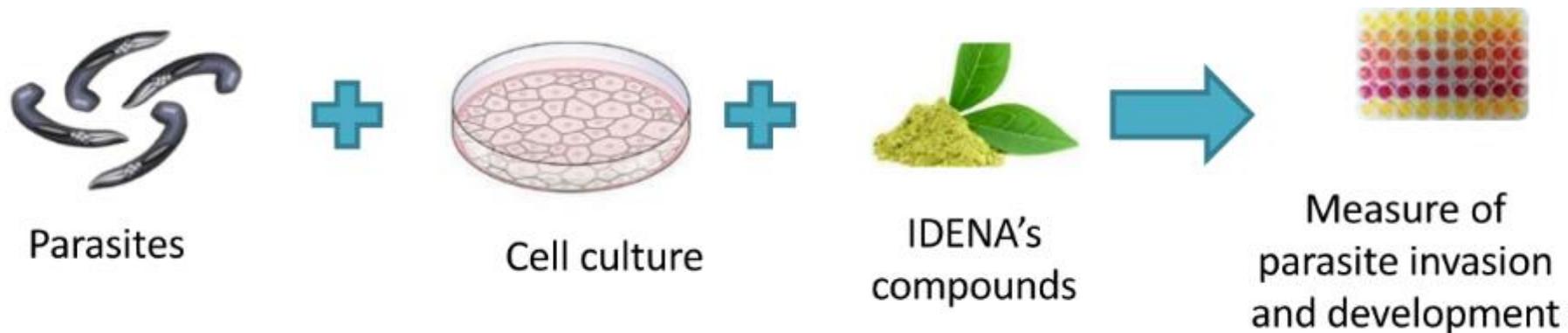


## Screening model



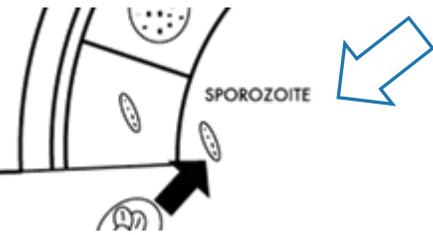
Development of a methodology to test the bioactives on the different phases of the Eimeria cycle:

- ✓ Oocysts sporulation
- ✓ Sporozoites invasion
- ✓ Development (schizogony)



# In vitro model coccidia

Biocidal effect on sporozoite



Use of different marking techniques to visualise the survival of protozoa

10 ppm

SE15401\_T Dilution : 0,05% to 0,005%    RD14020\_G Dilution: 0,05% to 0,0125%    RD14020\_M Dilution: 0,05% to 0,0125%

100 % mortality

Dead Sporozoite by heating  
Positiv control for mortality

Live Sporozoites

PREMIX01 0,05%

PREMIX02 0,05%

neg. control for mortality

RD14020\_A    RD14020\_D 0,05%    RD14020\_E    RD14020\_F 0,05%

RD14020\_N 0,05%    RD14020\_Q    SE15401\_U    SE15401\_Y 0,05%

no significativ mortality

RD14020\_O Dilution 0,05%

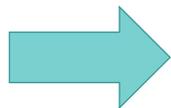
<50% mortality

# IN VITRO CELL-BASED MODEL – RESULTS (1)



		A	B	C	D	E	F	G	K	L	M	N	O	Q	R	H	I	J	S	T	U	V	W
SPORULATION	% efficiency'	97	13,8	6,8	93	97	78	88	NA	8,2	9	96,0	49,0	NA	5,3	100	18	7	23	9,7	38	52	8,5
INVASION	% efficiency	1	2	23	22	2	9	20	5	3	12	0	23	63	0	57	0	0	34	22	0	36	4
DEVELOPPEMENT	% efficiency	74	37	94	80	49	42	90	18	12	18	0	54	57	20	75	28	35	59	79	71	17	0
	Selectivity Index <sup>e</sup>	7,9	-	16,8	21,0	-	-	22,3	-	-	-	-	8,4	215,3	-	8,6	-	-	9,1	18,2	12,4	-	-

- Of the 37 compounds tested by IDENA => 20 actives or mixture of actives have an effect on at least one of the 3 phases of the parasite cycle
- SI (Selectivity Index = efficacy/ toxicity ratio) allows to select the most effective and least toxic mixtures for the epithelial cell



Optimisation of bioactive selection for the best control of biological cycle of *Eimeria*



## In vitro activity of botanical active ingredients on bacteria (*Enterococci*, *Clostridia*, *Lactobacilli*)



# ANTIBACTERIAL EFFECTS: CHOOSING THE RIGHT COMPOUNDS BY PERFORMING A RELEVANT SCREENING OF THE COMPONENTS



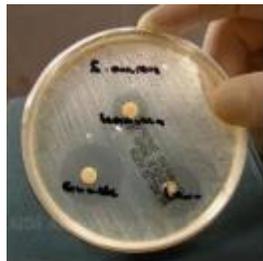
## EXAMPLE OF AGAR DIFFUSION RESULTS (MIC results- $\mu$ /ml)

- Screening of ingredients as part of a study conducted in partnership with a pharmacy university (Lille, France)



- Selection of active ingredients with efficacy on *Clostridium perfringens* with no effect on beneficial flora

### → Minimum Inhibitory Concentration (MIC) Measurements with agar diffusion technique



**A - No or little antibacterial effect = high MIC**

**B – Antibacterial against *Clostridium perfringens* but also against positive microflora**

**C – Antibacterial on *Clostridium* spp. and no action on positive microflora (e.g. *Lactobacillus* spp.)**

**D – Activity against *Clostridium* + negative bacteria (e.g. *Enterococcus* spp.)  
VERY GOOD POTENTIAL FOR THESE BIO-ACTIVES**

INGREDIENTS	Blend A	Blend B	Blend C	Blend D
<b>STRAINS OF <i>C. PERFRINGENS</i> (CP)</b>				
CP s1	>512	8	32	4
CP s2	>512	4	8	4
CP s3	>512	4	8	4
CP s4	>512	4	32	4
<b>STRAINS OF « NEGATIVE » BACTERIA (NB)</b>				
NB s1	>512	32	>512	8
NB s2	>512	16	>512	8
NB s3	>512	64	>512	8
NB s4	>512	8	>512	8
NB s5	>512	128	>512	8
<b>STRAINS OF « POSITIVE » BACTERIA (PB)</b>				
PB s1	>512	32	>512	>512
PB s2	>512	512	>512	>512
PB s3	>512	16	>512	>512
PB s4	>512	32	>512	>512
PB s5	>512	32	>512	>512
PB s6	>512	8	>512	>512
PB s7	>512	8	>512	>512
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>



**FORKEY<sub>LS</sub>**

**FORKEY LS : an original solution for turkey production**

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# EFFICACY OF HERBAL AND AROMATIC COMPONENTS IN VITRO SUGGESTS PREVENTION OF HISTOMONOSIS AND ENTERITIS PROBLEMS IN TURKEYS



J.M. Watier<sup>1</sup>, A. Mahieu<sup>2\*</sup>, A. Canin<sup>2</sup>, R. Domitile<sup>2</sup>, M. Aoun<sup>2</sup>, F. Laurent<sup>1\*</sup>, D. Liebhart<sup>3</sup>, D. Schuller<sup>3</sup>, A. Greuter<sup>3</sup>  
 1. INRAE, Université de Tours, ISP, AIM team, F-37380, Nouzilly, France - 2. IDENA, 21, rue du moulin, F-44880, Sautron, France  
 3. University of Veterinary Medicine, Veterinärplatz 1, A - 1210 Vienna, Austria

**INTRODUCTION** The main challenge for the turkey gut health is to fight against the main parasitic threats, mainly histomonosis and coccidiosis, against bacterial infections like *E. coli* and *Clostridium* and preserving a positive microflora.

**OBJECTIVES** To design a phytoproduct able to decrease the risks of histomonosis outputs and in the same time prevent the coccidiosis and the dysbiosis symptoms. To design this product, we worked with three different researchers teams on the different mode of action of the product.

## MATERIAL AND METHODS

**Histomonosis study:**  
 On ten substances (natural, synthetic and mixes) at two concentrations (100 and 500 ppm) were selected and provided by IDENA (Sautron, France).  
 The *in vitro* tests were performed at the Clinic for Poultry and Fish Medicine, University of Veterinary Medicine Vienna.



**Coccidia study:**  
 In collaboration with INRAE, we evaluated a wide range of bioactives against avian coccidiosis agents by carrying out an *in vitro* screening. It aimed at evaluating their capacity to limit the sporulation, invasion and/or the replication of the parasites in epithelial cells.



**Antibacterial study:**  
 The aim was to determine the Minimum Inhibitory Concentration (MIC) using agar dilution method, as antibiotic molecules. Around thirty combinations of active ingredients were tested.

## RESULTS AND CONCLUSION

**Efficacy on Histomonads:**  
 We have succeeded in choosing bioactives that have the same non-regrowth effect as dimetridazole on histomonads.

Example of results obtained *in vitro* on histomonads compared with the positive control (DMZ\*\*)

Substance to test	Bioactive concentration	Average number of H. meleagridis oocysts after 24, 48 and 72 h of inoculation at 18°C in histomonads				Regrowth
		0 h	24 h	48 h	72 h	
FORKEY LS	1/5 normal dose of use	100	82	100	74	YES
	normal dose of use	100	0	0	0	NO
DMZ**	0,4 ppm	100	0	0	0	YES
Hegabith control		100	154	162	201	YES

\*\* DMZ: Dimetridazole  
 At the FORKEY LS upper doses, *in vitro* tests showed a 100% activity to DMZ on histomonads oocysts.

**Antibacterial effect:**  
 With the right choice of the components, only the negative bacteria are affected. These bioactives have strong effect on *Clostridium*, on other negative (e.g. *Enterococcus spp*) bacteria but did not affect the positive bacteria (like *Lactobacillus spp*).

**CONCLUSION** It is possible to formulate an alternative solution able to stop the development of *Histomonas meleagridis*, breaking the parasitic cycle of *Eimeria*, and obtaining an antiseptic effect to fight bacterial infections (mainly *C. perfringens*).

For several years, some turkey production companies have been using FORKEY LS without encountering any clinical cases of histomonosis.

**Efficacy on coccidia:**  
 Three main substances were chosen to act on the different parts of the coccidia cycle:



**Making a micro-emulsion to enhance the global effectiveness of the product:**  
 FORKEY LS benefits from IDENA's ECHY Technology increasing its effectiveness.

Technological advantages	Zootechnical advantages
→ Fine dispersion	→ Palatability: reduces olfactory impact
→ Homogeneity: no phase shift	→ Safety: reduces toxicity to membranes
→ Active ingredients protection and retention	→ Efficacy: nanoparticles with a high surface activity
→ Stability over time	→ Economy: reduces doses

# 16th Turkey Science and Production Conference

Carden Park Hotel and Golf Resort  
 Chester, UK

March 6 - 8<sup>th</sup> 2024



The leading conference and meeting point for the European Turkey Industry

Efficacy of herbal and aromatic components in vitro suggests prevention of histomonosis and enteritis problems in turkeys.

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## FORKEY on field results



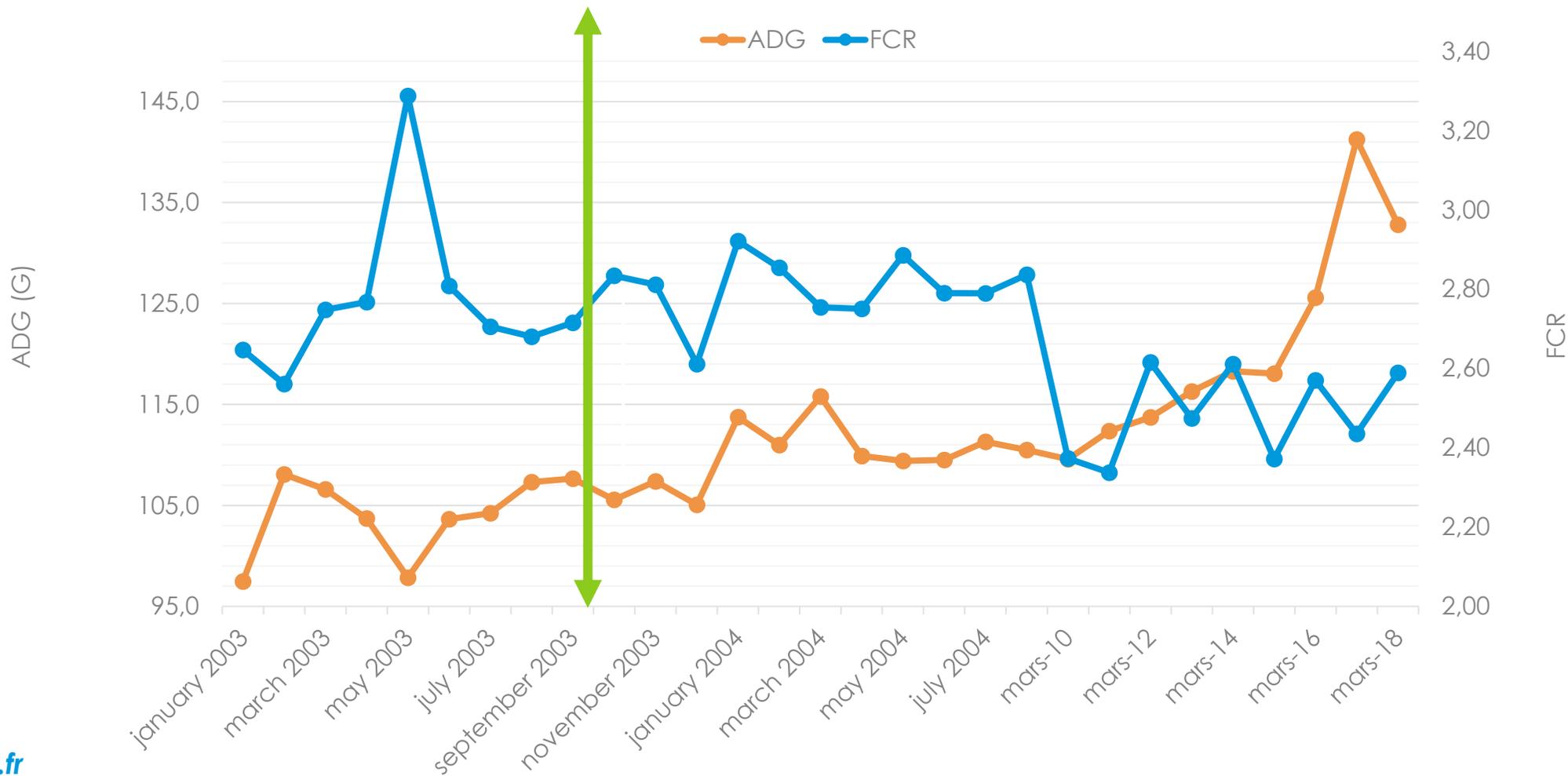
# FORKEY - HISTORICAL FIELDS RESULTS



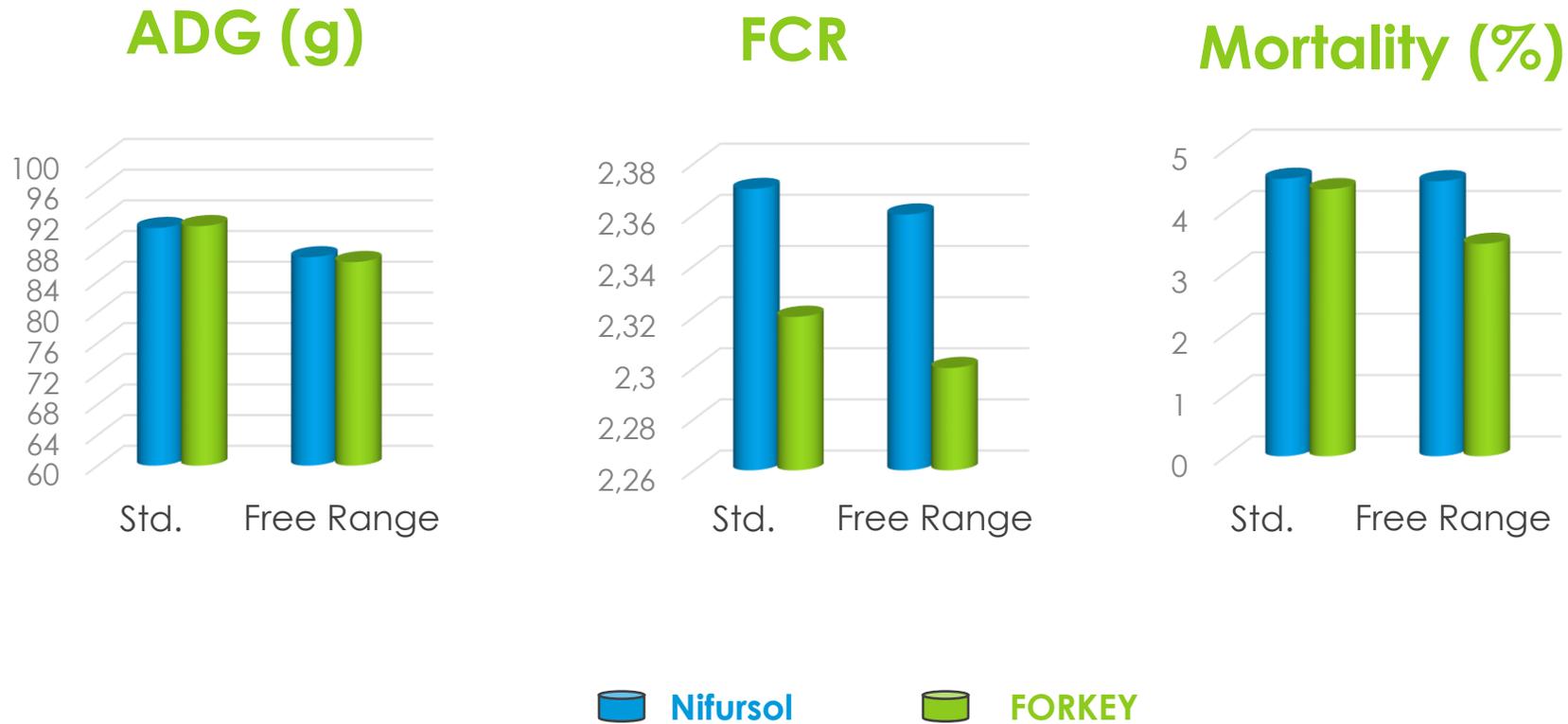
Turkey – 60 000 turkeys / week with FORKEY

South America

FORKEY in replacement of histomonostats IN THE FEED



## Turkey – Summary for standard and free range production Results during year 2003 – French poultry organization



# FORKEY Liquid: APPETENCY TESTS



# APPETENCY TESTS: MATERIAL AND METHOD

**Aim:** to evaluate FORKEY Liquid appetency on meat turkeys  
In collaboration with Chene Vert Vet Praticice

- **Material and method:**

- control flock:
  - ✓ 11 700 **female turkeys 52 days** of age
  - ✓ water given by the general line (Plassons)
- Test flock : 300 female turkey, same age, same building coming from the same flock,
  - ✓ grouped at 42 days In the trial barn (among the control flock)
  - ✓ Water distributed with the same kind of drinkers as the control flock (Plassons)
- **Forkey liquide** was distributed by independant tanks
- **for 5 days at 1 L/1000 Liters drinking water**

# COMPARAISON TRIAL AND CONTROL FLOCKS

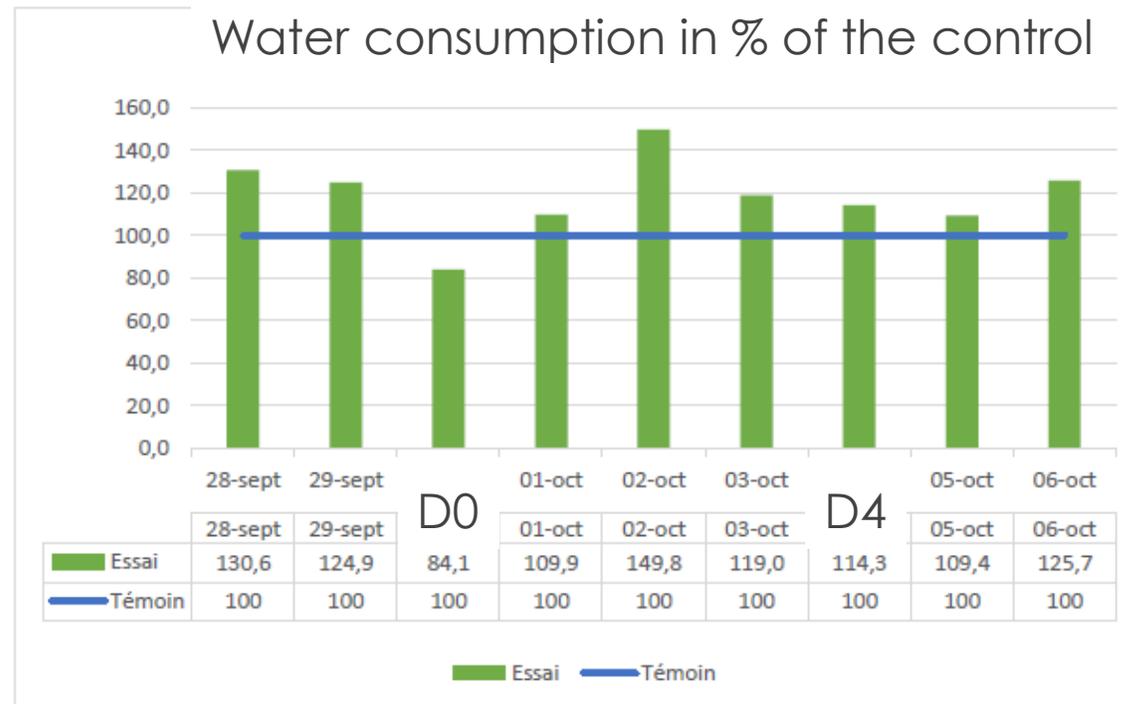
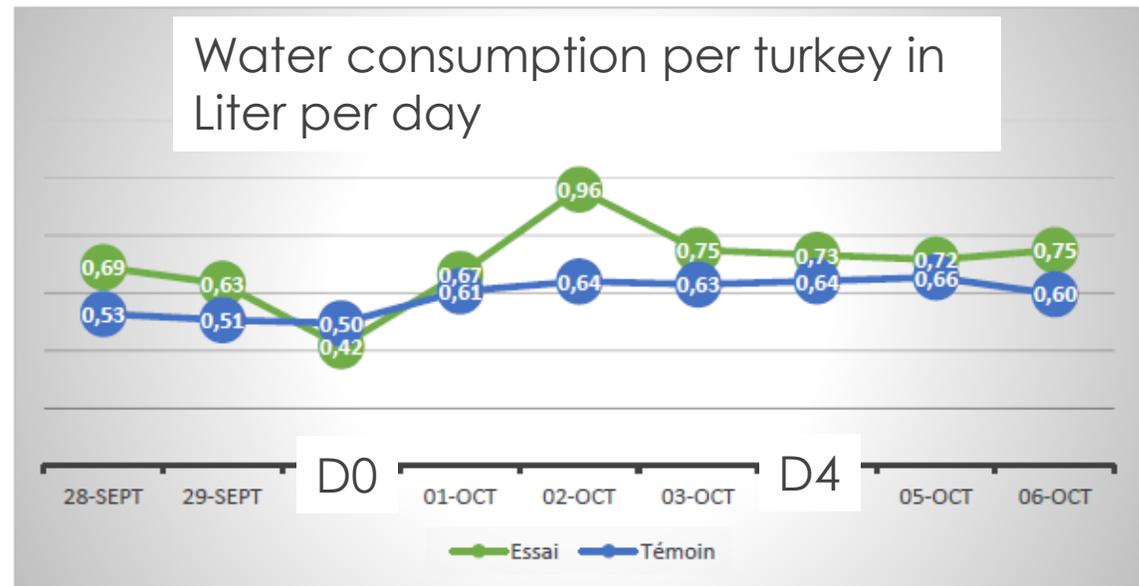
Days	Trial flock	Control flock
D-2		
D-1		
<b>D0</b>	<b>Forkey L</b>	Orniacid 0,5 L/1000L
<b>D1</b>	<b>Forkey L</b>	<b>Tylosine</b>
<b>D2</b>	<b>Forkey L</b>	<b>Tylosine</b>
<b>D3</b>	<b>Forkey L</b>	<b>Tylosine</b>
<b>D4</b>	<b>Forkey L</b>	Hydrocare 0,5 L/1000L
D5		Hydrocare 0,5 L/1000L
D6		Hydrocare 0,5 L/1000L

## Measurements:

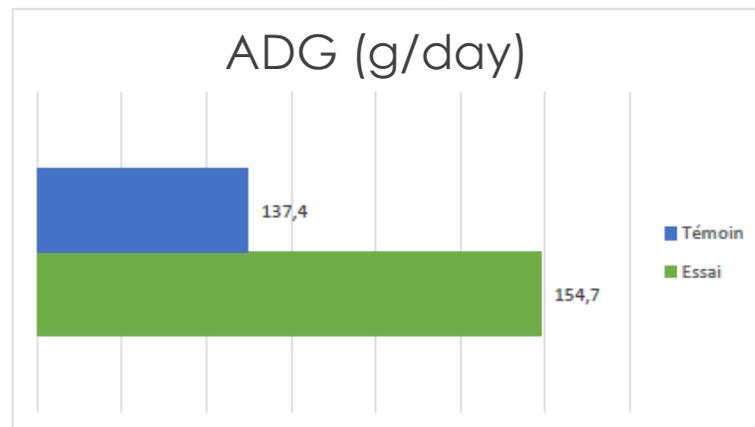
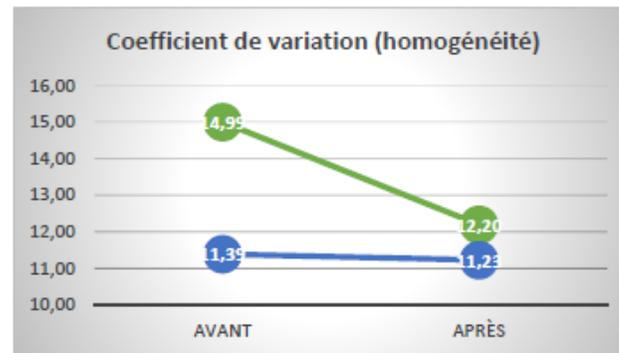
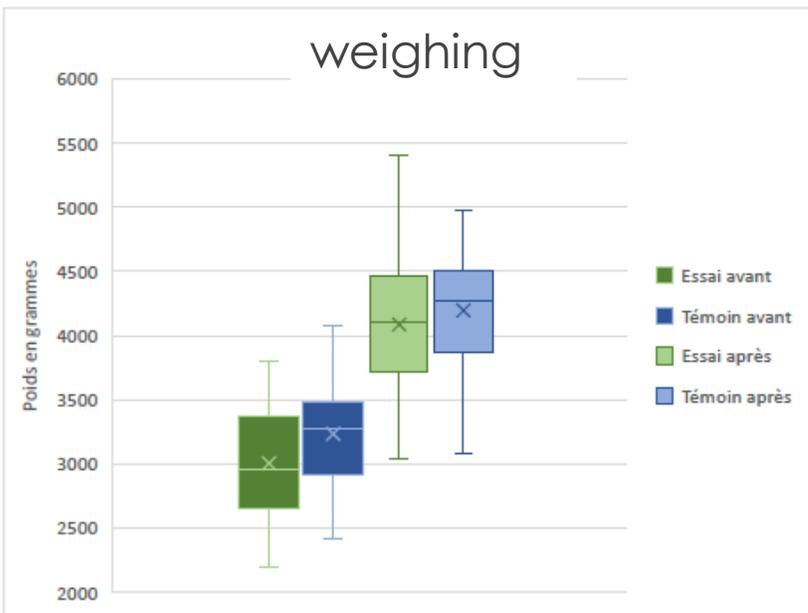
- 50 turkeys, from both groups, were **weighted** at the first day and at the end of treatment
- **Water consumptions** were recorded daily in both groups

# WATER CONSUMPTION

- Drop in water consumption the first day for the trial group, then recovering of normal intake
- No sign of dehydration noticed
- The stop of treatment the last day did not lead to overconsumption the days after



# WEIGHING AND LITTER RESULTS



- **Growth is better** on the trial group (despite the Tylosine treatment on the control group)
- The farmer noticed a **good litter which was improving** along the trial

# FORKEY: Field results



## FEEDBACK FROM ON FARM USERS (LIQUID FORM)

Use on meat turkeys  
flocks diagnosed  
with histomonosis

From slight improved situation  
to clearly stop of increased  
mortality

Use on other farms  
without  
histomonosis:

- Improvement in litter quality
- avoiding in antibiotics  
treatment use

No histomonosis outbreak during  
the lifetime of these flocks

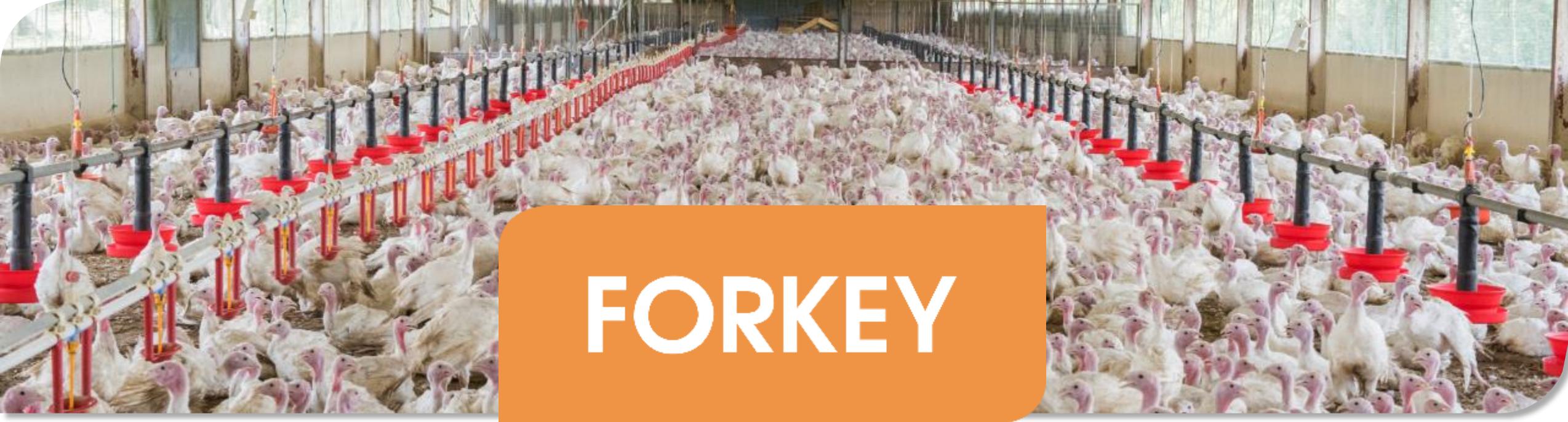
## CONCLUSION ON LIQUID FORM USE

Positive effect on  
histomonosis  
declared flocks:

stop in mortality  
increasing

turkeys continue to drink water  
whereas they have stopped to  
eat => possible action of liquid  
product

Unlike to powder form => visible effect on  
histomonosis disease



**FORKEY**

**FORKEY : application**

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PRODUCT	CODE	Rate of incorporation into drinking water	
		Standard turkeys	Free-range turkeys guinea fowl – game birds
<b>FORKEY LIQ</b>	<b>SV494L – can 5 Liters</b>	0,4 à 0,8 Liter/m <sup>3</sup>	0,4 à 1 Liter/m <sup>3</sup>

- Use in periods of risk or in case of digestive disorders.
- **5-day treatment in drinking water.**
- **Do not add FORKEY LS to feed and water at the same time (or adjust the dosage).**



To be added directly in the feed or to be mixed in the premix first

PRODUITS	Rate of incorporation into the feed	Turkey Hybrid or BUT
<p><b>FORKEY LS</b> (Large Spectrum) <i>To use without coccidiostat in feed</i></p>	<p>0.7 to 1 Kg/T</p> <p>0 to 10 weeks of life minimum</p>	<p>0.8 Kg/T from 0-50 days 0.7 Kg/T &gt;50days</p>
<p><b>FORKEY LS ION</b> (Large Spectrum and Organic) <i>To use without coccidiostat in feed</i></p>	<p>1.75 to 2.5 Kg/T</p>	<p>2 Kg/T from 0-50 days 1.7 Kg/T &gt; 50 days</p>



- Possible use of from 10 weeks to slaughter to maintain digestive balance.
- No contraindication to maintain an "economical" chemical coccidiostat from 0 to 5 weeks (only cost).

# FORKEY LS

Thanks for your  
attention!



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To prevent the risk of parasites  
and improve turkey performance.