ARISTOTLE UNIVERSITY OF THESSALONIKI

Probiotics as natural substances to control chicken cocidiosis

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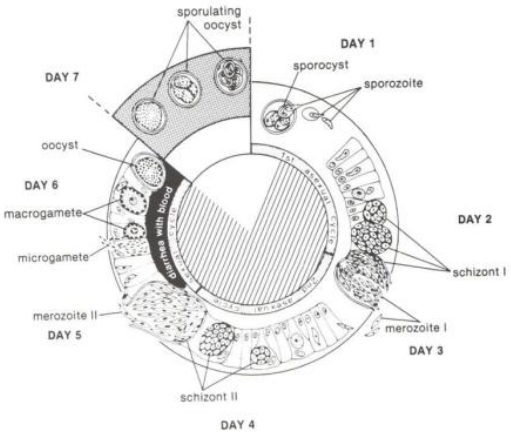
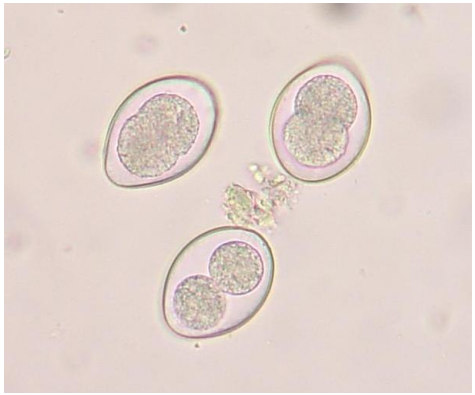
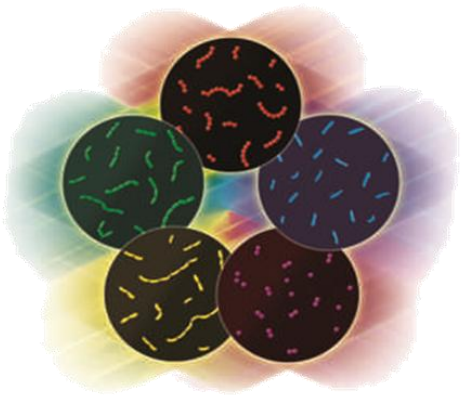






Presentation outline

- **Introduction**
- **Current interventions to control coccidiosis in poultry**
- **Probiotics and coccidiosis control**
- **Bioactive compounds and health implications**
- **Conclusion**

Introduction

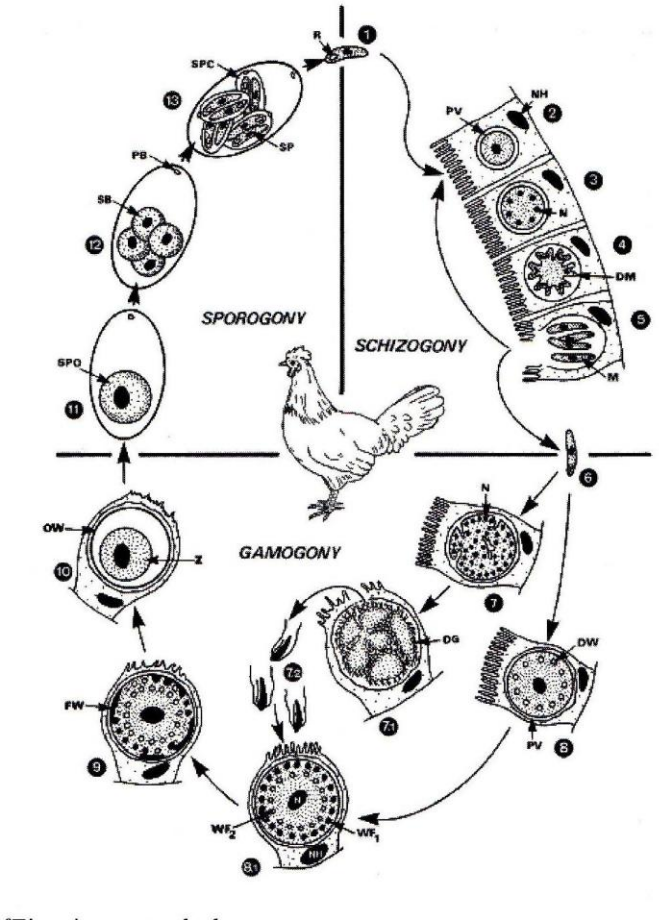
- Coccidiosis is a significant endemic disease in poultry

- The financial loss to the poultry industry worldwide circa 3 billion USD/year

- Mainly due to prophylactic or therapeutic in-feed medications and also as a result of the disease impact on birds' health

(Williams, 1999; Bozkurt et al., 2013)

Life cycle of *Eimeria*



Coccidiosis, although a common intestinal parasitic disease, causes economic losses (Stevens, 1998; Naidoo, 2008).

E. acervulina

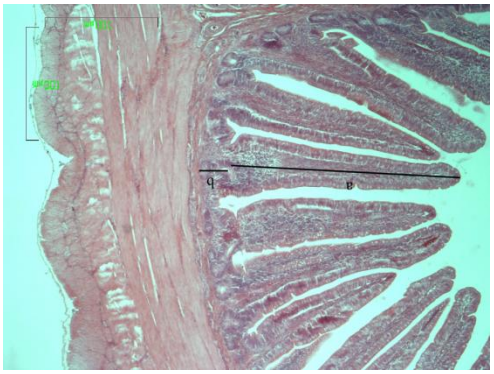
E. tenella

E. maxima

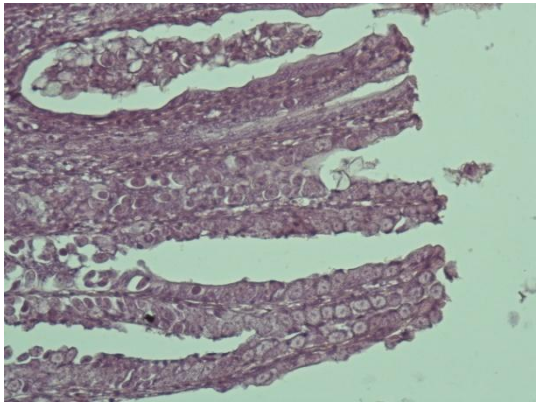
these 3 possess 90% of the pathogenicity

Effects of *Eimeria* coccidia on intestines

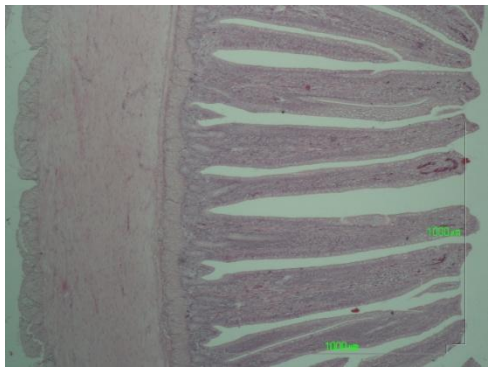
Effects of *Eimeria* coccidia on intestinal villus

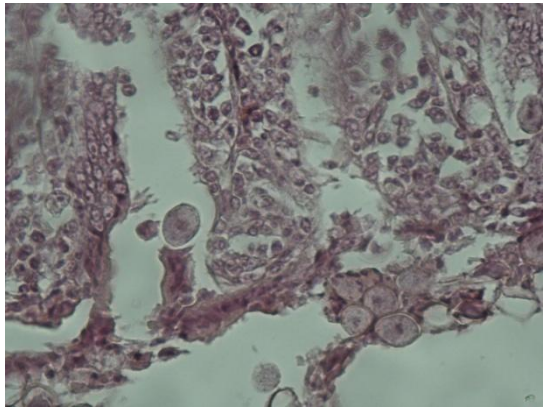


Normal villae



Cocci – affected villae

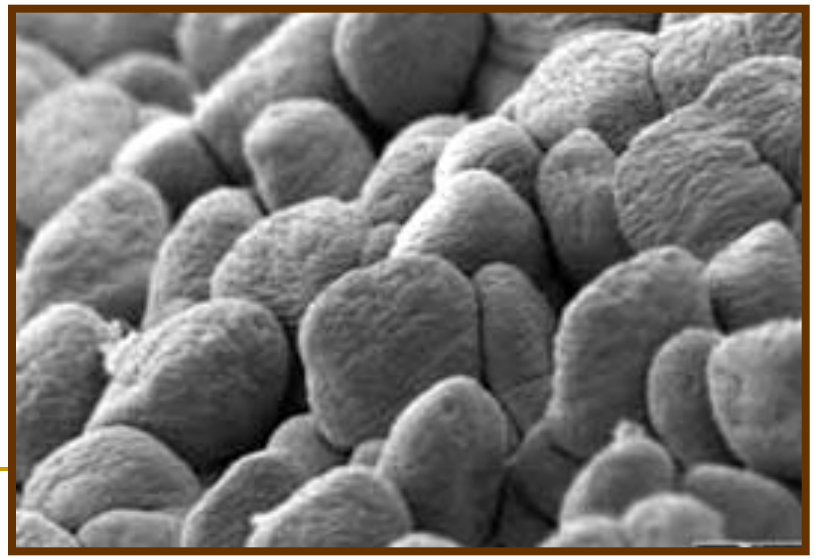




Effects of *Eimeria* coccidia on intestinal villus

Normal villae

Cocci – affected villae



Current interventions to control coccidiosis in poultry

- Chemotherapeutics (antibiotics - chemicals)
- Control of coccidia has been greatly dependent on the use of chemotherapeutics agents
- It has proven success in many parts of the world, due to its ease of use and the ability to provide uniform treatment and prevention.

Current interventions to control coccidiosis in poultry

However..

- On some occasions, might be toxic to the birds

- The constant pressure to reduce the dependence on antimicrobials including the anticoccidial drugs
  - High cost
  - Drug residue in poultry meat, eggs and its byproducts are of a public health concern
  - Resistance development to drugs by *Eimeria* species

Resistance to anticoccidial drugs worldwide

- *Approved drugs= Ionophores, synthetic chemicals, sulfonamides, mixed products*
- *Resistance* "the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended"
- Development of resistance to all anticoccidial drugs
- Widespread occurrence in US, EU, South America, South Africa, China (1974-2004)
- Cross resistance to new substances with similar mode of action!

Anticoccidial drugs and mode of action

Anticoccidial drugs	Affected life cycle stages	Mode of action	*Eimeria* species studied
Sulfonamides	2nd and later schizonts	Inhibition of folic acid pathway	*E. tenella*
Amprolium	2nd schizont	Thiamine antagonist	*E. tenella*
Decoquinate	Sporozoite	Inhibit respiration by blocking electron transport in the parasite mitochondrion	*E. tenella*
Clopidol	Sporozoite	Affects electron transport	*E. tenella*
Benzeneamines	Sporozoite	Oxidative stress	Various species
Monensin	Sporozoite	Influx of sodium ions	*E. tenella*
Robenidine	Multiple stages	Bind to proteins and affects parasite mitochondria	Various pecies
Halofuginone	Asexual stages	Alkaloid, may suppress oocyst production	Various species
Salinomycin, ionophores	Sporozoites and merozoites	Cation transport across cell membrane	Various species
Diclazuril	Sexual and asexual stages	Nucleotide analogue	*E. tenella*
Toltrazuril	Multiple stages	Act against plastid like genome	Various species

Current interventions to control coccidiosis in poultry

- Vaccines …relevant success
- Limited by
  - the possibility of adverse effects on weight gain and feed conversion
- high production costs especially when these vaccines includes more than one *Eimeria* speciesVaccines are still the most potential solution to control coccidiosis in poultry.
- The industry has to consider other alternatives such as phytochemicals or probiotics to be used with anticoccidial drugs

(Dalloul and Lillehoj, 2006)

Current interventions to control coccidiosis in poultry

- Novel control strategies - more knowledge on the interaction between *Eimeria* species and the birds' immune system
- Gut microflora - birds' immune system as it is considered one of the first lines of defense against pathogens.
- Substances that may help immunity

E. tenella in vitro ::: optimisation

Figures 4 - 6: *In vitro* production of *E. tenella* merozoites (Mz) in dependence of varied cultivation conditions. Groups that possess different letters (a, b, c) are significantly different.

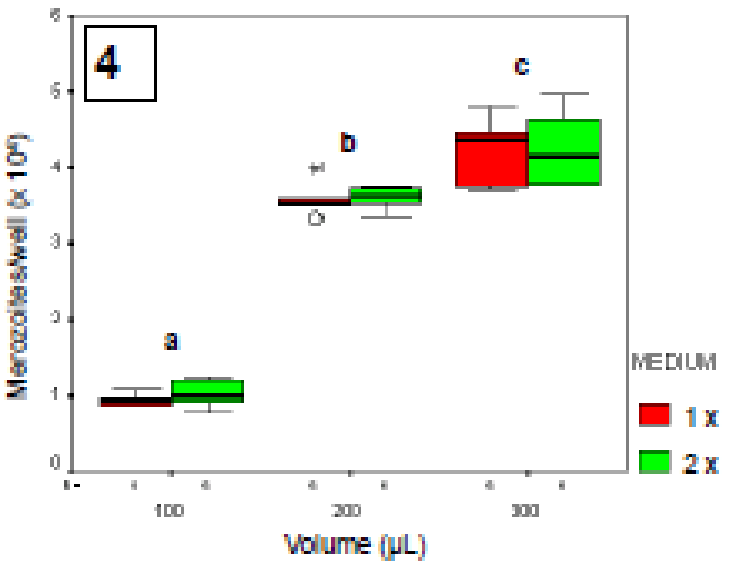
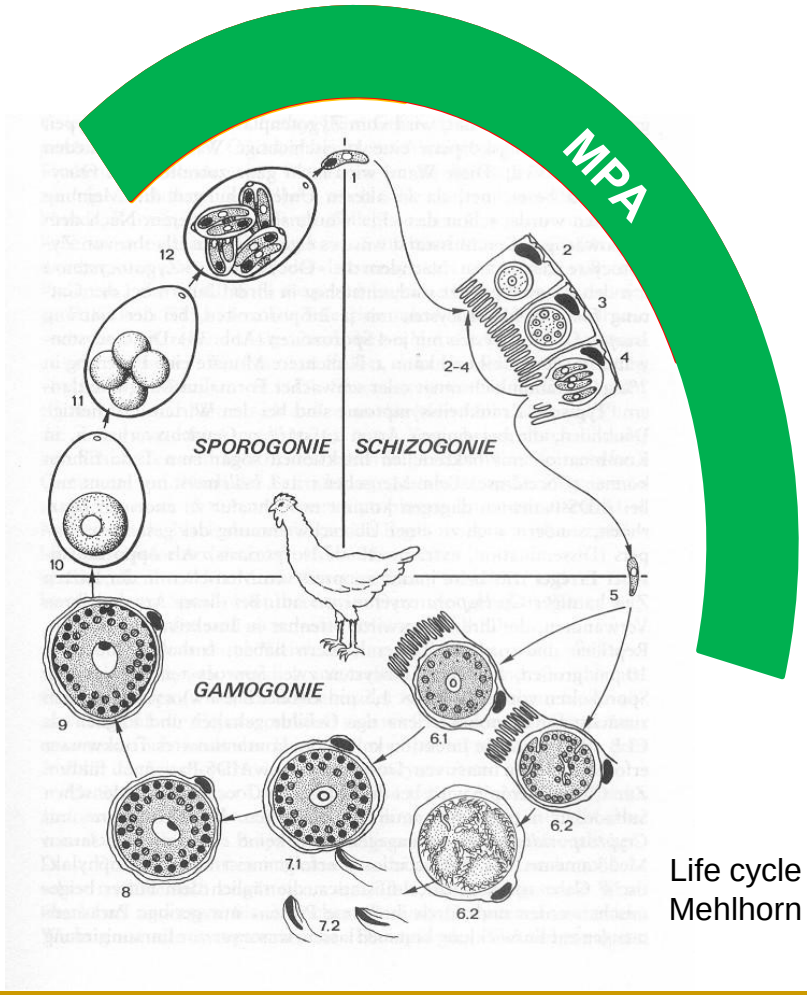


Figure 4: culture medium volume and concentration.

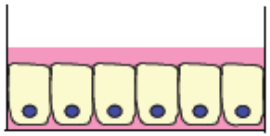
E. tenella in vitro ::: optimisation



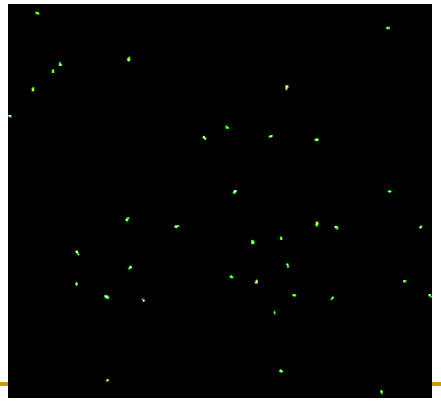
Life cycle of *Eimeria tenella*
Mehlhorn & Piekarski, 2001

E. tenella in vitro ::: invasion assay

1. Cultivation of MDBK cells



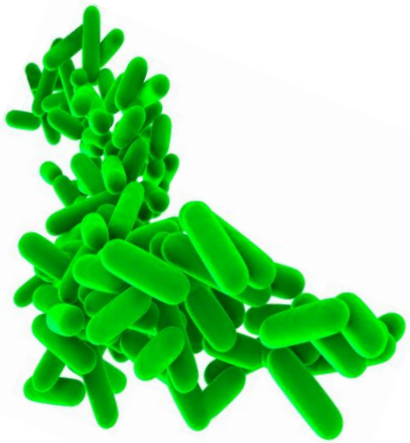
According to Schubert *et al.* (2005)
CFDA-SE labelling

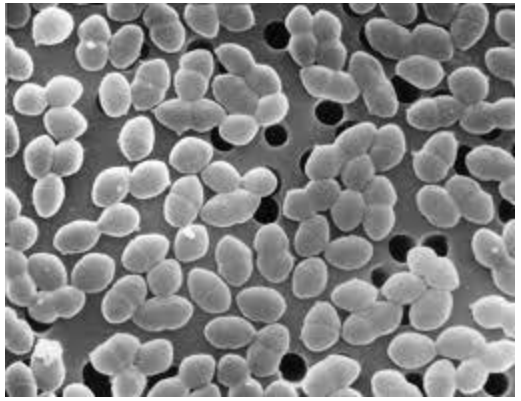


Integrated assay for host cell viability (WST-1, Roche)

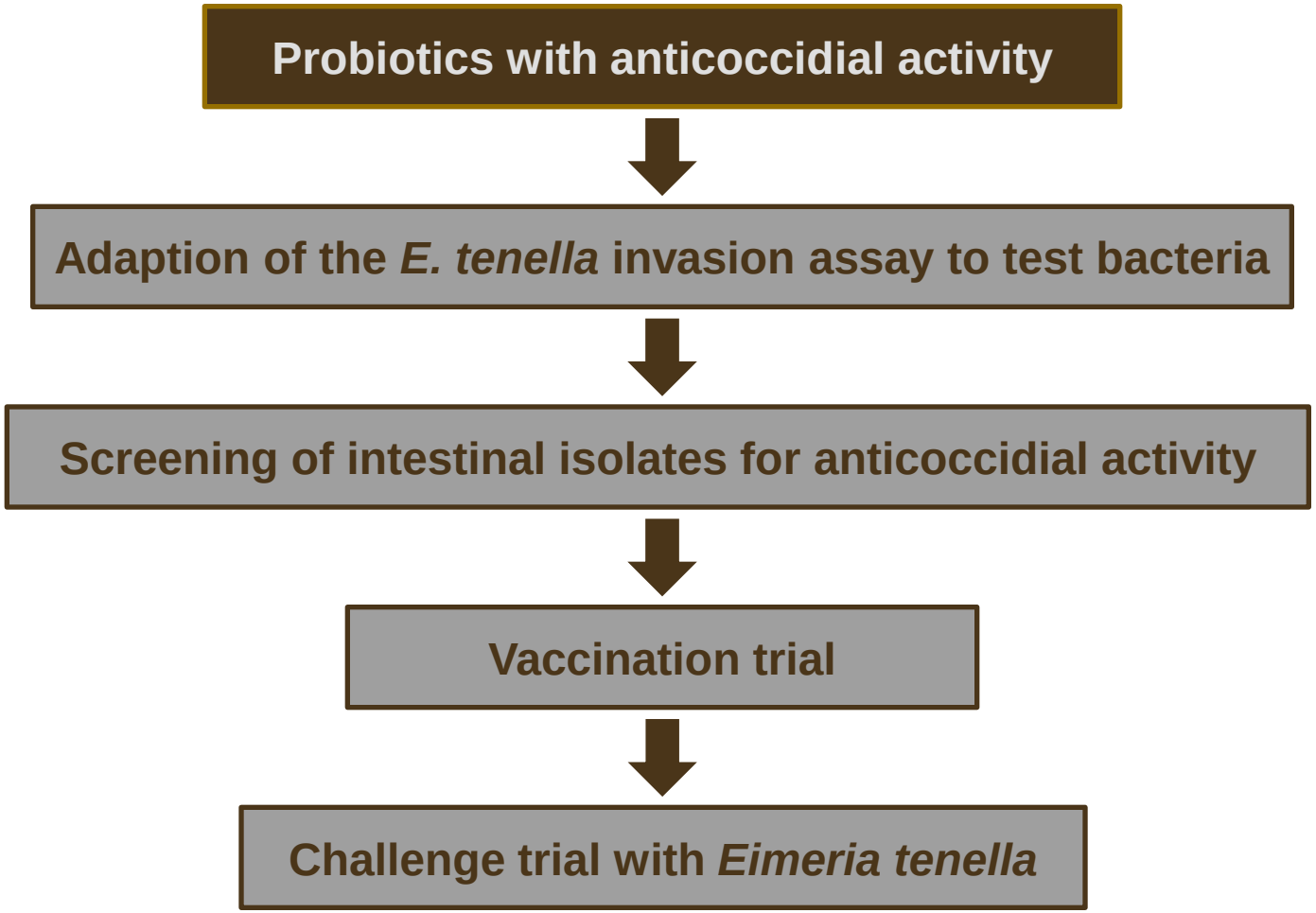
E. tenella in vitro ::: invasion assay

Probiotics





Aim


Probiotics with anticoccidial activity
Adaption of the *E. tenella* invasion assay to test bacteria
Screening of intestinal isolates for anticoccidial activity
Vaccination trial
Challenge trial with *Eimeria tenella*

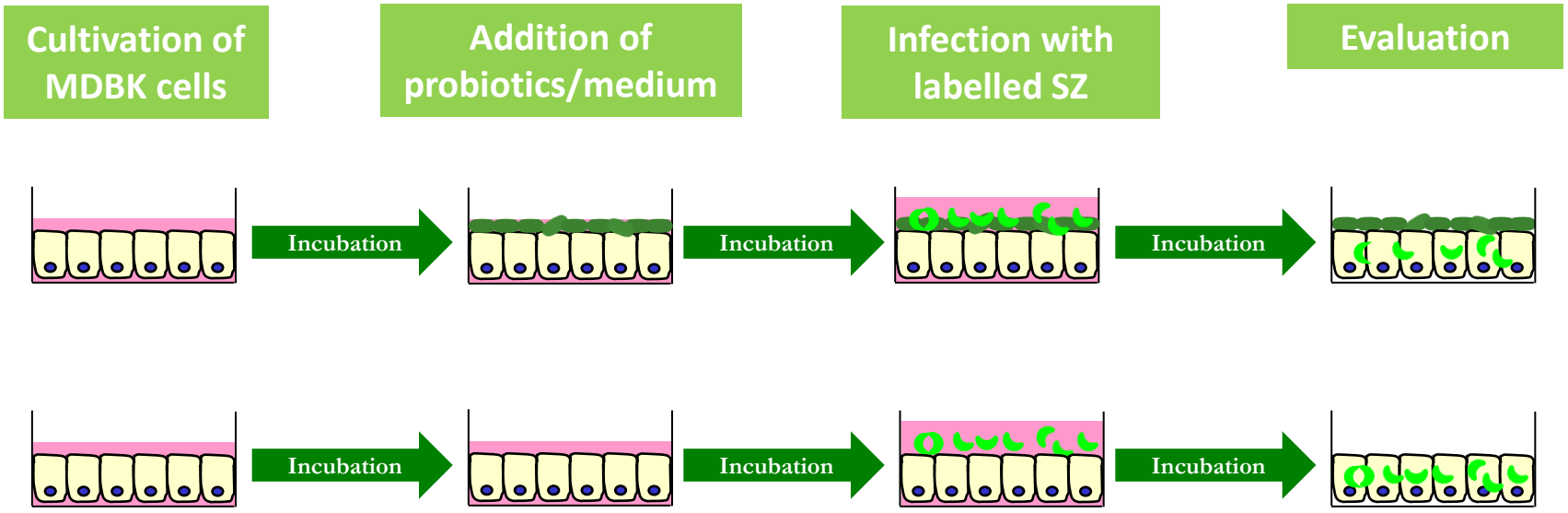
What makes a probiotic?

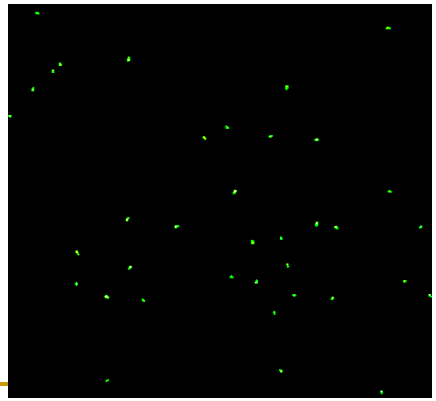
- Probiotics are "live microorganisms which when administered in adequate amount confer a health benefit on the host (FAO 2001).“
- Requirements for a microbe to be considered a probiotic (according to FAO)
  - identified and deposited in an internationally recognised culture collection
  - alive when administered
  - documented health benefit
  - safe
  - good technological properties

Test strains

No	Strains
1	*Bacillus subtilis* #588
2	*Bifidobacterium animalis* #503
3	*Enterococcus faecium* #589
4	*Enterococcus faecium* #497
5	*Enterococcus faecium* BIO34
6	*Lactobacillus reuteri* #514
7	*Lactobacillus salivarius* #505
8	*Pediococcus acidilactici* #499

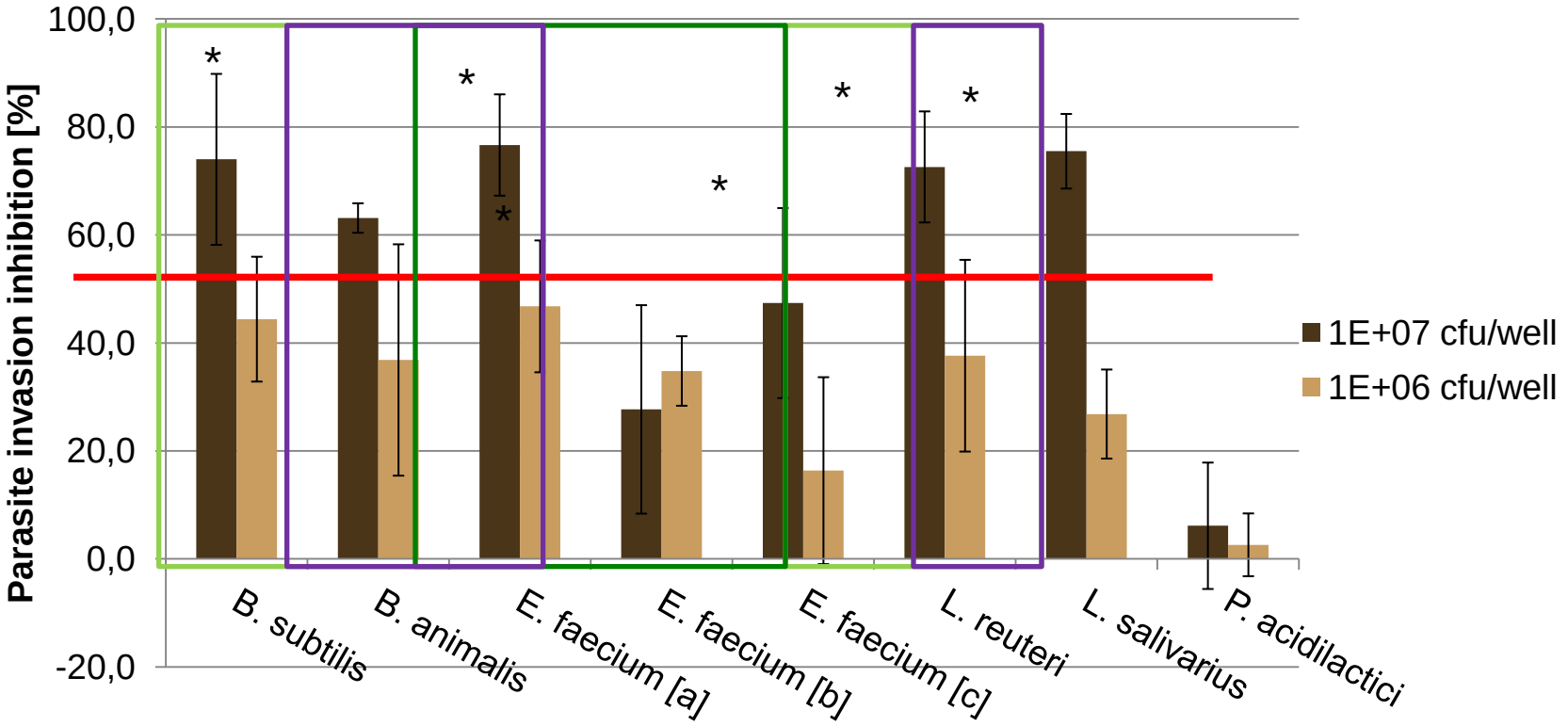
Invasion assay





According to Schubert *et al.* (2005)
CFDA-SE (Carboxyfluorescein diacetate succinimidyl ester) labelling

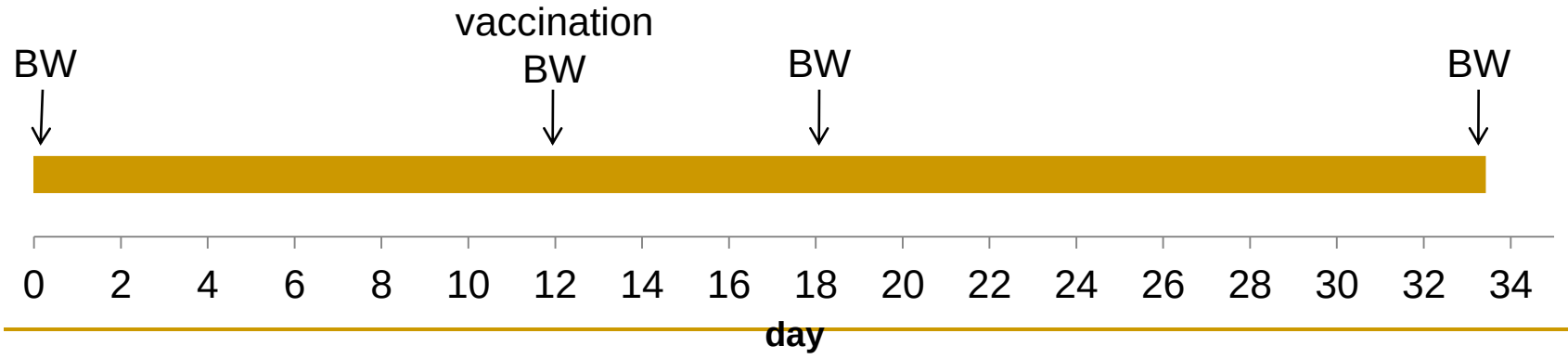
Anticoccidial activity of test strains *in vitro*



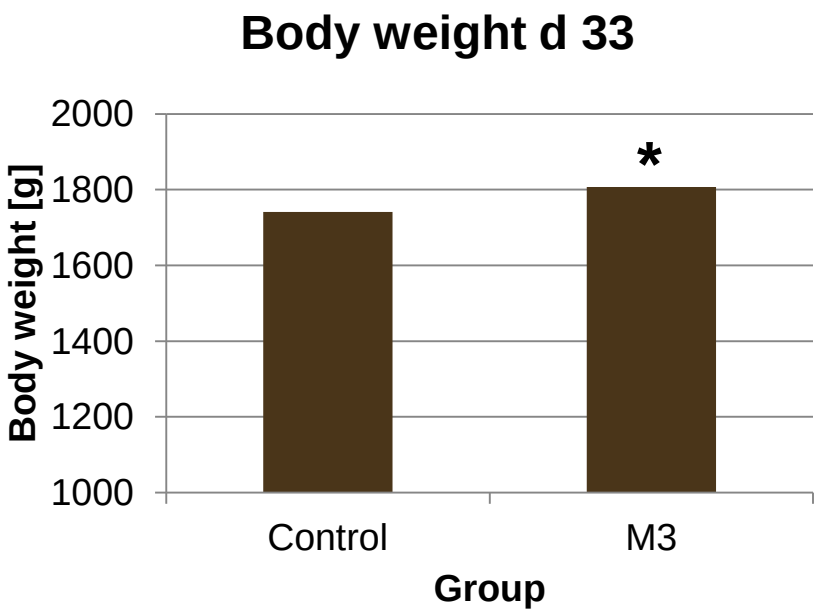
* significantly different from the control ($p < 0.05$, ANOVA, Dunnett test)

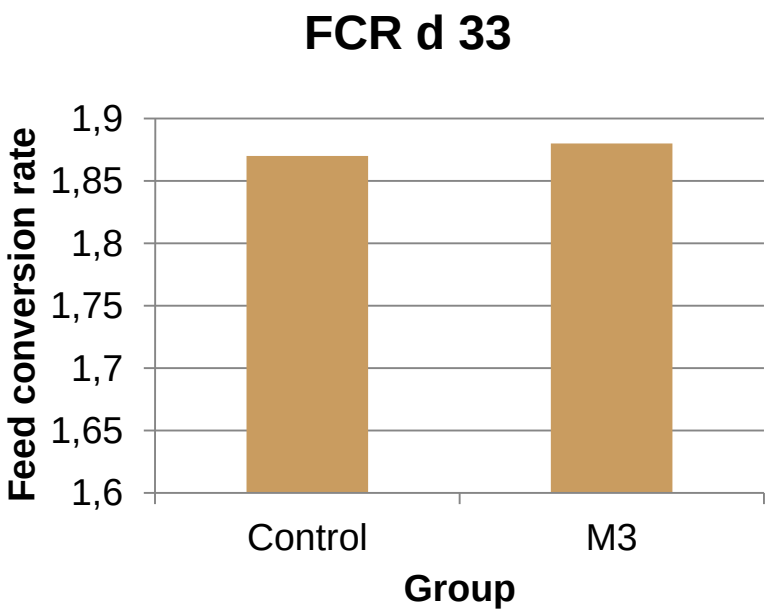
Vaccination trial

- 320 Ross broiler, 1 day old, male
  - 2 groups
    - Group A: Control
    - Group B: M3 – mixture of *E. faecium, L. salivarius*, *B. animalis* 5E+08 cfu/kg feed
  - 8 replications per group
  - 20 birds per replication
- vaccination with 10-fold dosis Paracox®-5 (crop intubation)
  - *E. acervulina* HP 500
  - *E. maxima* CP 200
  - *E. maxima* MFP 100
  - *E. mitis* HP 1000
  - *E. tenella* HP



Performance parameter

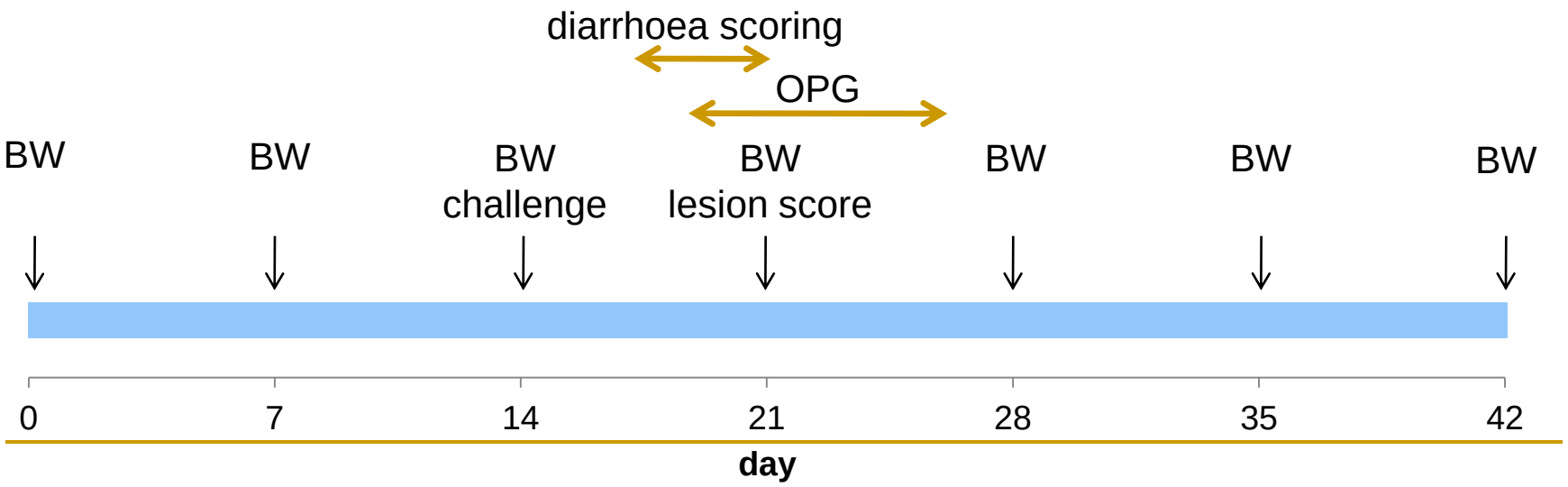




* p-values ≤ 0.05 (Mann-Whitney *U* test)

Challenge trial

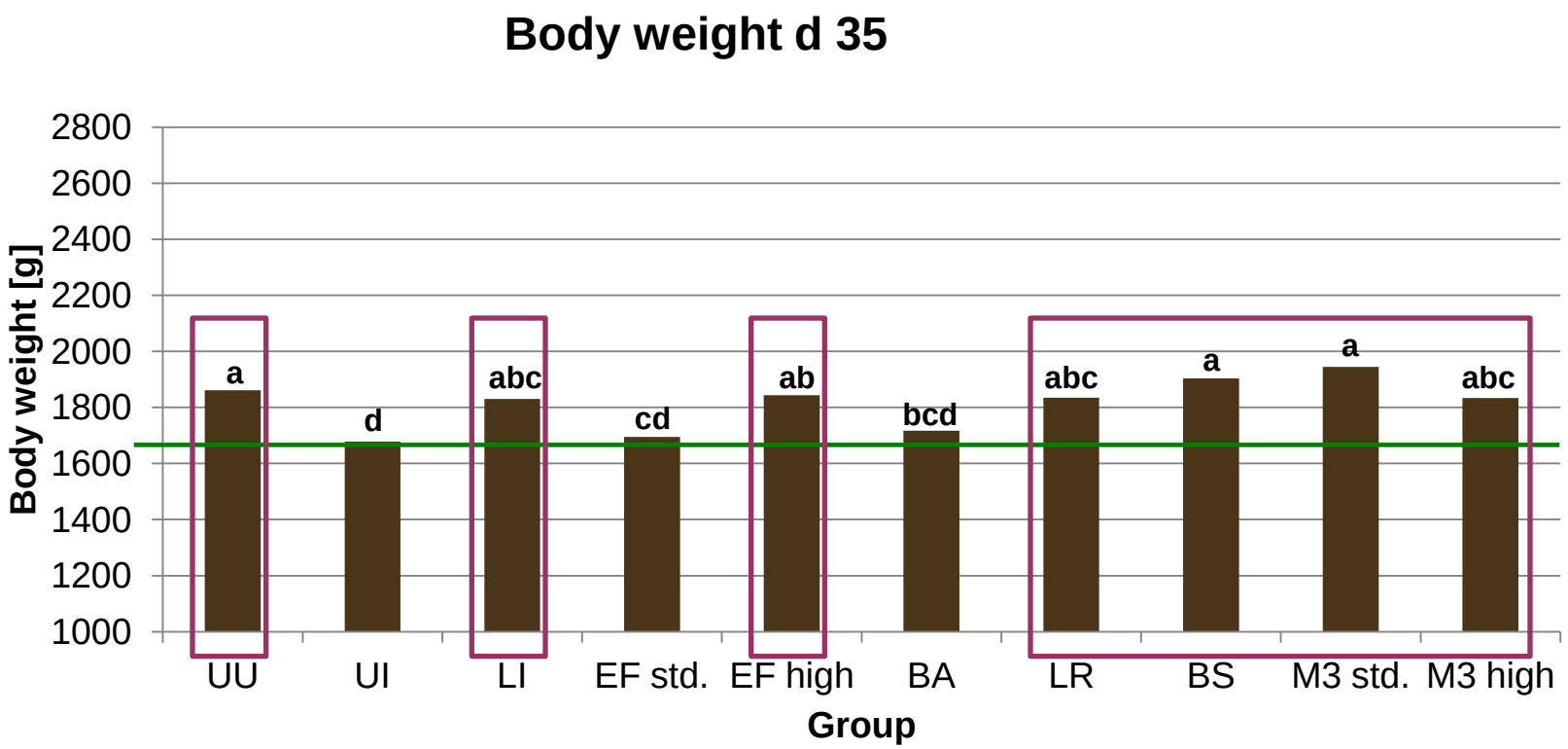
- Laboratory of Animal Nutrition and Husbandry, Veterinary Faculty, University of Thessaly, Greece
- 300 Cobb500^{TM} broiler, 1 day old
  - 10 groups
  - 3 replications per group
  - 10 birds per replication
- challenge with *E. tenella* Wis 2E+04 oocysts per bird at day 14 (crop intubation)



Groups

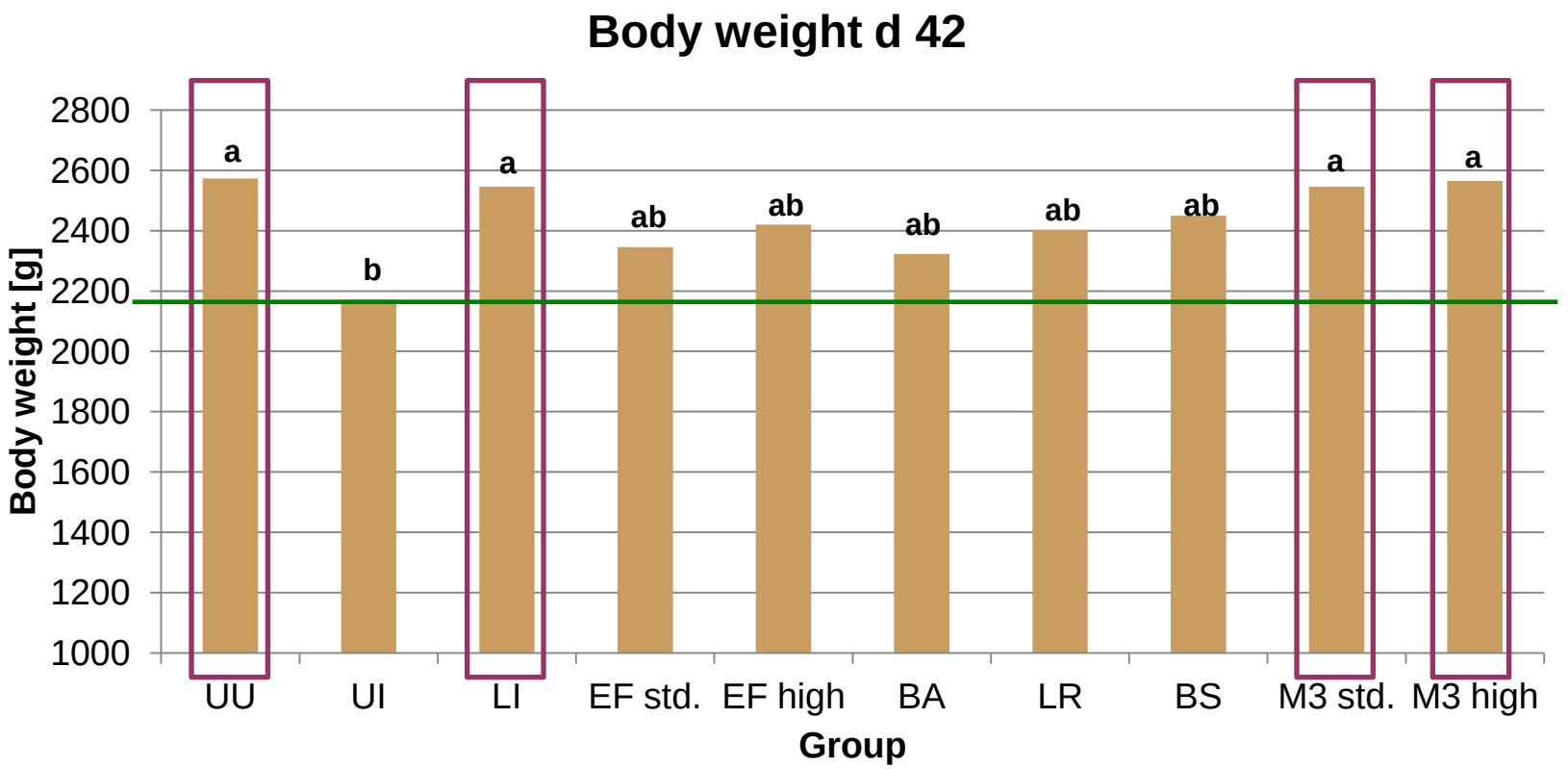
Group	Code	Description	Inclusion rate [cfu/kg feed]
1	UU	untreated, uninfected	
2	UI	untreated, infected	
3	LI	lasalocid, infected	
4	EF std.	*E. faecium*	5E+08
5	EF high	*E. faecium*	5E+09
6	BA	*B. animalis*	5E+08
7	LR	*L. reuteri*	5E+08
8	BS	*B. subtilis*	5E+08
9	M3 std.	Mixture of EF/BA/LS	5E+08
10	M3 high	Mixture of EF/BA/LS	5E+09

Performance parameters I



[a,b,c,d] different letters in data series indicate different significances ($p > 0.05$, Tukey's test)

Performance parameters II



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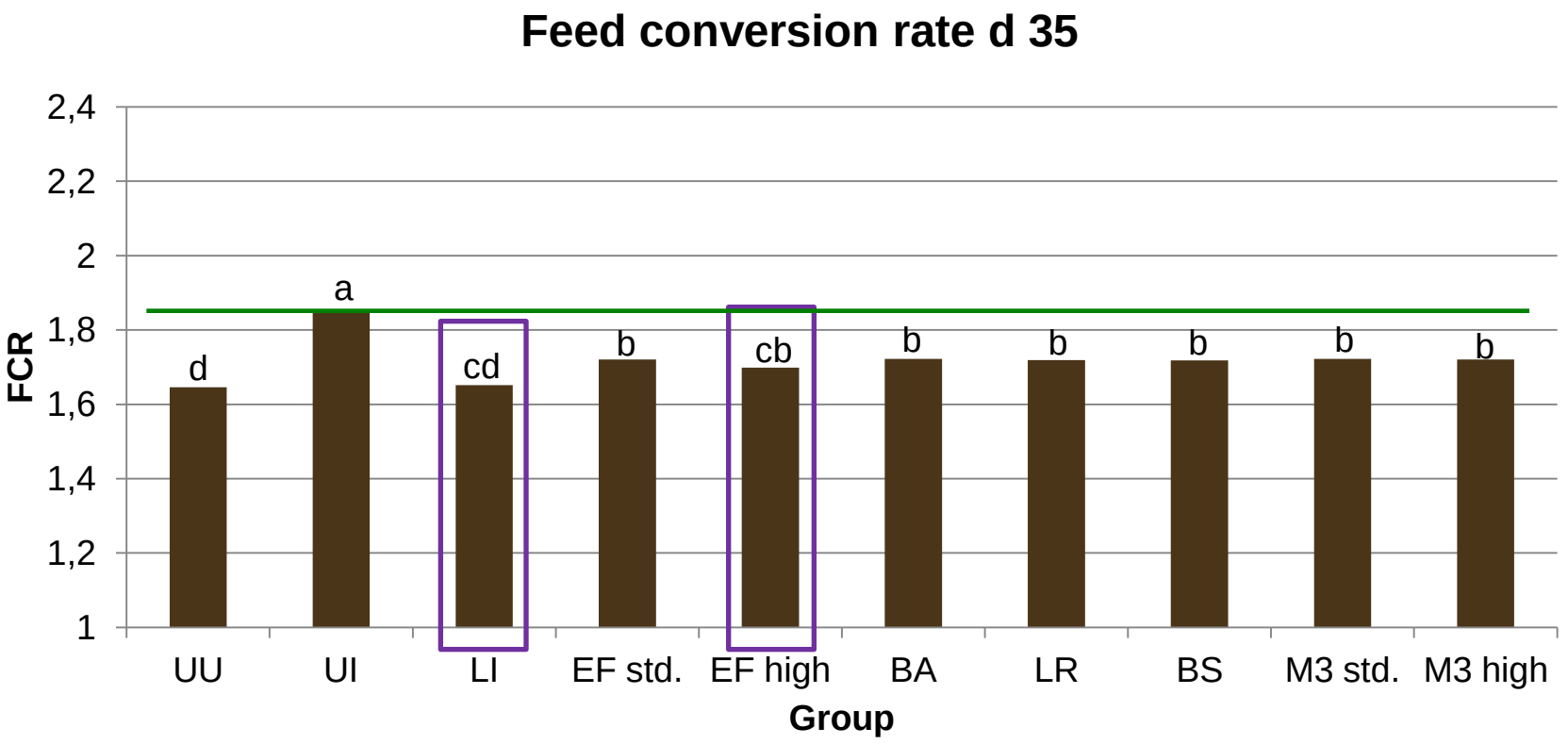
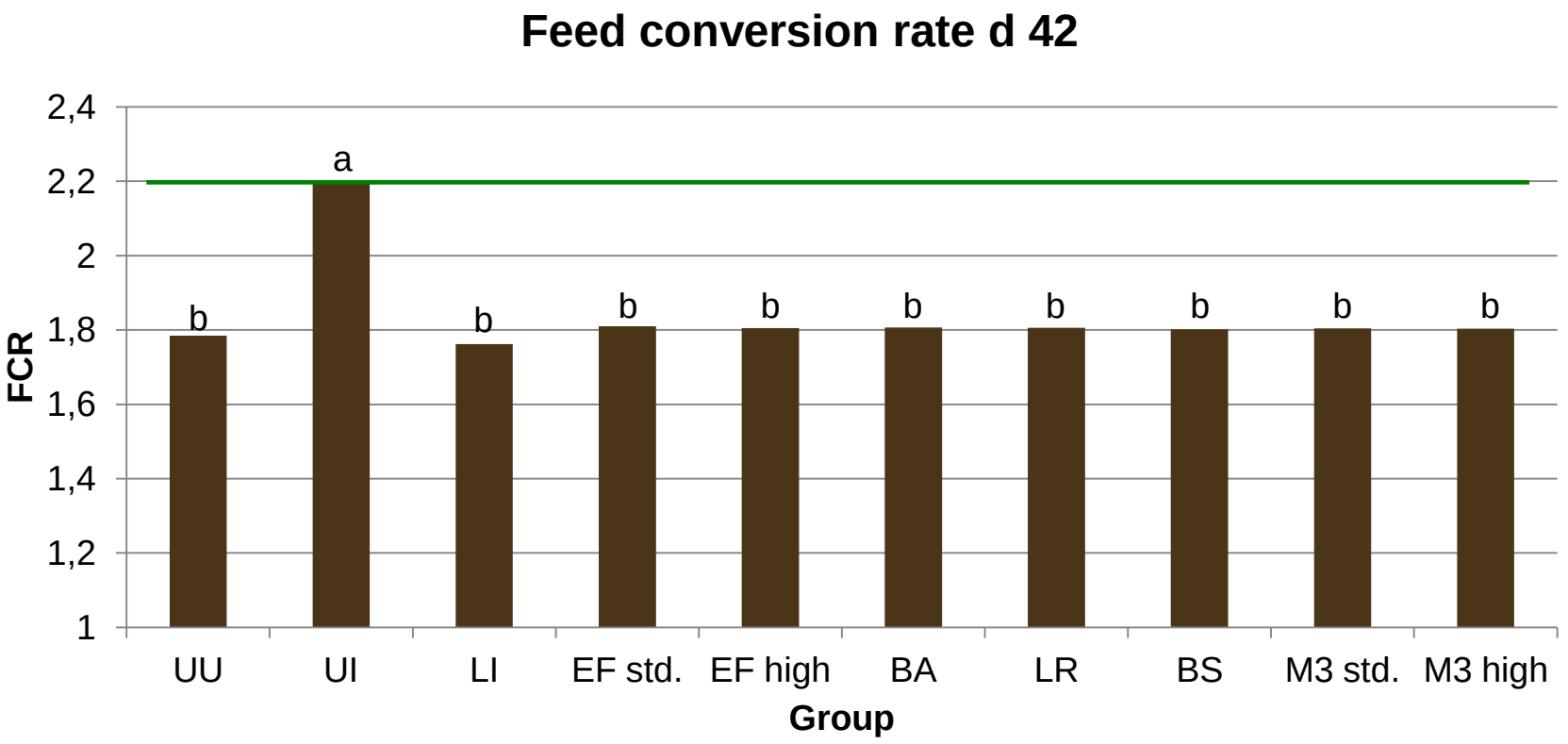
Performance parameters III



[a,b,c,d] different letters in data series indicate different significances ($p > 0.05$, Tukey's test)

Performance parameters IV



[a,b,c,d] different letters in data series indicate different significances ($p > 0.05$, Tukey's test)

Parasitological parameters

	UU	UI	LI	EF std.	EF high	BA	LR	BS	M3 std.	M3 high
Lesion score d 21	0 ^{d}	3.33 ^{a}	1.67 ^{c}	2.75 ^{abc}	2.08 ^{abc}	2.58 ^{abc}	3.33 ^{a}	3.08 ^{ab}	1.75 ^{bc}	2.17 ^{abc}
Faecal score	0	3+	2+	3+	3	3	4	3+	2+	2+
Oocyst reduction [%]		0 ^{a}	98 ^{d}	45.1 ^{b}	71.4 ^{c}	46.6 ^{b}	86.4 ^{d}	89.6 ^{d}	71.1 ^{c}	56.8 ^{b}
Total mortality	0	6	2	3	4	4	3	4	3	2

Lesion score according to Johnson and Reid, 1970
Faecal score accroding to Youn and Noh, 2001

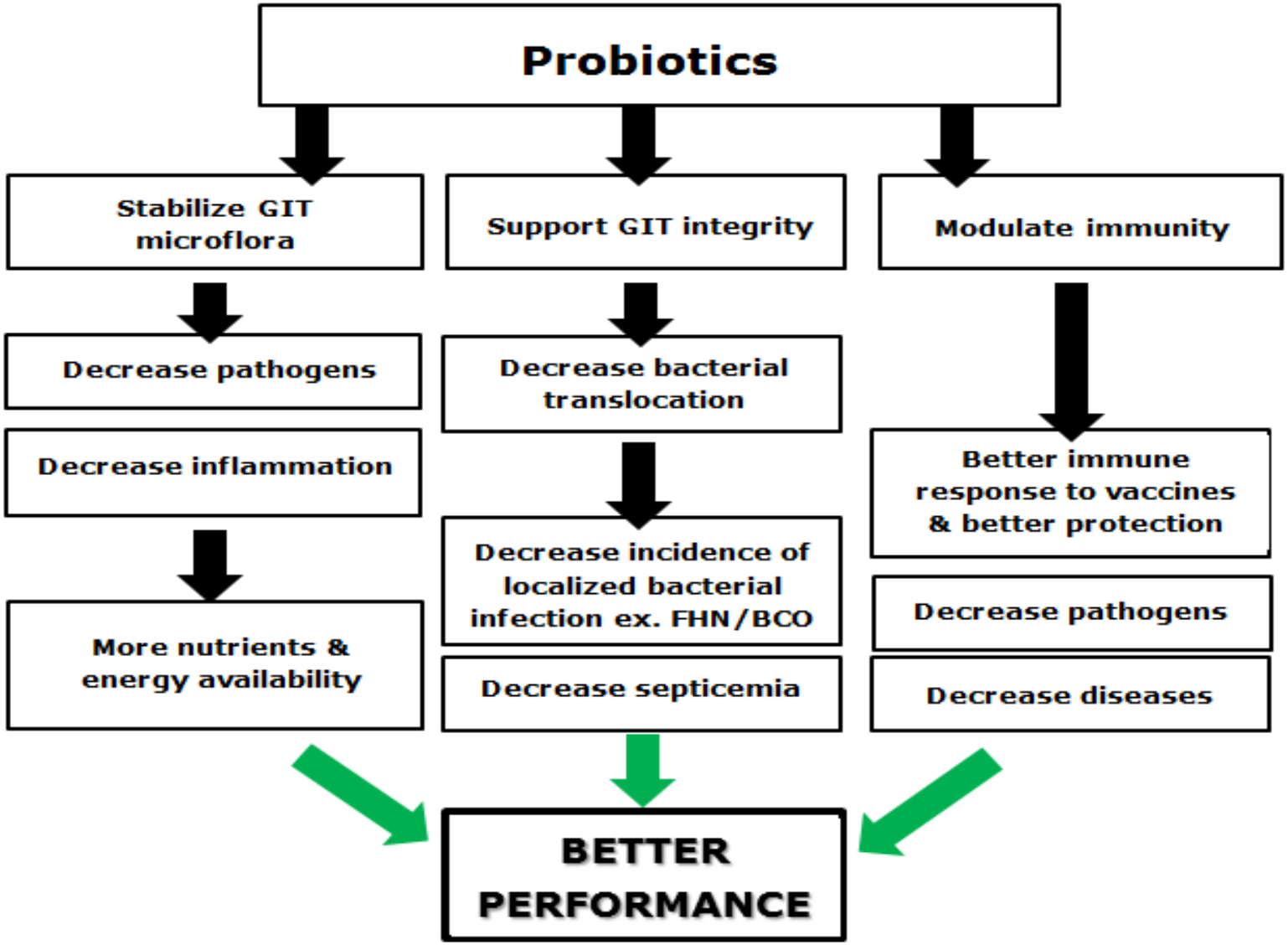
Conclusion & Outlook

- invasion assay suitable for testing anticoccidial activity of probiotics
- anticoccidial activity is strain specific
- *in vitro* data were confirmed in feeding trials
- performance parameters did not always correlate with parasitological parameters
- be

5 out of 8 tested strains showed anticoccidial activity ✓

- study mode of action *in vitro*
- more *in vivo* studies under practical conditions
- activity against other *Eimeria* species

Probiotics and coccidiosis control



Probiotics and coccidiosis control

- Paucity of the literature discussing probiotics use to control parasitic diseases such as coccidiosis

- Most studies have focused on the effects of probiotics killing pathogens at the gut level, however *in vivo* studies on non-gut pathogens such as *Trypanosoma* support a remote effect of probiotics possibly through a non-specific immune modulation

Bera et al., 2010; Dalloul et al., 2003 and 2005; Ghasemi et al., 2010; Giannenas et al., 2012; Hume et al., 2011; Lee et al., 2007 and 2010; Stringfellow et al., 2011; Travers et al., 2011; Taherpour et al., 2012

Another study

- To investigate the potential effect of feed probiotics on broilers' performance and gut health experimentally challenged with sporulated oocysts of
  - ***Eimeria acervulina*,**
  - ***Eimeria maxima* and**
  - ***Eimeria tenella*.**

Design

- 150 Day-old Ross 308 male broiler chicks
- 5 groups - 3 replicates
- Challenged by oral gavage at day 14

  5 10^4 sporulated oocysts of *E. acervulina*,
  2 10^4 sporulated oocysts of *E. maxima*
  2 10^4 sporulated oocysts of *E. tenella*

Design

Code	Description	Incl. Rate	Infected
UU	Untreated		No
UI	Untreated		Yes
LI	Lasalocid	75 ppm	
PS1	Non-encapsulated multistrain probiotic (EF, BA, LS)	5E+08 [cfu/kg]	
PS3	Encapsulated multistrain probiotic (EF, BA, LS)	5E+08 [cfu/kg]	

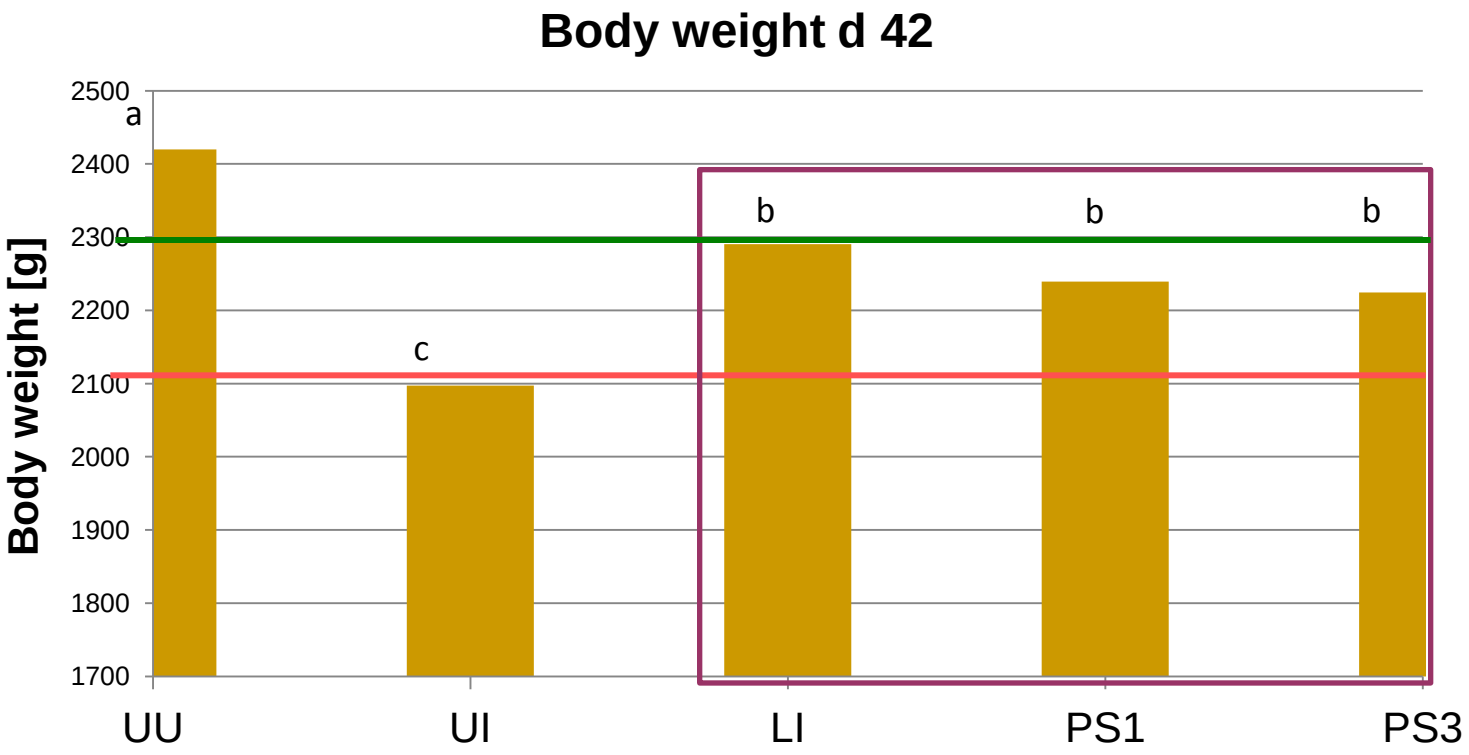
Study parameters

- Trial period - 42 days.
- Body weight, feed intake, FCR, lesion score (21), bloody diarrhoea (17-21) and oocysts count (7 ,14, 20,21-26, 35 ,42) were recorded and calculated.
- At the end of the experiment, duodenum, jejunum and ileum samples were subjected to morphological evaluation.

Results

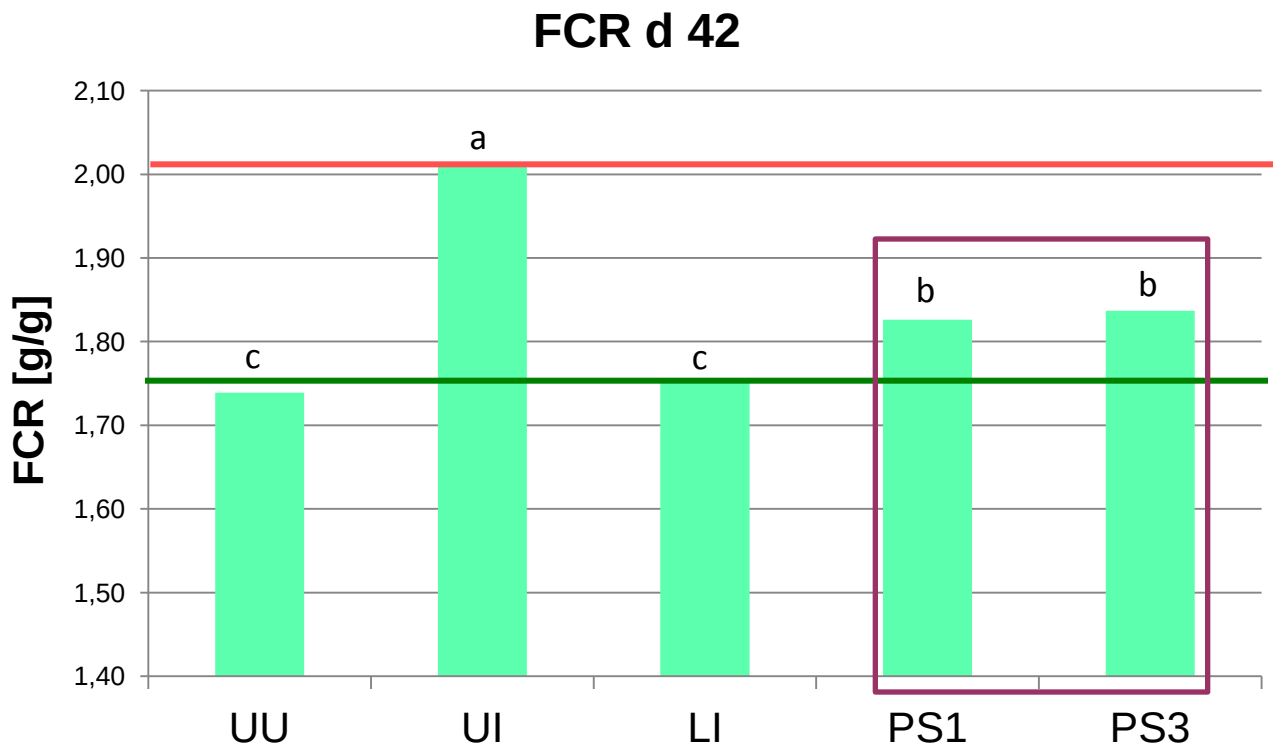
- The results of the study showed that probiotics supplementation exerted a coccidiostatic effect against *Eimeria* species, reflected on birds' performance that was similar to lasalocid ($P<0.05$).

Results



[a,b,c] significant differences ($p < 0.05$, ANOVA+Duncan test)

Results



[a,b,c] significant differences ($p < 0.05$, ANOVA+Duncan test)

Results

	UU	UI	LI	PS1	PS3
Lesion score ileum d21 [0-4]	0.0 ^{a}	3.3 ^{b}	**1.6** ^{ac}	**2.6** ^{bc}	**2.6** ^{bc}
Lesion score cecum d21 [0-4]	0.0 ^{a}	3.0 ^{b}	**1.6** ^{ac}	**2.7** ^{bc}	**2.4** ^{bc}
Fecal score [0-4]	0	3+	**1+**	**2+**	**2+**
OPG reduction [%]	-	0	**99**	**70**	**72**
Duodenum VH/CD	8.98^{b}	9.93ab	10.01^{ab}	**10.73^{ab}**	**11.13^{a}**
Jejunum VH/CD	6.74^{b}	6.53^{b}	6.70^{b}	**6.81^{b}**	**9.53^{a}**
Ileum VH/CD	6.76^{a}	6.75^{a}	6.37^{a}	**8.53^{bc}**	**7.93^{ac}**
Total mortality [n/30]	0	4	**1**	**2**	**2**

^{a,b,c} significant differences ($p < 0.05$, ANOVA+Duncan test)

Lesion score according to Johnson and Reid, 1970
Fecal score according to Youn and Noh, 2001

In conclusion,

- **Probiotics**
- showed improvement in both growth performance and intestinal health in birds challenged with *Eimeria* species, in comparison to control infected birds and similar improvement to that exhibited by lasalocid

- can be used as a complementary approach in shuttle and rotation programs with anticoccidials, vaccines to reduce the disease incidence, performance reduction and the intensity of *Eimeria* species resistance to the anticoccidial drugs

Perspectives

New investigations are warranted on the following topics:

• mechanisms of action for promising compounds (e.g. bacteriocins from various probiotics, substances with health benefits, functional amino acids, etc.)

• bioavailability and pharmaco-kinetics of these substances

• zootechnical and biological effects of BLENDS of alternatives based on the fact that GIT disorders post-hatching display highly complex aetiology and mechanisms

• negative interactions between feed components and added alternatives OR among alternatives must be understood

"Attempts to rear chickens in the absence of coccidial infections are in general ill advised."

Tyzzer, 1932