Antibiotic Free Research Design: Optimize product understanding through thoughtful design

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Southern Poultry Research Group
SPRG

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BACKGROUND:

- Undergraduate – Avian Biology and Bio. Sci. at UGA (2011)
- Graduate School – Department of Poultry Science (2011-2018)
  - Applied nutrition – intestinal health
- UGA College of Vet. Medicine (2012 – 2016)
- AZOMITE Mineral Products- Research and Tech. Serv. (2018-2019)
  - Feed manufacturing
- SPRG – Applied, health-based poultry research
  - Nutritionist and Veterinarian

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BACKGROUND:

- SPRG
  - Challenge models with much focus on Nutritional Health.

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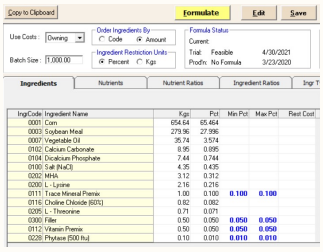
  - Challenge models with much focus on Nutritional Health.



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BACKGROUND:

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What kind of Experiments does SPRG conduct?

- Enteric Health
  - Coccidiosis: *E. acervulina, E. maxima, E. tenella*
  - Necrotic enteritis: mild, moderate, severe
    - Modulate cocci and/or *C. perfringens*
- Food Safety
  - *Salmonella*: Enteritidis, Typhimurium, Heidelberg, Infantis, Kentucky
  - *Campylobacter jejuni*
- Applied poultry performance
- Digestibility
- Others: Wire ramps, wire floor, *Pasteurella multocida,* intratracheal *E. coli* gavage, environmental experiments, Feed manufacturing stability

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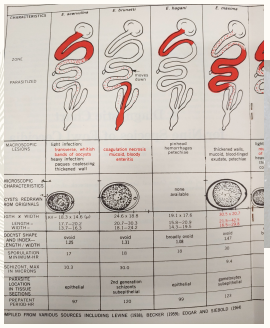
Applied ABF Poultry Experiments



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Applied ABF Poultry Experiments

1. Where do we begin when designing a research trial?
   *2 vital questions to answer early in process
2. What can we do to optimize the design?

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WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

A. What is the end goal of the experiment?

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WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

A. What is the end goal of the experiment?
- Internal use
- Marketing
- Publishing

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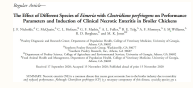
WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

A. What is the end goal of the experiment?
- Internal use
  - May not need a negative control
  - Applied aspect is not necessarily as important
- Marketing
  - Ensure specific questions are answered for the target audience
  - Focus on applied aspect of design
- Publishing
  - Negative controls

For Internal Use Only



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WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

B. If we can only learn ONE thing, what would it be?

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WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

B. If you could learn ONE thing, what would it be.

- Dietary Stress
- Environmental Stress
- *Eimeria* infection

} Pick One

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WHERE DO WE BEGIN IN DESIGNING A RESEARCH TRIAL?

B. If you could learn ONE thing, what would it be.

It seems like a good economical way to learn a lot in a single study, but as a study becomes more complex the focus is lost and the outcome is more difficult to interpret.

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THE PROGRESSION:

- There are exceptions, but there is a general progression of studies as you answer one question at a time.

Internal Use Marketing Publishing/Academic

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THE PROGRESSION:

- Are you screening **products**, or do you have a product identified?
  - Screening → *In Vitro* → Battery cages

Internal Use

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THE PROGRESSION:

Why battery cages?
- More replicates
- Control
- Quick turnaround

Internal Use

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THE PROGRESSION:

- So, you have a product, do you know what **dose** you need to use?
  - Dose titration → Battery cages

Internal Use

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THE PROGRESSION:

- So, you have a product, do you know what dose you need to use?
  - Administration **route** (water, feed, gel, spray, etc.)→ Battery cages

Internal Use

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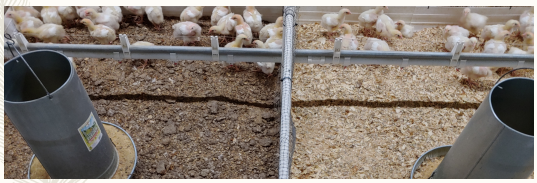
THE PROGRESSION:

- Do you have recent applied data?
  - If not, a floor pen model may be an appropriate next step



Marketing

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THE PROGRESSION:

- What is the primary metric we are considering and how effective is the product?
  - This is going to guide challenge
  - This will also be important to ensure enough replicates
  - Consider the target audience

Marketing

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THE PROGRESSION:

- If we have all "yes" up to this point,
  - Do we know how the product works?
  - If not, back to tightly controlled battery cages
  - Test a specific hypothesis

Publishing/Academic

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THE PROGRESSION:

- Continued understanding of the product,
  - How is it interacting with different therapeutics?
    - Coccidiosis vaccine
    - Chemicals
  - How is it interacting in a diet with _____.
  - Does conditioning temperature influence product stability?

Marketing
Publishing/Academic

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SUMMARY

- Product
- Inclusion/Dose and Route
- Recent applied data
- MOA
- Special circumstances

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MAXIMIZING RESEARCH BY DESIGN

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MAXIMIZING RESEARCH BY DESIGN

- Standardization
- Statistical Design
- Challenge Design

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Maximizing Research by Design

- Standardization
  - Environment
    - Same bedding
    - Same number of birds/square foot
    - Stir fans

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Maximizing Research by Design

- Standardization
  - Test subjects
    - Chick sorting – select healthiest chicks
    - Single sex experiments – sexual dimorphism

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Maximizing Research by Design

- Standardization
  - Diet
    - Basal diet manufacturing
    - CVs - Optimal Mix time

	3-minute mix time	5-minute mix time	7-minute mix time
CV	13.6	9.75	7.13

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Maximizing Research by Design

- Standardization
  - Labels

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Maximizing Research by Design

- Standardization
  - Biosecurity

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Maximizing Research by Design

- Statistical Design
  - Consult a Statistician
  - Get feedback in the design of the experiment, not after data is collected

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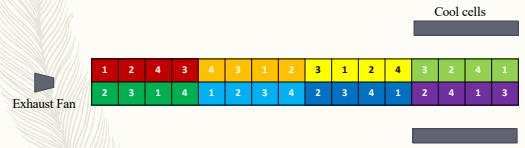
Maximizing Research by Design

- Statistical Design
  - Randomized block design – account for facility/spacial variation.



*4 treatments and 8 blocks

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Maximizing Research by Design

- Statistical Design
  - Power analysis – a guide to number of replicates needed under specific circumstances
    - As you increase replicates you increase statistical power.
    - Generally, greater bird numbers will lower the standard deviation

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Maximizing Research by Design

- Statistical Design
  - Power analysis – a guide to number of replicates needed under specific circumstances

Challenge	Parameter/Day	Replicates	Power
None	Weight gain/42	3	0.37
None	Weight gain/42	6	0.64
None	Weight gain/42	8	0.76
None	Weight gain/42	10	0.85
None	Weight gain/42	12	0.90

Assume 2.5 kg average wt. of control and product will provide 100g (4%) increase.
Assume a standard deviation of 0.075 kg
Assume a P-value of 0.05

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Maximizing Research by Design

- Statistical Design
  - Power analysis – depends on model

Challenge	Parameter/Day	SD	CV
None	FCR/28	0.0250	1.7423
None	Wt/28	0.0477	3.5318
None	FCR/42	0.0253	1.5314
None	Wt/42	0.0603	2.4791
Cocci	FCR/28	0.1250	7.3159
Cocci	Wt/28	0.0976	9.7674
Cocci	FCR/42	0.1902	9.4062
Cocci	Wt/42	0.1830	9.7108

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Maximizing Research by Design

- Statistical Design
  - Power analysis – depends on model

Challenge	Parameter/Day	SD	CV
None	Weight gain/42	0.0603	2.4791
Cocci	Weight gain/42	0.1830	9.7108

No Challenge
~100 grams (4.0%):
$\alpha = 0.05$ → Power = **0.96**
$\alpha = 0.10$ → Power = **0.98**

Eimeria Challenge
~100 grams (4.0%):
$\alpha = 0.05$ → Power = **0.23**
$\alpha = 0.10$ → Power = **0.34**

Assume 2.5 kg average wt. of control and product will provide 100g (4%) increase.
Assume 10 replicates

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Maximizing Research by Design

- Statistical Design
  - Power analysis – depends on "margin of gain" (or loss)

Improvement	Parameter/Day	Replicates	Power
None	Weight gain/42	8	**0.02**
2% (50g)	Weight gain/42	8	**0.27**
4% (100g)	Weight gain/42	8	**0.76**
6% (150g)	Weight gain/42	8	**0.98**
10% (250g)	Weight gain/42	8	**1.00**

Assume 2.5 kg average wt. of control
Assume a standard deviation of 0.075 kg
Assume a P-value of 0.05

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Maximizing Research by Design

- Statistical Design
  - Benchmarking
    - Antibiotics – can be a good benchmark because the influence is well characterized and accepted.
    - ABF you are not competing. It is more of a positive control.

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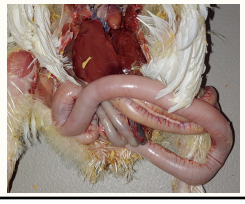
Maximizing Research by Design

- Challenge Design
  - Mild, moderate, or severe (ranges)
  - Often mild challenges do not elicit enough response to measure
  - Severe challenges often overwhelm host defenses



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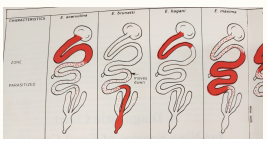
Maximizing Research by Design

- Challenge Design
  - *Eimeria sp.*



Treatment	Body weight gain (kg)	Feed conversion	Necrotic enteritis mortality (%)	Necrotic enteritis lesion score (0–3)
CP only	0.272a	1.480g	0.0a	0.0a
E. acervulina	0.162def	2.100cde	0.0a	0.0a
E. acervulina + CP	0.144f	2.362bc	0.7a	0.11a
E. maxima	0.174cdef	2.316bcd	0.0a	0.0a
E. maxima + CP	0.153ef	2.575b	2.0a	0.94a
E. tenella	0.190cd	2.025def	0.0a	0.0a
E. tenella + CP	0.176cde	2.101cde	0.0a	0.11a
E. necatrix	0.227b	1.762fg	0.0a	0.06a
E. necatrix + CP	0.202bc	1.971ef	0.3a	0.50a
E. brunetti	0.147ef	2.339bcd	0.0a	0.0a
E. brunetti + CP	0.112g	2.937a	1.8a	0.78a

[A]Each value represents the treatment group mean from six replicate cages.
[B]Differing lowercase letters (a–g) indicate $P < 0.05$.

Nicholds *et al* 2021

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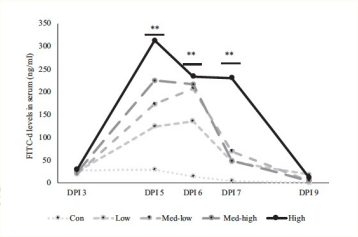
MAXIMIZING RESEARCH BY DESIGN

- Challenge Design - *Eimeria*
  - Pathogen behavior



Teng *et. al* 2020

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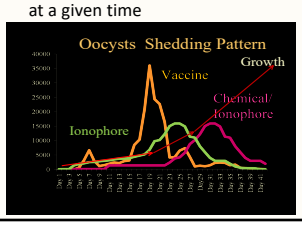
MAXIMIZING RESEARCH BY DESIGN

- Challenge Design - *Eimeria*
  - *Eimeria* lesion score
  - OPG (oocysts/gram)
    - Oocyst production does not always reflect the lesions at a given time



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MAXIMIZING RESEARCH BY DESIGN

- Challenge Design-NE
  - Parameter of interest: clinical disease vs. performance

Performance Data (0-42 Days) and Necrotic enteritis challenge results				
Description	Adjusted FCR (0-42 days)	Body Weight (kg) (0-42 days)	Percent NE Mortality	NE Lesions
Coccidiosis vaccine + CP on DOT 14, 21, 28	1.63^{A}	2.35^{A}	21.33^{A}	0.75^{A}
Coccidiosis vaccine + CP on DOT 14, 15	1.65^{A}	2.34^{A}	20.00^{A}	0.83^{A}
Litter from a previous NE challenge	1.66^{A}	2.24^{B}	1.00^{B}	0.50^{A}

Corresponds to data submitted to AAAP 2021

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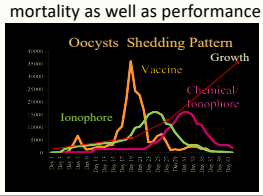
Maximizing Research by Design

- Challenge Design-NE
  - Necrotic enteritis mortality
  - Necrotic enteritis lesion score
    - Metric indicating challenge status
    - Additional birds evaluated for lesions may impact NE mortality as well as performance outcomes.



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Summary

- In order to conduct optimal research, identify a singular outcome of interest [peer reviewed publication, marketing piece, internal understanding] and focus on one aspect at a time.
- Once the goal is established, limit variation between groups through standardization, considering power analysis to maximize opportunity to declare a difference, and optimize challenge/sampling to answer the core questions.

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Questions

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