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From research trials to field application: alternatives to antibiotics

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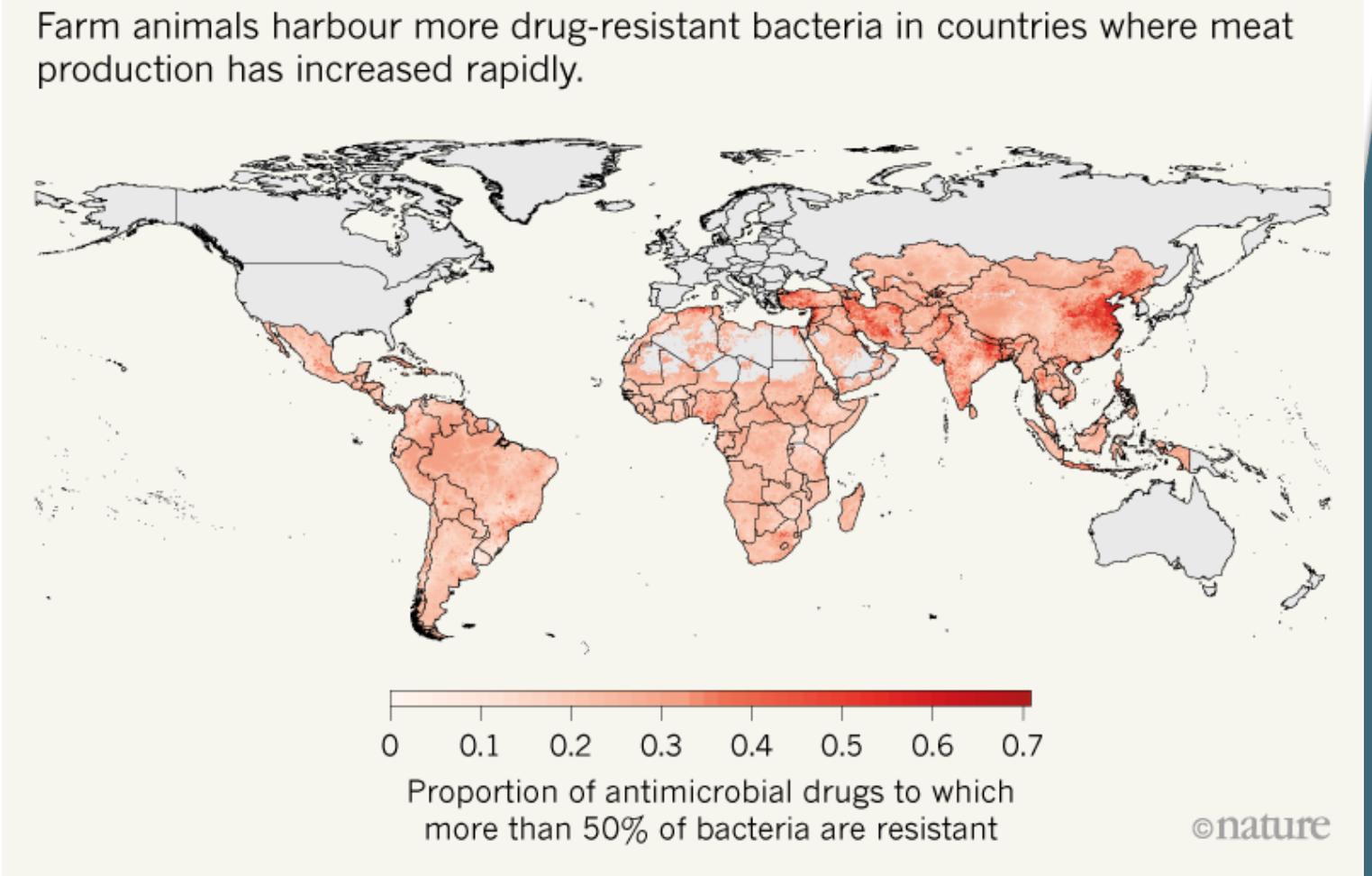
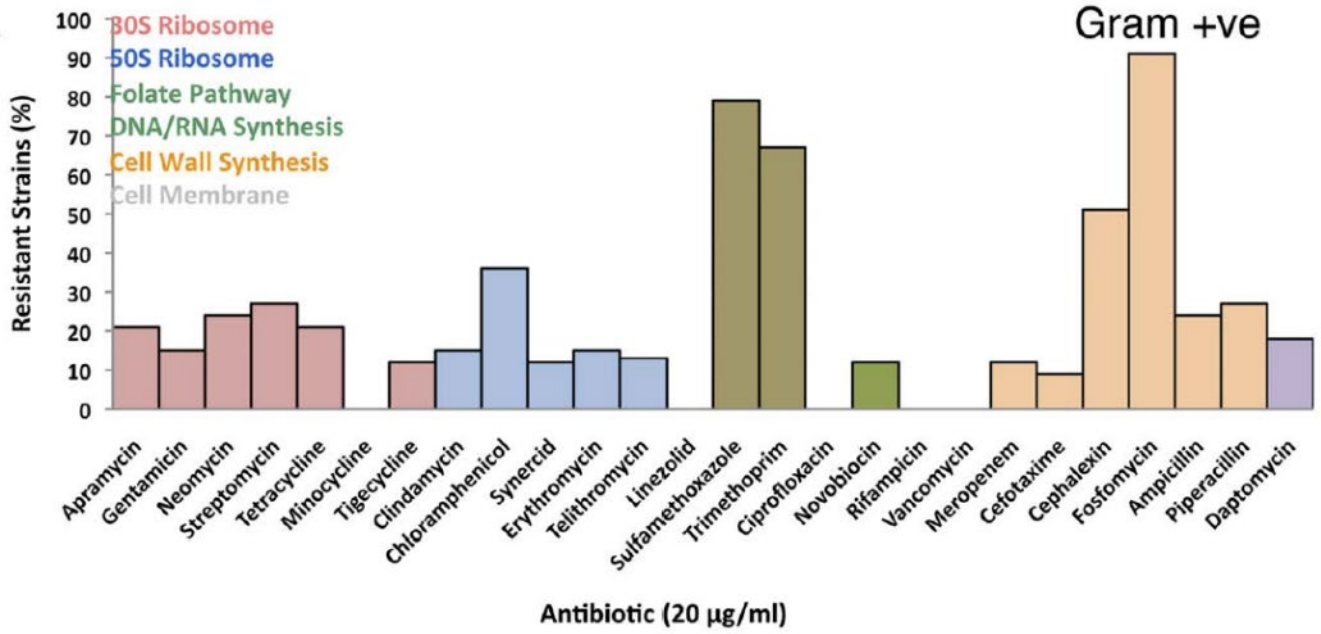
Antimicrobial use – setting the context

- **Global move away from prophylactic/growth promotion use of antibiotics**
  - **Mostly due to concerns over antimicrobial resistance**

T. P. Van Boeckel et al., Science 365, eaaw1944 (2019). DOI: 10.1126/science.aaw1944



Antibiotic resistance in cave bacteria



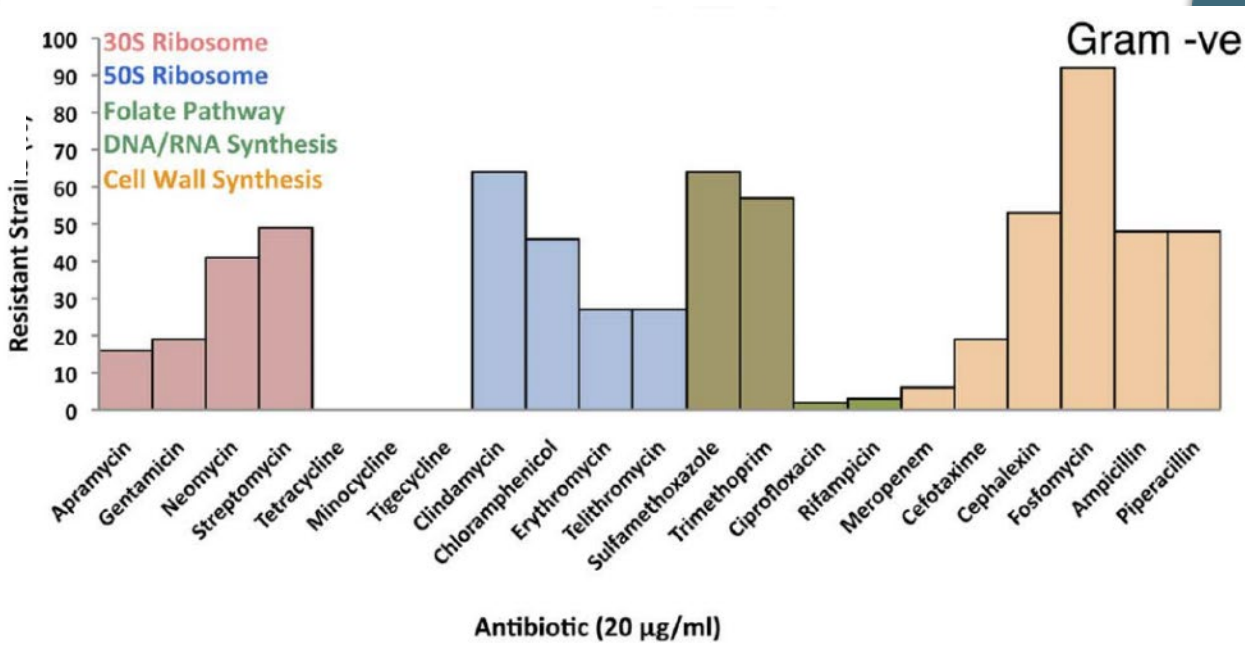


Figure 2. Resistance levels of Lechuguilla cave bacteria at 20 mg/ml against various antibiotics: (top) Gram-positive strains (bottom) Gram-negative strains. Antibiotics are grouped according to their mode of action/target, where each color represents a different target.
doi:10.1371/journal.pone.0034953.g002

Bhullar, K., et al. (2012). "Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome." PLoS One **7: e34953.**

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Antimicrobial use – setting the context

- **Global move away from prophylactic/growth promotion use of antibiotics**
  - **Early adopters**
    - **E.g. European Union**
  - **Niche markets and advancing consumer pressure**
    - **Avoid legislative approach**
      - **E.g. USA, Canada**
  - **Exporting countries**
    - **Match requirements of importing countries**
  - **Developing countries**
    - **Legislation, consumer demands, pressure from large purchasers (e.g. chain restaurants)**

Antimicrobial use – setting the context

- **Move towards non-antibiotic control of bacterial disease**
  - **Niche markets vs industry-wide adoption**
    - **Product differentiation (US, Canada, etc.)**
      - **May command a premium**
    - **Legislation (EU, exporters to EU, etc.)**
      - **AGP-free is the standard to be able to sell chicken**
      - **No premium**
  - **In either case, the objective is to maintain:**
    - **Animal health**
      - **Production efficiency**
      - **Profitability**
    - **Food safety**

Antimicrobial use – setting the context

- **Removal of AGP from poultry diets has real consequences**
  - **Cardinal et al., 2019 Poultry Science 98:6659–6667**

Withdrawal of antibiotic growth promoters from broiler diets: performance indexes and economic impact

Katia Maria Cardinal,[1] Marcos Kipper, Ines Andretta, and Andréa Machado Leal Ribeiro

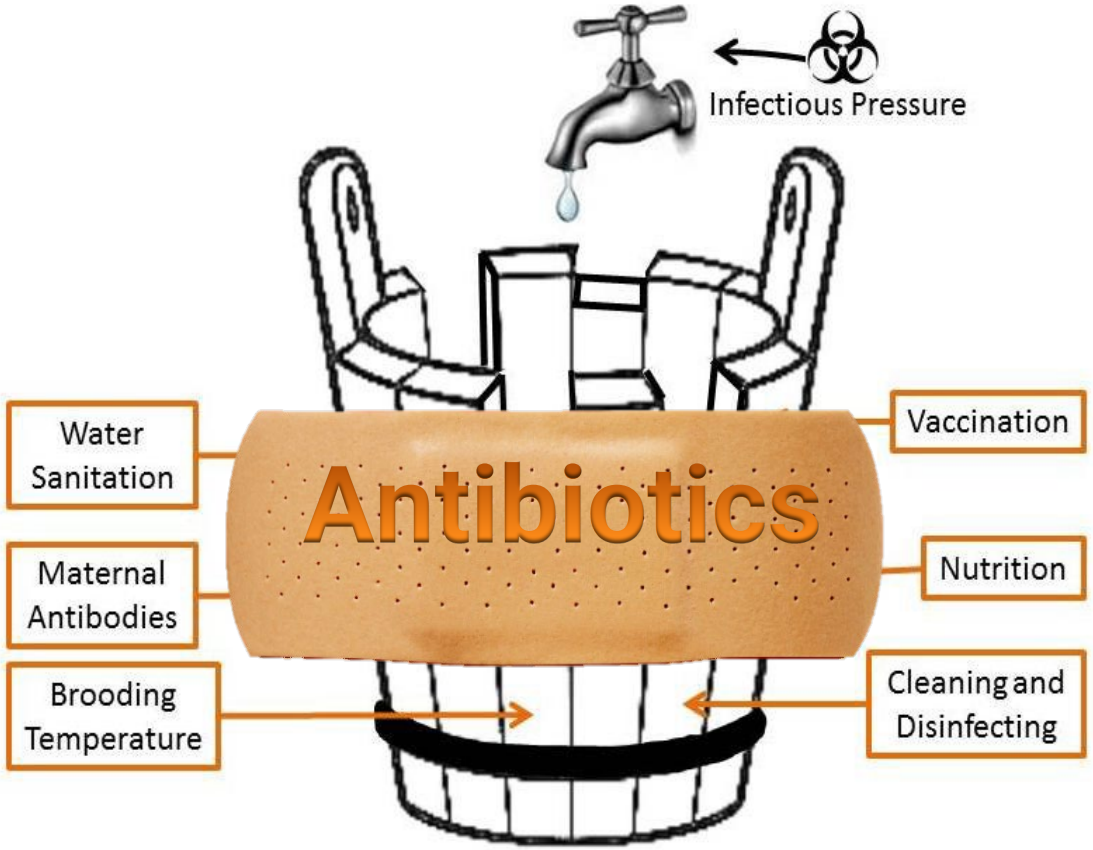
Department of Animal Science, Universidade Federal do Rio Grande do Sul, Porto Alegre, Av. Bento Gonçalves, 7712 – Agronomia, CEP: 91540-000, Rio Grande do Sul, Brazil

  - **Meta-analysis (174 articles; 183 experiments; 121,643 broilers), extrapolated to Brazilian industry (5,840,000,000 broilers in 2017)**
    - **FCR (1-42 d) increased from 1.66 to 1.72**
    - **AGP removal increased costs by $0.03 USD/bird; $183,560,232/year due to additional feed required**
    - **Greater impact early in life**

Alternatives to antibiotics

- **Removal of AGP presents opportunities for a wide range of potential pathogens**
- **Want to reduce negative interactions between the host and gut microbes**
  - **Local and systemic effects**
    - **Disease**
    - **Reduced performance**
- **Protect humans from food-borne illness**
  - **Immunological tolerance by the bird**

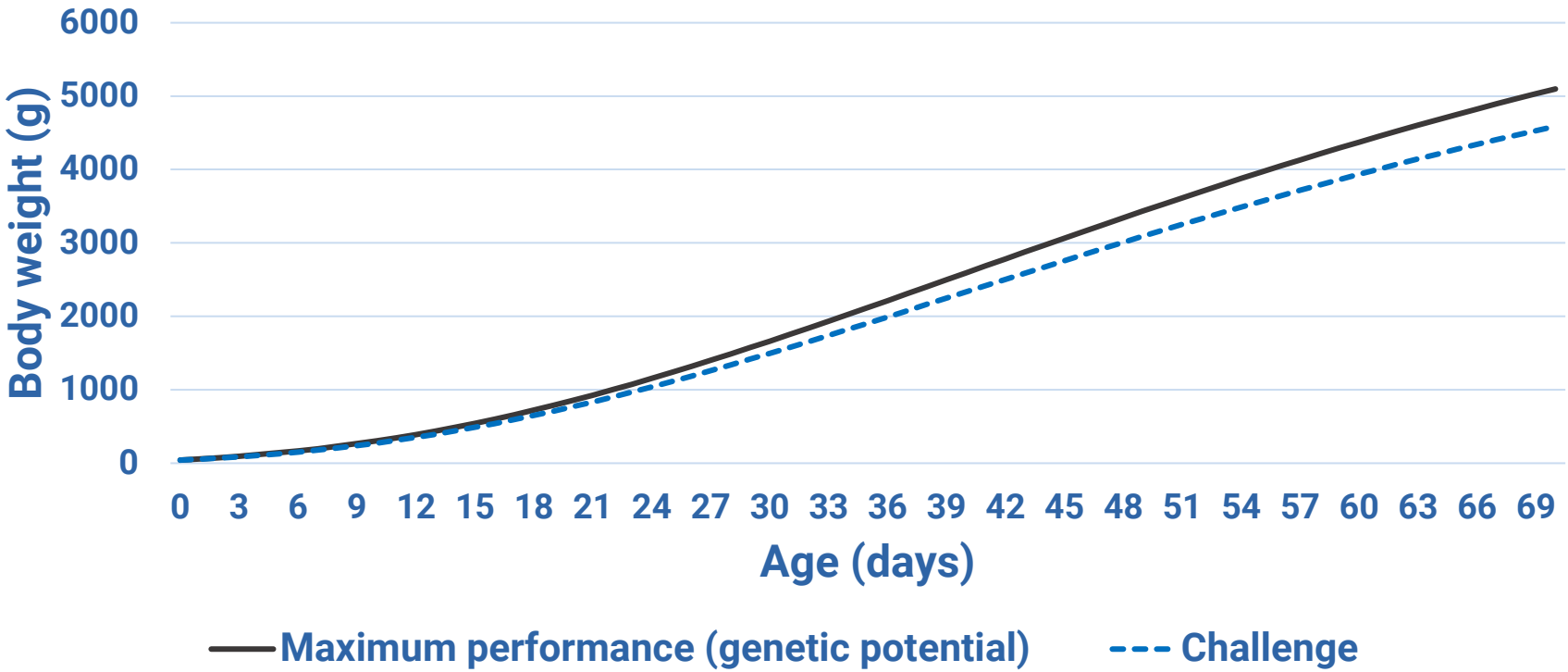
Alternatives to antibiotics



- **Antibiotics covered a wide range of problem organisms**
  - **Multiple predisposing factors**
    - **Incubation**
    - **Stress**
    - **Feed quality**
    - **Biosecurity**
    - **Environment**
  - **Greater response with increased (bacterial) infectious pressure**

AGP – reducing losses in performance

- **Growth-promoting antibiotics are actually growth-permitting antibiotics**
- **What is a reasonable expectation of AGP effects?**
- **What is a reasonable expectation of AGP alternative effects?**



How did growth-promoting antibiotics work?

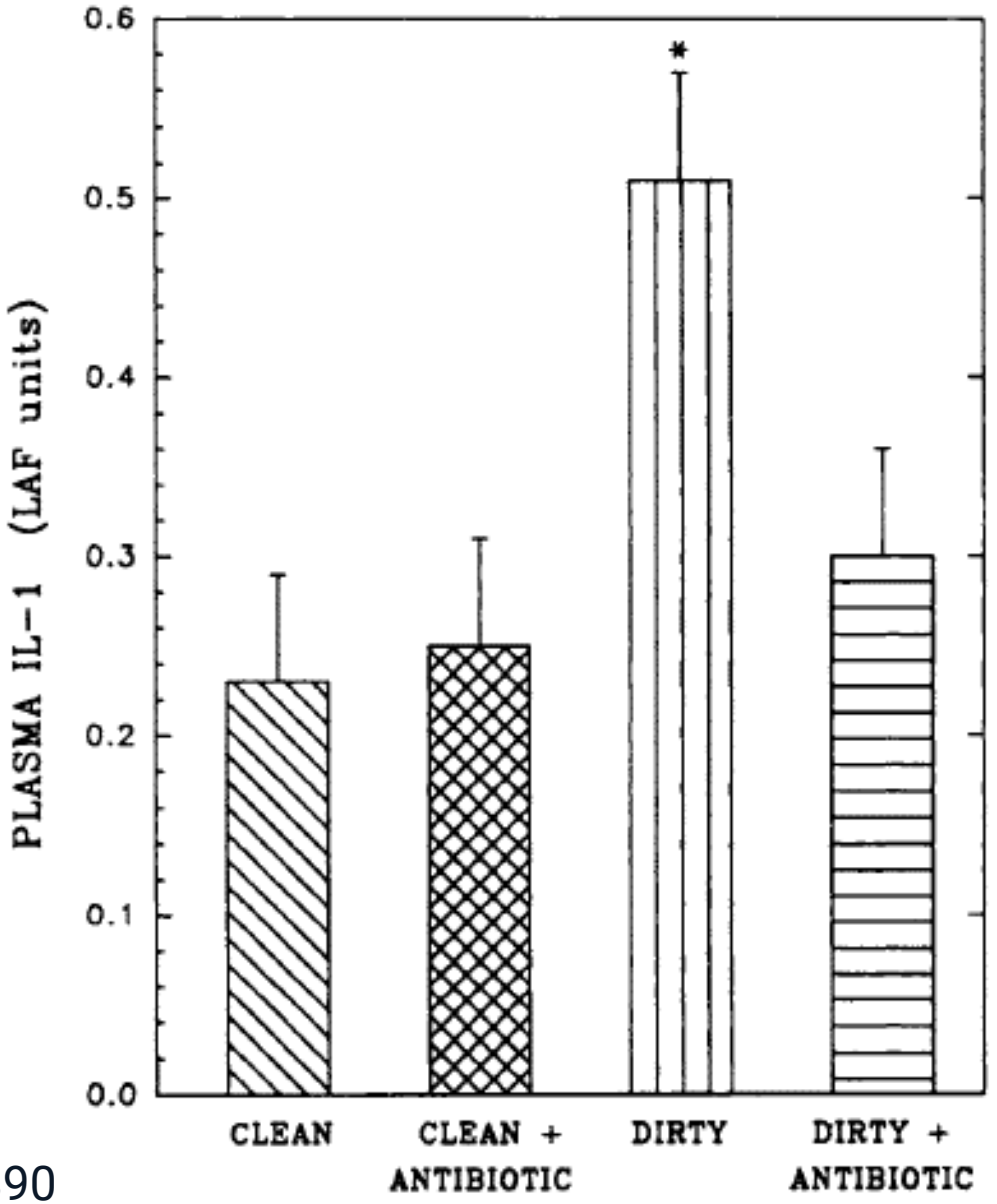
- **Multiple likely mechanisms**
  - **Inhibit growth of organisms that produce excessive amounts of ammonia and other toxic nitrogenous compounds in the intestines**
  - **Increased availability or absorption of specific nutrients**
  - **Favor the growth of nutrient-synthesizing microbes or suppress nutrient-destroying microbes**
- **Directly and/or indirectly reduce or prevent inflammatory interaction between host and immunogen**
  - **Reduce maintenance cost associated with turnover of the intestinal epithelium**
  - **Reduce inflammation-induced growth depression**
    - **Effect on feed or water consumption, or both**
    - **~~...and inefficient nutrient utilization~~**

Influence of environment and antibiotics (AB) on weight gain and feed efficiency in chicks (Experiment 1)[1]

Treatment[2]	Weight gain	Feed efficiency
	g/(chick·d)	*g gain/g feed*
Clean	12.65[a]	0.66[a]
Unsanitary	12.10[b]	0.54[b]
Clean + AB	12.72[a]	0.67[a]
Unsanitary + AB	12.57[a]	0.63[a]
Pooled SEM	0.14	0.02

[1]Means in a column with different superscript letters are significantly different ($P < 0.05$).

[2]Sixty-four chicks were raised for 14 d (from 3 to 17 d of age) in each of the two different environments (clean or dirty) and fed diets either without antibiotic or with streptomycin (100 mg/kg) and penicillin (100 mg/kg).



Roura et al., J. Nutr. 1992 122:2383-2390

Evaluating an antibiotic alternative strategy

- **Characteristics of a viable AGP alternative**
  - **be efficacious**
  - **economically feasible**
  - **simple to apply consistently under field conditions**
  - **be accepted by consumers**
  - **not promote microbial resistance**

Evaluating an antibiotic alternative strategy

- **AGP increased growth and efficiency by 3-5%**

Dahiya et al., 2006

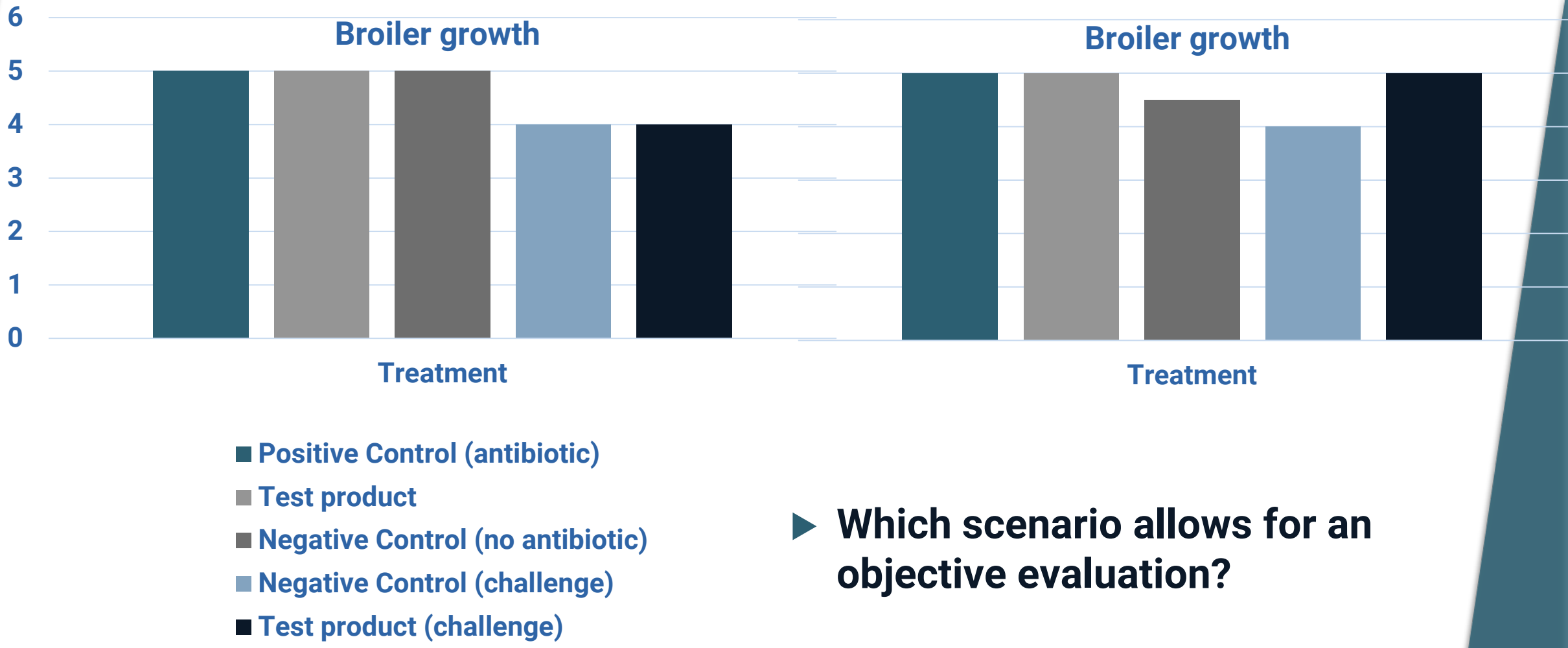
- **Positive response 72% of the time**

Rosen, 1995

- **Compared to what?**
  - **…not using AGP**

- **Shouldn’t we evaluate alternatives to AGP in the same context?**

A common problem in AGP-replacement studies…



- **Which scenario allows for an objective evaluation?**

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Extrapolating data from in vitro to in vivo, and the transition of animal trial data to field observations

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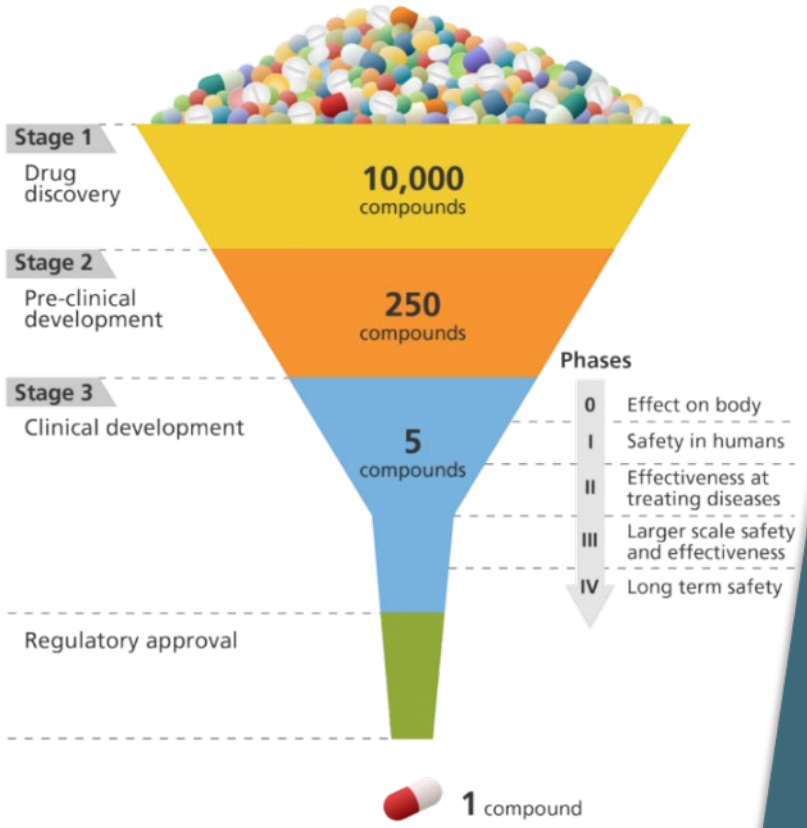
Evaluating alternatives – the research continuum

- **Bringing an AGP alternative to market**
  - **In vitro, small scale in vivo under lab conditions, artificial situations**
  - **Scale-up – medium scale (university broiler facilities)**
    - **Mimic commercial conditions**
    - **Small pens, replication**
    - **Pen cleanliness, stocking density, feed (pellets/mash)**
    - **Cage vs litter floor**
  - **Small scale commercial conditions**
    - **Research barns**
    - **Pens within commercial barns**
- **Commercial implementation**
  - **Convincing customers to try the product**



How are drugs designed and developed? | Facts | yourgenome.org

Evaluating alternatives – the research continuum

- **Biological systems are complex**
- **Housing environment is complex**
- **Inputs into poultry production are complex**
- **The gut environment is exceedingly complex**

- **Conclusion: there isn't a simple way to extrapolate from in vitro to in vivo**

...but there <u>is</u> a process

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Extrapolating data from in vitro to in vivo, and the *transition of animal trial data to field observations*

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Evaluating an antibiotic alternative strategy

- **Many proposed AGP alternatives have evidence of some direct or indirect influence on inflammation or antimicrobial activity**
  - **Cell culture/in vitro antimicrobial activity**
  - **Tissue gene expression**
  - **Plasma/tissue mediators of inflammation**

- **Are there links to effects on performance?**
  - **Efficacy**

Evaluating an antibiotic alternative strategy

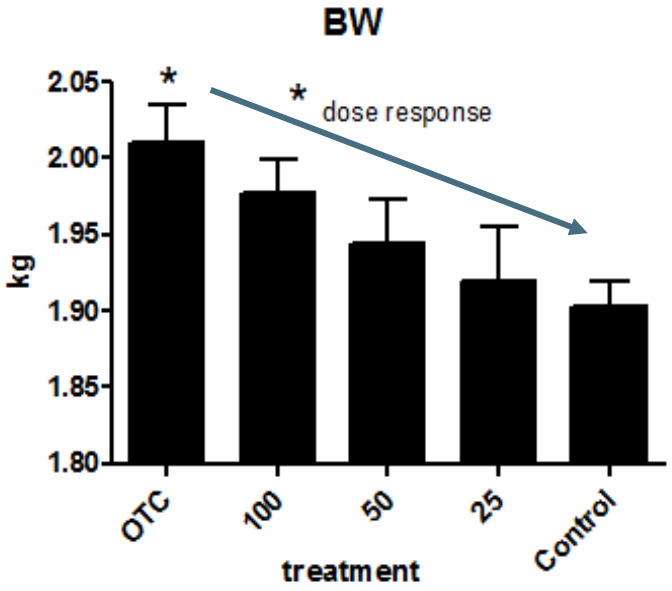
- **Clear research question (hypothesis)**
  - **A limited number of variables**
- **Experimental design**
  - **Controls**
    - **Positive and negative**
    - **Presence of a challenge: PC vs NC**
  - **Replication of treatments**
  - **Blocking**
  - **Sampling**
- **Conducting the study**
  - **Appropriate measurements**
  - **Quality control**
  - **Response to errors**
- **Objective evaluation**
  - **Statistics**
- **Interpretation**
  - **Is the product effective in the face of a challenge?**
  - **Is the performance of the PC birds relevant to commercial production?**

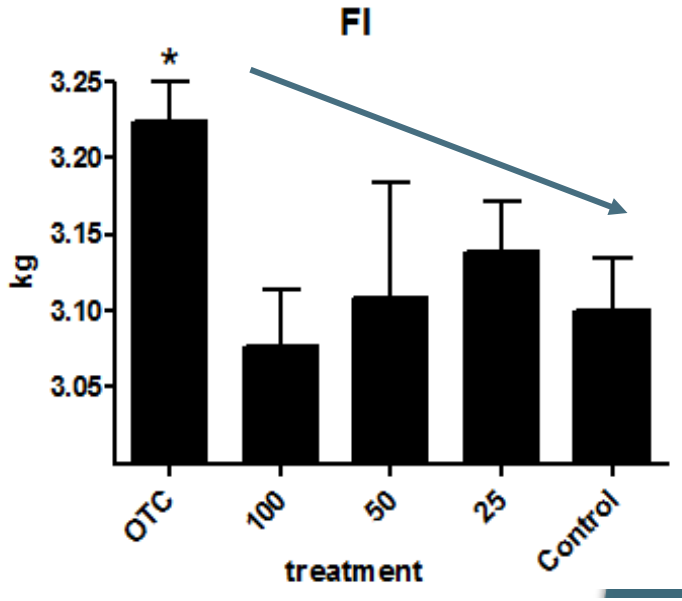
Evaluating an antibiotic alternative strategy

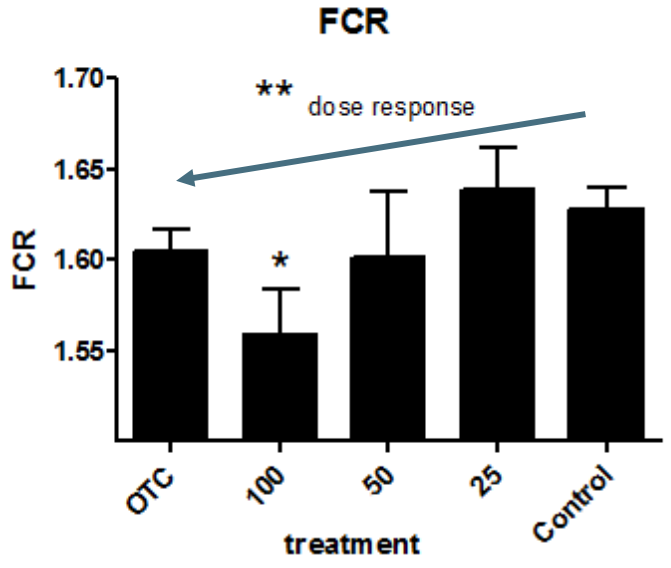
- **Experimental challenge models**
  - **Do the experimental studies reflect what happens in the field?**
    - **Single vs multiple challenges**
      - **Which pathogens?**
    - **Natural vs artificial challenges**
      - **Effect of challenge model on response**
    - **Clinical vs sub-clinical challenges**
      - **Prophylactic vs therapeutic uses**
    - **What about the environment/location?**

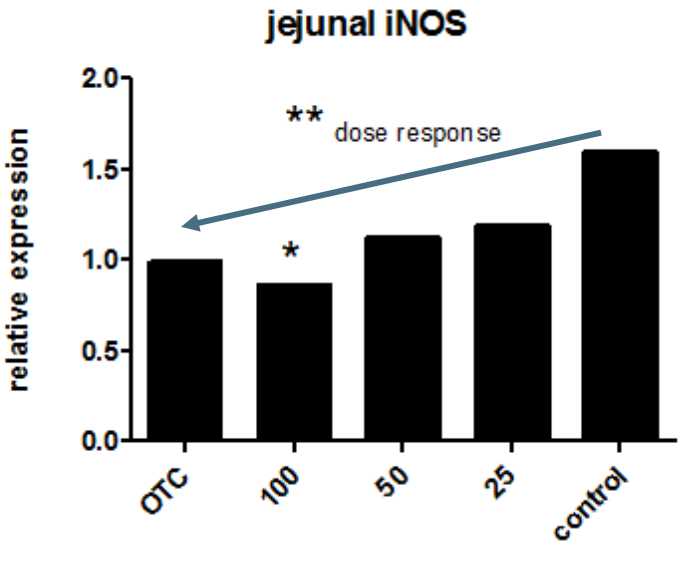
Evaluating an antibiotic alternative strategy

- **Sound experimental design**
  - **Positive control (with AGP) negative control, (no AGP)**
    - **Loss of performance in NC**
  - **NC plus experimental treatments**
    - **Full or partial recovery of performance**









Growth promotion in broilers by both oxytetracycline and *Macleaya cordata* extract is based on their anti-inflammatory properties. Khadem et al. 2014. Br. J. Nutr. 112, 1110–1118

Evaluating an antibiotic alternative strategy - university

- **University – small scale, intensive, highly controlled research facilities**

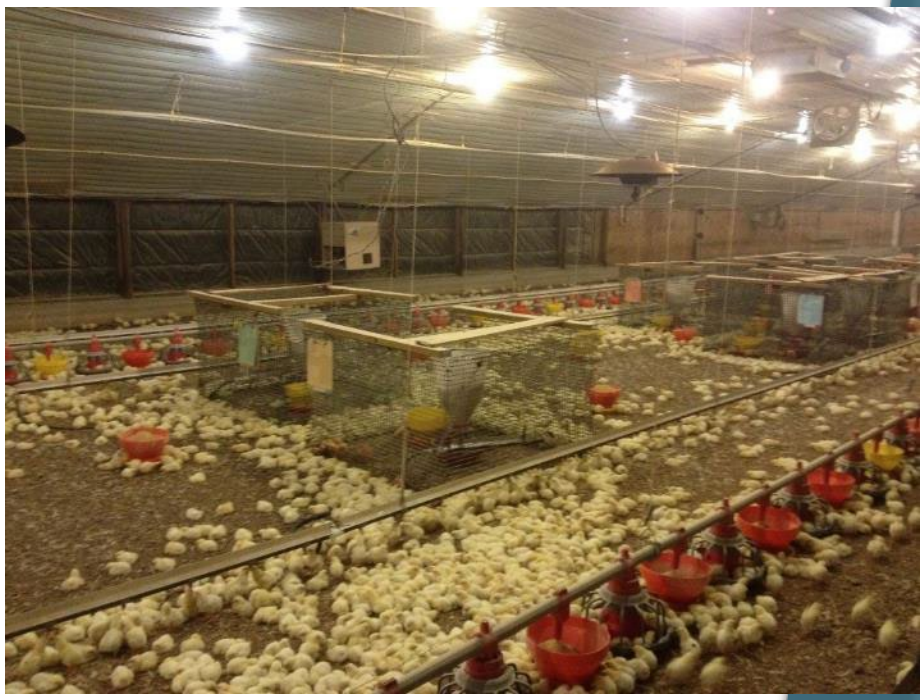


- **Are research results applicable to the real world?**
  - **Do the experimental ideas work?**
  - **Are they feasible?**

Evaluating an antibiotic alternative strategy - commercial

- **Company-owned research farms**
  - **Smaller scale commercial units, subdivided into pens**
    - **Many similarities to university-level research trials**
    - **Company-specific management, feeding programs, veterinary programs, etc.**

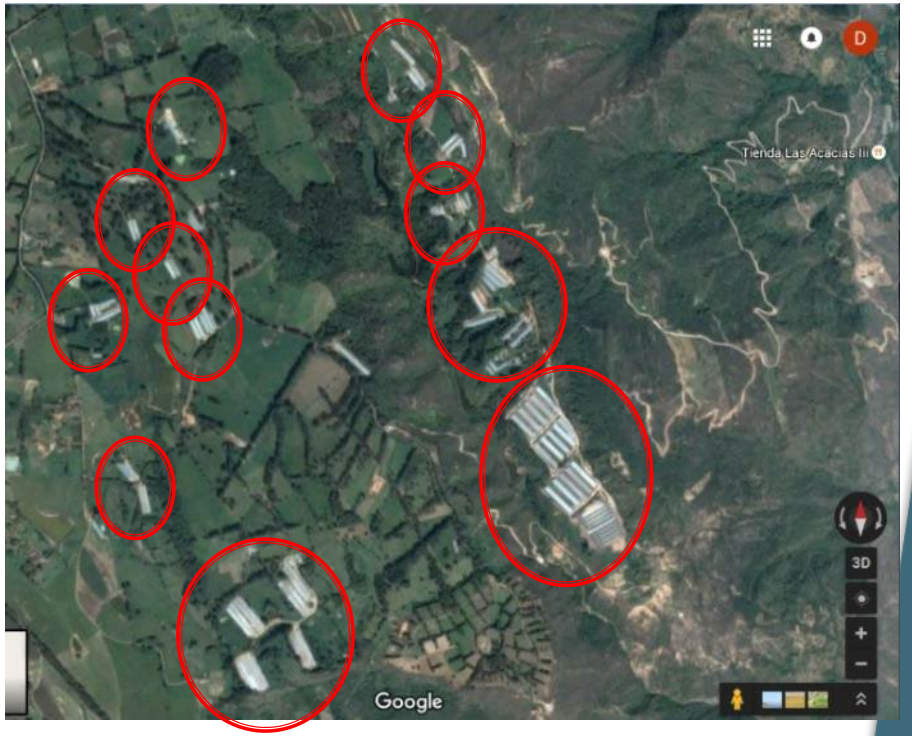
- **Research pens within a full-sized commercial barn**
  - **Same environment as commercial birds, replication within the barn**



University of Arkansas Broiler Farm
https://uabroilerfarm.wordpress.com/

Evaluating an antibiotic alternative strategy – field trials

- **Advantages**
  - **Large numbers of barns - replication on a farm/over time/across a large number of facilities**
  - **Real-world conditions (including challenges)**
  - **Economics in the context of the company**



Evaluating an antibiotic alternative strategy – field trials

- **Limitations**
  - **Lack of negative controls**
  - **Variation**
    - **Barn to barn**
    - **Farm to farm**
    - **Geographical location**
    - **Statistical differences vs trends**




Evaluating an antibiotic alternative strategy – field trials

- **Production realities**
  - **Disease outbreaks**
  - **Staff time/attention to detail**
    - **Production takes priority over research**
  - **Large number of steps/people involved**
    - **E.g. getting feed into the right bin/barn**
      - **Coloured feed bags/tags/bin labels**
  - **Responsibility for maintaining experimental protocol**
    - **What happens when a mistake is made?**
    - **What happens when someone changes things?**

Dietary Treatments

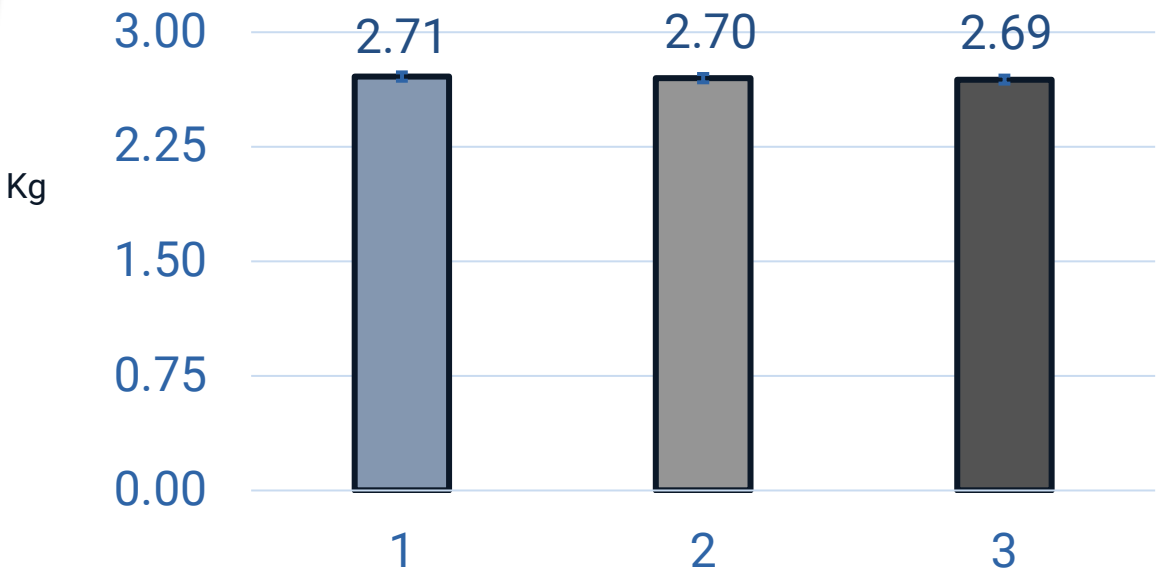
	T1	T2	T3
Basal Diet**	Integrator	Integrator	Integrator
AGP	✓	✗	✗
Butyrate	✓	✓	✗
MOS (Mannan-oligosaccharides)	✓	✓	✗
Essential Oil + Benzoic Acid	✓	✓	✓
Enterococcus faecium	✓	✓	✓
Bacillus subtilis – Bacillus licheniformis	✗	✓	✓
Protease + Xylanase + Amylase	✓	✓	✓
Phytase (1000 FYT/g)	✓	✗	✗
Phytase (2500 FYT/g)	✗	✓	✓

** Pre-starter, starter, grower and finisher (mash form)

Sanabria et al., International Poultry Scientific Forum February 11, 2019, Atlanta, GA, USA

Alternatives to antibiotics – commercial trial

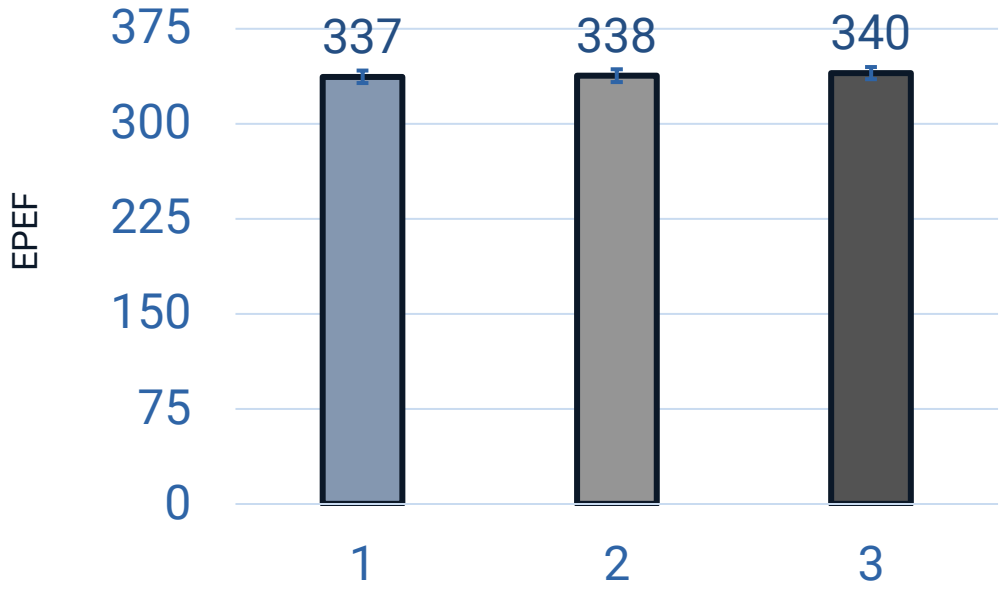
Body weight D44*



P = 0.8613

* Kg/m² and age at slaughter as covariates

European Performance Efficiency Factor D44*



P = 0.8801

Sanabria et al., International Poultry Scientific Forum February 11, 2019, Atlanta, GA, USA

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Evaluating an antibiotic alternative strategy – economics

Traditional supplements

- **E.g. phytase vs inorganic phosphate**
  - **What alternatives will achieve the same performance at the same or lower cost?**

Antimicrobial alternatives

- **Multiple products/approaches to replace a single product**
  - **Higher costs**
  - **Will I be able to sell my product or not?”**
    - **Legislation, export markets**
    - **Consumer preferences**

Evaluating an antibiotic alternative strategy – economics

- **Cost of implementation**
  - **Removing a single product (AGP)**
  - **Replacing with a group of different products, each with its own cost**
  - **Often an "insurance policy"**

- **Additional costs**
  - **Vaccinations**
  - **Reduced performance**
  - **Lower stocking density**
  - **Longer down times between flocks**
  - **Increased biosecurity/improved facilities**
  - **Feed supplements**
  - **Immune modulation**
  - **Feed quality**
    - **Nutrients, contaminants and mycotoxins**

Why don’t good ideas always work?

- **Characteristics of a viable AGP alternative (revisited)**
  - **be efficacious**
  - **economically feasible**
  - **simple to apply consistently under field conditions**
  - **be accepted by consumers**
  - **not promote microbial resistance**

Why don’t good ideas always work?

- **Why don’t in vitro results always translate to in vivo effects?**
  - **Isolated cells vs complex organism**
    - **Tissue, organ, organism**
    - **Regulation, endocrine/exocrine controls**
- **Why don’t in vivo effects observed in one situation (e.g. research trials) translate to commercial effectiveness?**
  - **University trials vs commercial**
    - **Sanitation**
    - **Animal care standards**
    - **Replication - multiple pens per treatment vs multiple barns**
    - **Adherence to research protocol**
  - **Other (biological) factors**

Why don’t good ideas always work?

- **Why don’t good results on one farm necessarily translate to good results on a different farm?**
  - **Variation in responses – sources of the microbiome**

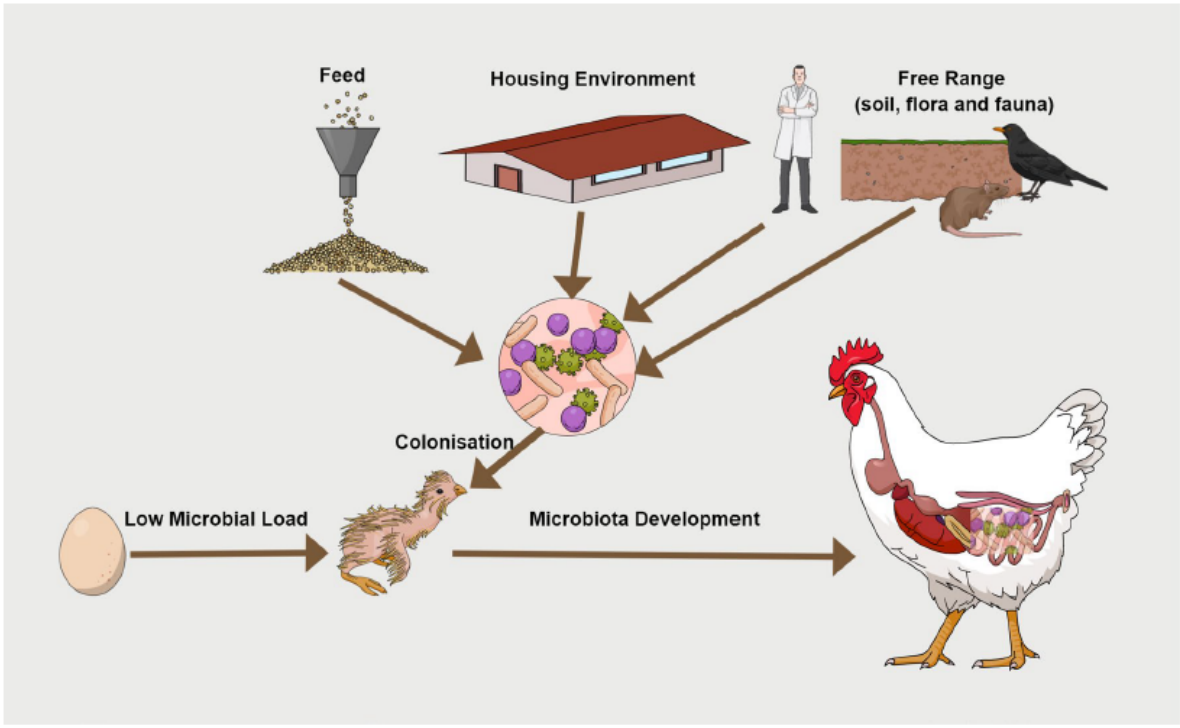
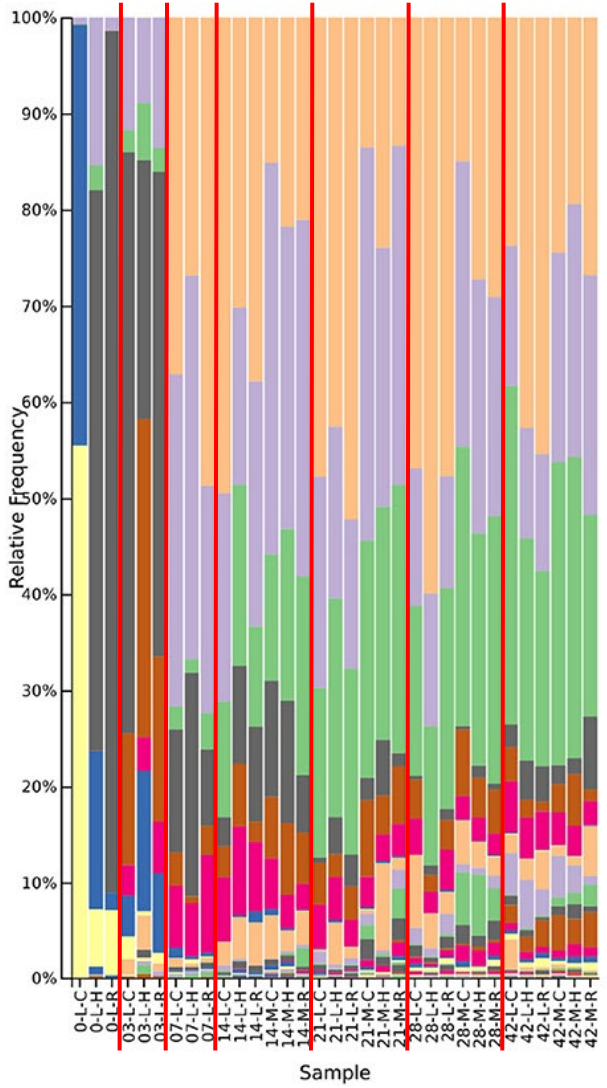


Fig. 6 Feed contains a diverse microbial community and is an active bacterial coloniser in poultry

Haberecht *et al. AMB Expr (2020) 10:143*
https://doi.org/10.1186/s13568-020-01077-5

Variation in response – age and strain

- **Ross, Cobb, Hubbard**
- **0, 3, 7, 14, 21, 28, and 42 days post hatch**
- **Cecal samples**
  - **Mucus (M)**
  - **Lumen (L)**



Richards et al.,
Front. Vet. Sci. 6:201. doi: 10.3389/fvets.2019.00201

Conclusions

- **From in vitro to commercial success**
  - **Necessary for the poultry industry**
  - **Time-consuming process**
    - **Many steps, candidate products can be eliminated at any step along the way**
  - **Advances in scientific methods allow for better testing along the way**
    - **Greater confidence**
  - **The gut microbiome is exceedingly complex, and we're only starting to understand it**
    - **Different challenges, environments likely require different approaches**
    - **A successful strategy will likely require multiple products with multiple mechanisms**

Conclusions

- **From in vitro to commercial success**
  - **The good news…**
    - **Many effective products have been developed**
    - **With more experience, chances of success increase**
      - **Farm-level evaluation of successful strategies**
      - **Continual evaluation**

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