

Goat and Sheep Helminth Responses to aIL-10

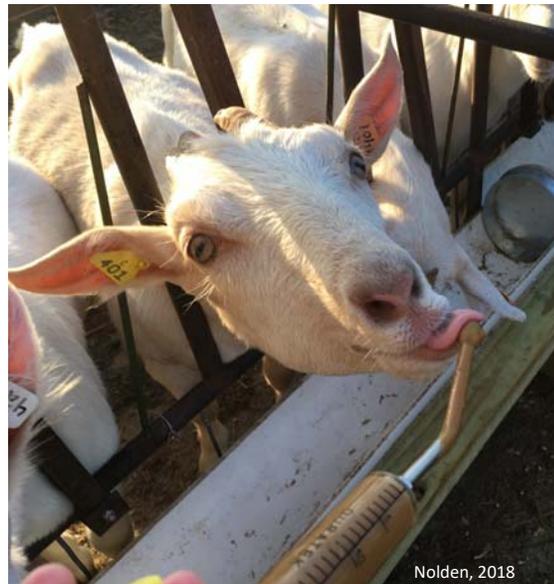
Research Update

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3/19/2020

1

Outline

- Goat Study
 - Animals
 - Timeline
 - Coproculture
 - Dosing
 - Antibody production
 - Diet
 - Fecal Counts
 - ELISAs
 - Results
- Sheep Study
 - Details
 - Results
- Comparisons



2

Timeline

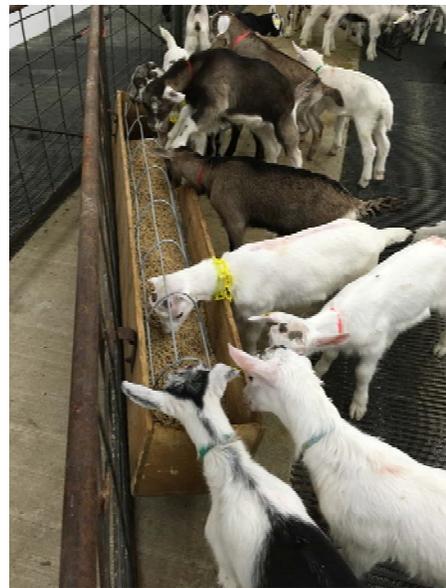
- 3/29/2018 – Pick up 90 bucklings in Chilton, WI (1 week old)
- 3/31/2018 – Kids drinking from LacTek machines
- 4/5-4/18 – Surgical castrations and disbuddings
- 4/18/2018 – Kids offered pellets free-choice
- 4/27/2018 – Kids reduced to access for 12 hours/day (5 weeks old)
- 5/1/2018 – 37 bags of Kid-O consumed; 50 lbs/d, 0.57 lb/kid/d (9 oz)
- 5/4/2018 – Powder added to pellets to encourage consumption, plus baleage, iodized salt and pails of electrolytes
- 5/16/2018 – Kids weaned (8 weeks old); ~1 lb Pellets/kid/d consumed
- 5/24/2018 – Collected goat fecal material and set up coproculture
- 5/30/2018 – Moved goats to Arlington
- 6/7/2018 – Collected coproculture L3 larvae (14 days)
- 8/7/2018 – Dosed 84 goats with 3000 L3 larvae each (138 days old)
- 9/12 & 9/13 – Initial weights, fecal counts, blood
- 9/15/2018 – Day 1 of treatment dosing (177 days old, 39 days post parasite inoculation)
- 9/27 & 9/28 – Day 13 & 14 weights, blood, fecals
- 10/29/2018 - Day 47 FECs, blood, weights



115 g/L NRV Kid-O formula, offered 24/7, warm, on-demand, 18 nipples



Pellets and baleage



Pellets, 10 kg, April 2018

growing kid		intact male		Dairy Goat		Booth Least Cost 5.0	
lb batch	Ingredients	%	Inclusion		ME, kcal/kg	Costs \$/cwt	
			min	max			
548.95	Corn	54.90	0.00	0.00	3.20	\$5.960	
150.00	Soybean Meal (49% CP)	15.00	0.00	0.00	3.10	\$15.000	
75.00	Exceller Meal	7.50	0.00	0.00	3.41	\$17.213	
150.00	Wheat Midds	15.00	0.00	0.00	3.00	\$6.950	
0.00	Soy Hulls	0.00	0.00	0.00	2.80	\$6.380	
2.00	Bk Sheep Base 25	0.20	0.00	0.00	0.00	\$224.000	
20.70	Calcium carbonate	2.07	0.00	0.00	0.00	\$6.300	
1.10	Magnesium oxide	0.11	0.00	0.00	0.00	\$22.200	
25.20	EZ Flo Glo Molasses (fat	2.52	0.00	0.00	0.00	\$19.500	
5.00	Salt	0.500	0.00	0.00	0.00	\$13.700	
0.080	Cupric sulfate (25.2% Cu	0.008	0.00	0.00	0.00	\$96.600	
0.17	Deccox 6%	0.017	0.00	0.00	0.00	\$78.300	
0.30	Ferrous Sulfate	0.030	0.00	0.00	0.00	\$26.200	
11.00	Vitamin E	1.100	0.00	0.00	0.00	\$55.450	
5.00	Vitamin A	0.500	0.00	0.00	0.00	\$36.600	
5.00	Ammonium Chloride	0.50	0.00	0.00	0.00	\$33.700	
0.50	Dynamate (KMgS)	0.05	0.00	0.00	0.00	\$30.400	
1000.00	Diet:	100.000			2.93	\$10.02	Body Weight (kg) 10
	% Match	100					

Body Weight Gain = 100 g/d Dry Matter Intake = 0.440 kg/d
 ME req = 1.39 Mcal/d Feed Cost = 0.097 \$/d

Pellet analysis

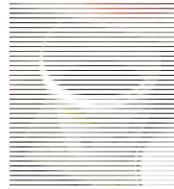
	TDN (%)	ME (mcal/kg)	Crude Protein (%)	DP (%)	MP (%)	UIP (%)	DE (mcal/kg)	NE _m (mcal/kg)	NE _g (mcal/kg)	NE _t (mcal/kg)	Fiber (%)	ADF (%)	NDF (%)	eNDF (%)	EE (%)	Ash (%)
Diet, unit																
DIET, total	73.66	2.93	18.97	11.75	13.29	10.89	3.35	2.02	1.31	1.87	3.16	4.98	13.43	36.69	3.67	3.37
Diet, at NRC unit	0.32	1.29	18.97	51.72	58.46	47.94	1.47	0.89	0.58	0.82	1.39	2.19	5.91	16.14	1.62	1.48
NRC, unit	kg/d	Mcal/d	%	g/d	g/d	g/d										
NRC, requirement	0.39	1.39	21	35	46	69										
X NRC	0.8	0.93	0.90	1.48	1.27	0.69										
max						40.00										

Macro Mineral Composition, %								Acid: base balance			
	Ca	P	Na	Cl	K	Mg	S	Ca : P	Cations - Anions	DEB	CAD
Diet, unit	%	%	%	%	%	%	%				
DIET, total	0.98	0.492	0.20	0.67	1.03	0.09	0.23	2.00	0.50	0.55	-0.01
Diet, at NRC unit	4.33	2.16	0.87	2.97	4.51	0.40	1.03	2.00			
NRC, unit	g/d	g/d	g/d	g/d	g/d	g/d	g/d				
NRC, requirement	3.60	1.60	0.39	0.4	1.9	0.38	1.0	2.2			
X NRC	1.2	1.4	2.2	7.4	2.4	1.1	1.0	0.9			
max, %		0.60	3.00	5.00	2.00	0.40	0.30	4			

Positive is goodnot definednot defined

Coproculture

- Modified Yazwinski protocol
- 3 gallons of goat manure collected from Nolden farm
- 1 gallon water added and stirred
- Mixed with 4 gallons vermiculite – tacky, not waterlogged
- Incubate covered at 72 F (22 C) for 14 days - Livestock Lab
- Place in zipper cotton pillowcases in 13 gal (51 L) Rubbermaid bins
- Add tap water to cover the pillowcase
- Wait 6 hours
- Elevate pillowcase and let drain into bin
- Rinse sides of bin into water (*Haemonchus* climb)
- Store refrigerated 40 F (4 C) until use, up to 6 months
- With a stirbar, sample 0.8 mL, count moving L3 on Mini-FLOTAC (average 80 larvae; 100 L3 strongyles/mL)



9

Dosing Goats with L3 Nematodes

- 8/7/2018
- 30 mL oral dose
- 2,000 L3 per dose
- Repeating dose syringe
- Bucket on a stir plate
- Chute system at Bookhout
- Wait 28 days for shedding
- Fecal count confirmation

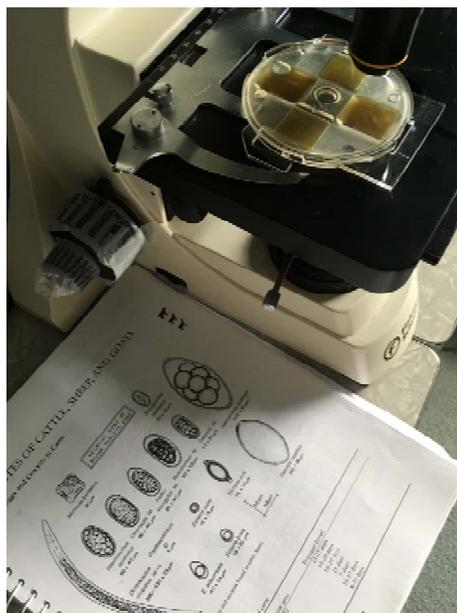


10

Fecal Counts

- Mini-FLOTAC

- 5 g feces
- 45 mL MgSO₄ fecal float solution
- Dilution ratio 1:10
- Fill chambers, 2 mL
- Wait 5-10 minutes
- Rotate disc
- Count strongyle eggs
- Multiply by 5 for eggs per gram



Gringoli, 2017

13

Vaccinate Hens to make Egg Antibody

Vaccine:

PBS (phosphate buffer saline)+
FIA (Freunds Incomplete Adjuvant)+
CpG-ODN (C-phosphate-guanosine oligodeoxynucleotides)+
BGG (bovine gamma globulin with glutaraldehyde)
Peptide (8 aa sequence from bovine IL-10) VMPQAENG-conjugated to BGG

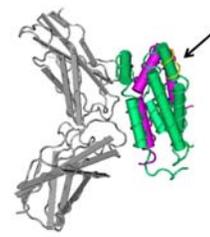
Each chicken received 1.0 mL IM of the vaccine
0.25 mL each breast
0.25 mL each thigh

Vaccinations:

1 primary, 5/18/2017
2 boosters, 6/1/17, 6/15/17

60 Peptide hens
20 Control (no peptide) hens
8 Blank (PBS only) hens

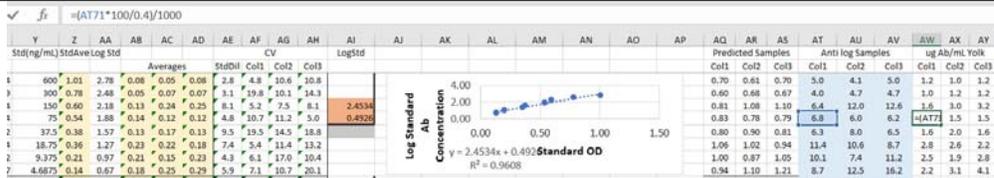
Yolks separated and frozen



Vaccine Reference Marcq et al., 2015

14

ELISAs



- Coat plate: ova-cow peptide
 - Acidify egg w acidified PBS
 - Wash & block plate
 - Add standards and samples in triplicate
 - Wash
 - Goat anti-chicken IgY
 - Incubate
 - Wash
 - Add substrate TMB
 - Add stop solution
 - Read plate
- Create standard curve
 - Standards 600-4.68 ng/mL, halved
 - Log (standard [Ab]) by ave OD reading of standard
 - Get formula- linear curve fit
 - Solve formula for each sample, averaged
 - $Y = 2.45x + 0.492$ where OD ave is x
 - 10^Y to get antilog
 - Calculate titer
 - $(\text{antilog} * 100 / 0.4) / 1000$ to get ug Ab/mL

15

Goats Assigned to Treatments

- Weights taken 9/12 and 9/13/2018
- Treatments assigned
 - Weight balanced
 - Breed stratification
 - 14 goats per treatment
- 9/15/2018 Treatment dosing, Day 1
 - Positive Control – Levamisole w Fenbendazole, once
 - Low 120 ug/hd/d, 14d
 - Med 240 ug/hd/d , 14d
 - High 480ug/hd/d , 14d
 - Blank egg, 10 mL, 14d
 - Negative Control – no dewormer

Breed	Count
Alpine	15
LaMancha	9
Nubian	6
Saanen	39
SaanenX	6
Toggenburg	9
Grand Total	84

16

Bookhout Facility, Arlington, WI



17



18

Iso-Egg

- Highest titer treatment set egg volume for all treatments
- Lower titer treatments diluted with Blank egg yolks to make volume match
- Molasses added to make the yolk flow; custard texture otherwise
- Same amount of molasses in all treatments
- 10 cc/goat/day for 14 d



Hypothesis:

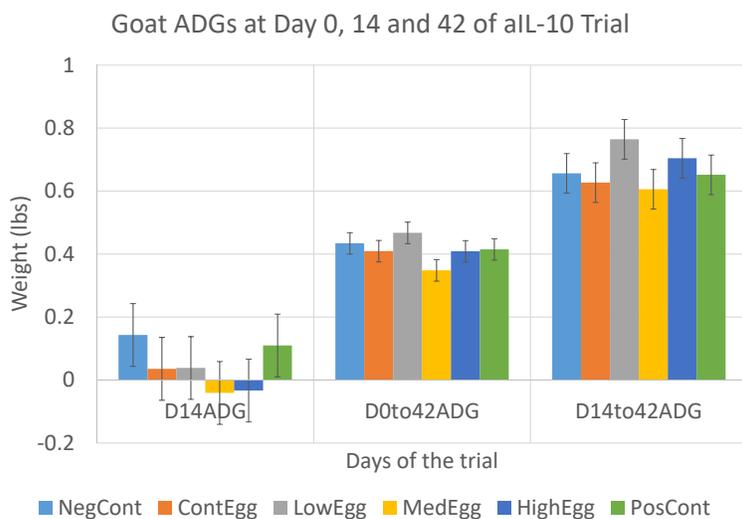
- Feeding an antibody to IL-10 will bind the IL-10 recruited by the parasites
- Removes the immunosuppressive effects of host IL-10 upregulation
- Host's innate immune system mounts normal short-term response
- Host's adaptive immune system develops long-term immunity

Statistics

- Mixed model ANOVA
- Completely randomized design
- 84 EUs, 6 treatments, 14 EUs per treatment
- Proc MIXED
- Class variable – treatment and breed
- Random effect - breed
- Response variables – ADG, FEC, Weight
- Model response = treatment
- Lsmmeans for treatment means
- Data were normally distributed, no correction needed

21

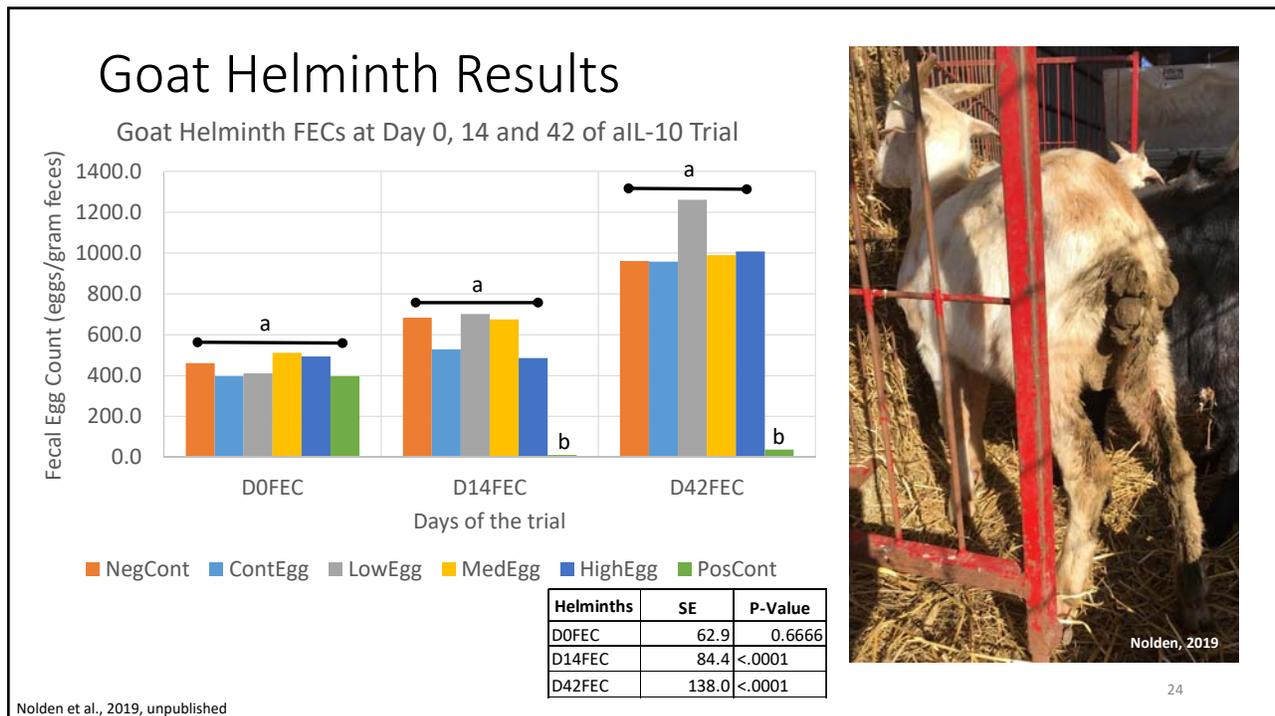
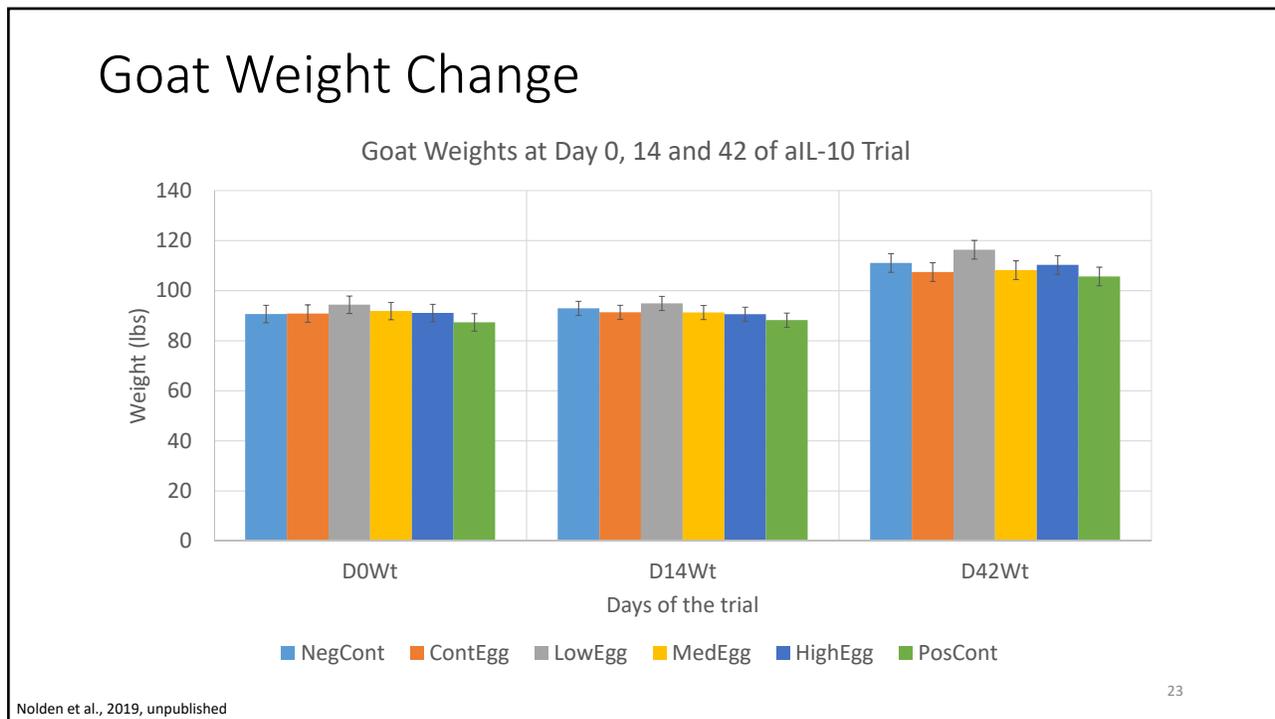
Goat Helminth Results



- ADG = measured from start of treatments until end (42 days).
- FEC = fecal egg count, means are of strongyle type eggs seen.
- Positive control = Levamisole combined with Fenbendazole oral drench at the label dose for group average wt.
- aIL-10 was individually dosed for 14 days at 120, 240 and 480 $\mu\text{g ab/hd/d}$.
- *Starting weights avg 90 lbs
- 84 head

Nolden et al., 2019, unpublished

22

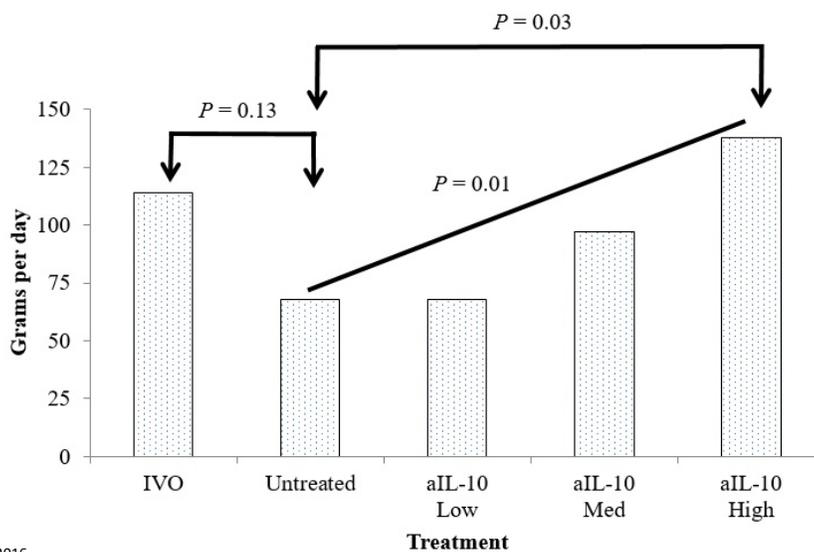


Mitch Schaefer Lamb Project

- 100 lambs, 4 breeds, age range 118-278 days old
- Confinement reared, then grazed 35 days on contaminated pasture
- 14 day treatment period
 - IVO and Untreated co-grazed, other groups separate paddocks
 - 0.45 kg grain daily, ad libitum forage
 - Egg treatment groups – dried yolk mixed with grain
 - 0, 0, 0.6, 1.1, 2.3 g dried pasturized frozen egg yolk/lamb/d (IVO, Un, L, M, H)
- 35 days long; D14 grazed, no grain
- aIL-10 was fed for 14 days on pasture at 30, 60, and 120 µg ab/hd/d.
 - Pasturized, dehydrated egg yolk
- Positive control = Ivermectin oral drench at 2X the label dose for group average wt.
- Starting weights avg 104 lbs

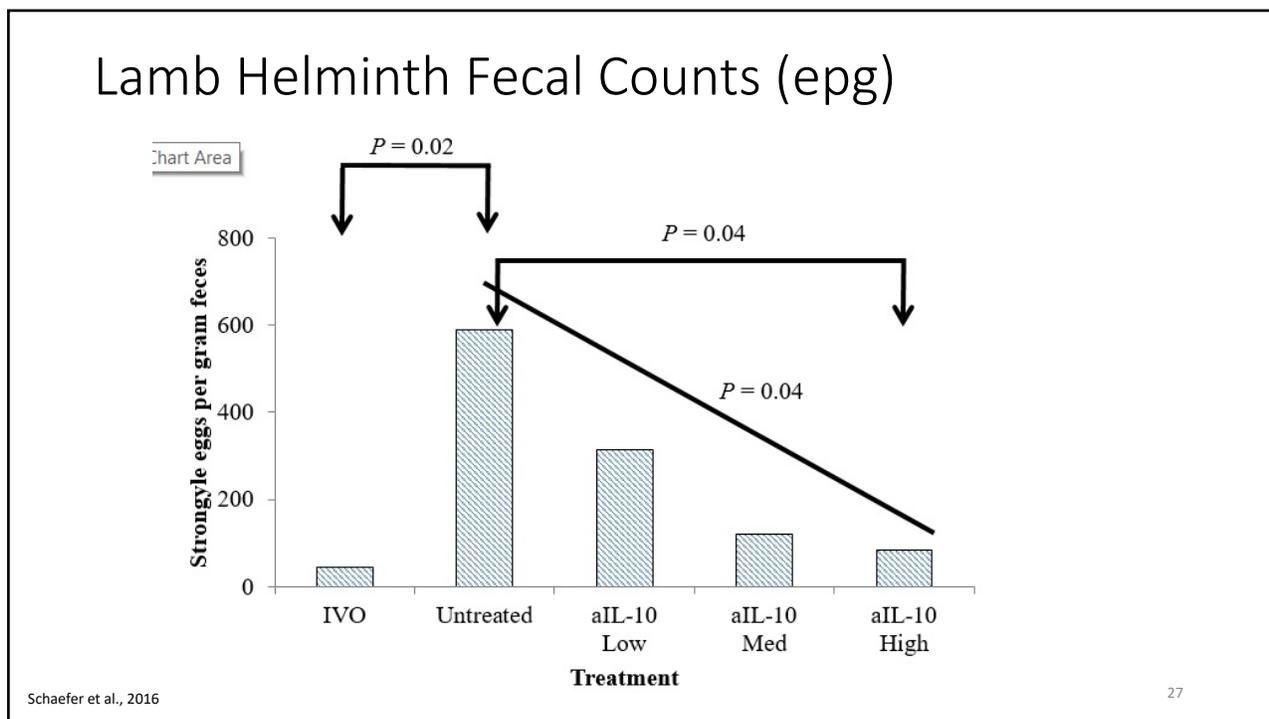
25

Lamb Average Daily Gain (g/d)



Schaefer et al., 2016

26



Comparison of Projects

• Similarities:

- Yolks frozen
- Egg yolk, 4 levels
- 14 day dosing
- Small ruminants
- Many breeds
- 39 vs 35 days post infection
- Age @ d0: 177d vs 118-278d

• Differences:

- Lyophilized, pasteurized
- Natural infection vs Coproculture
- More variable ages
- Larger weight
- Sheep vs goats
- Sheep vs goat GINs
- Grazed vs confinement
- No 35 day goat data
- More goat breeds
- 30,60,120 vs 120,240,480 ug/hd/d
- No grain post day 14