Runting Stunting Syndrome (RSS) in Broilers: *in vivo* Studies

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This presentation on runting and stunting syndrome (RSS) summarizes some of the recent research performed in Dr. Guillermo Zavala's laboratory at the University of Georgia.

Contents

- Characterization of RSS (GA)
- Experimental models for RSS:
  - Environmental thermal treatment
  - Delayed exposure, age susceptibility
  - Brooding temperatures
  - Role of breed cross and gender
  - Role of CAV, REO, IBD maternal antibodies
  - Role of EPEC
  - Anti-protozoal and anti-anaerobic drugs
  - Preliminary studies on vertical transmissibility

The contents of this presentation include unpublished results of studies regarding experimental reproduction of runting and stunting syndrome (RSS) in commercial broilers, using a model in which susceptible broiler chicks are reared on litter obtained from commercial broiler houses experiencing RSS in multiple consecutive flocks.

Clinical and Pathological Characteristics of RSS

- Severely delayed growth → low uniformity
- Lethargy, huddling (hypothermia?)
- Watery diarrhea → soiled vents → damp litter
- Delayed feed consumption
- No early mortality; late mortality may be high
- Losses due to ↑culling and ↑FC and ↓ADG
- Small liver + enlarged gall bladder
- Thin, pale intestinal wall + watery contents
- Cystic enteropathy → enteritis
- Pancreatitis → pancreatic degeneration/fibrosis

The most important and commonly observed characteristics of RSS are listed.

“Normal” Breed Variation

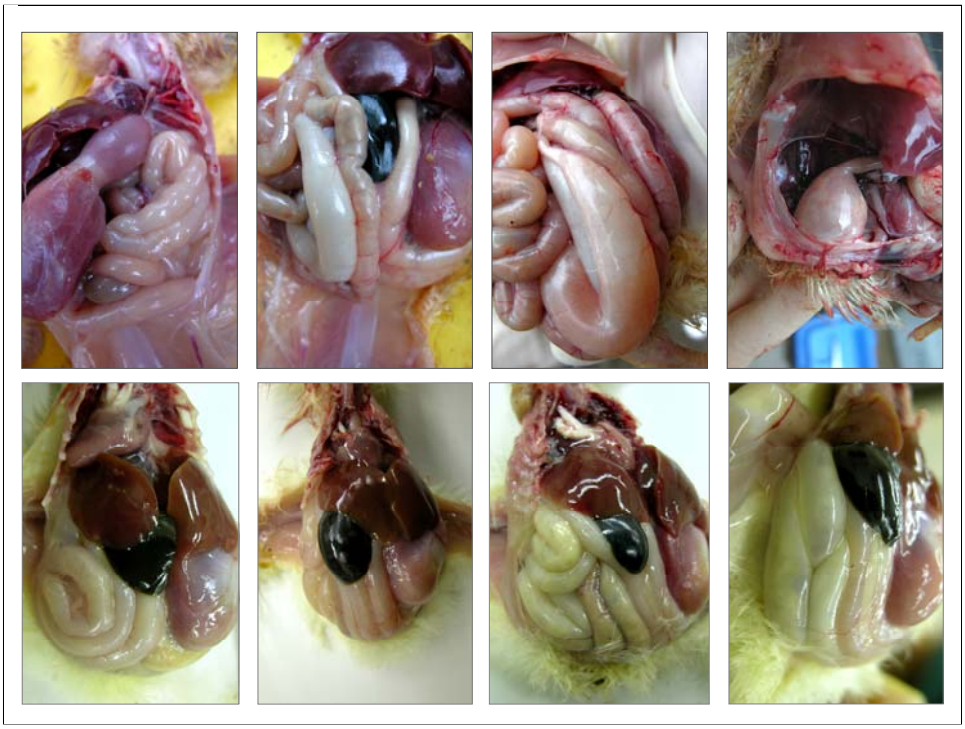
Control broilers housed in isolators from hatch until 13 days of age.

It is important to place genetic variation in context. Multiple companies complain about “lack of uniformity” in young broilers. However, it is not uncommon to observe poor uniformity in clinically healthy high yielding breeds. This photograph depicts the variation in growth of young broilers reared in isolators (not exposed to any infectious agents).

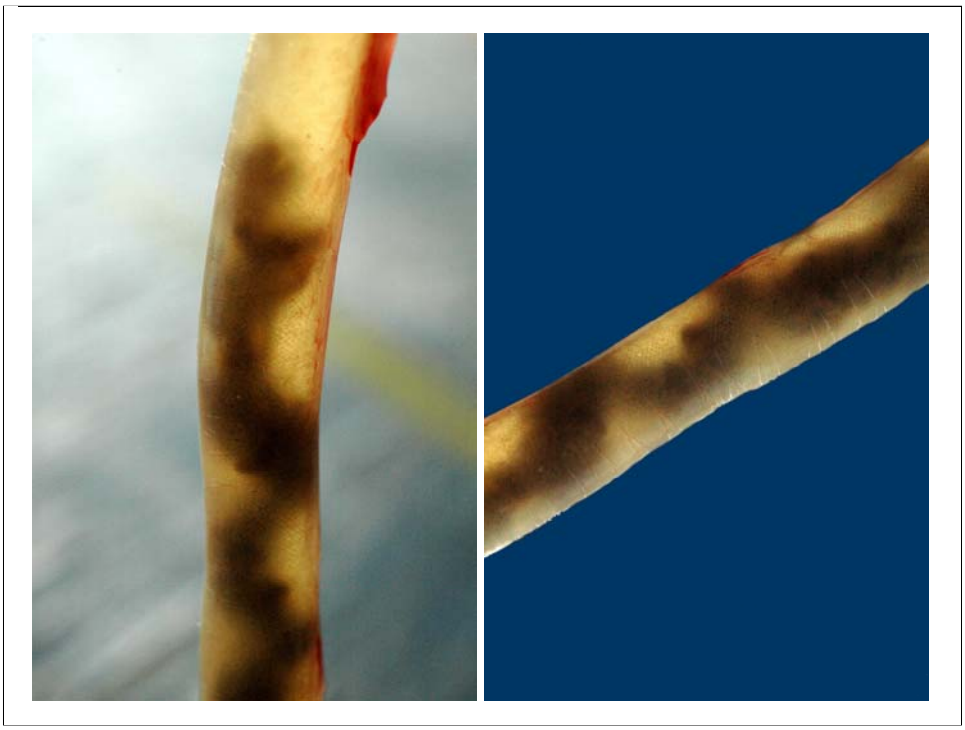
Field Case Lesions:

- Thin, pale intestines
- Watery, foamy contents
- Cloacal impaction

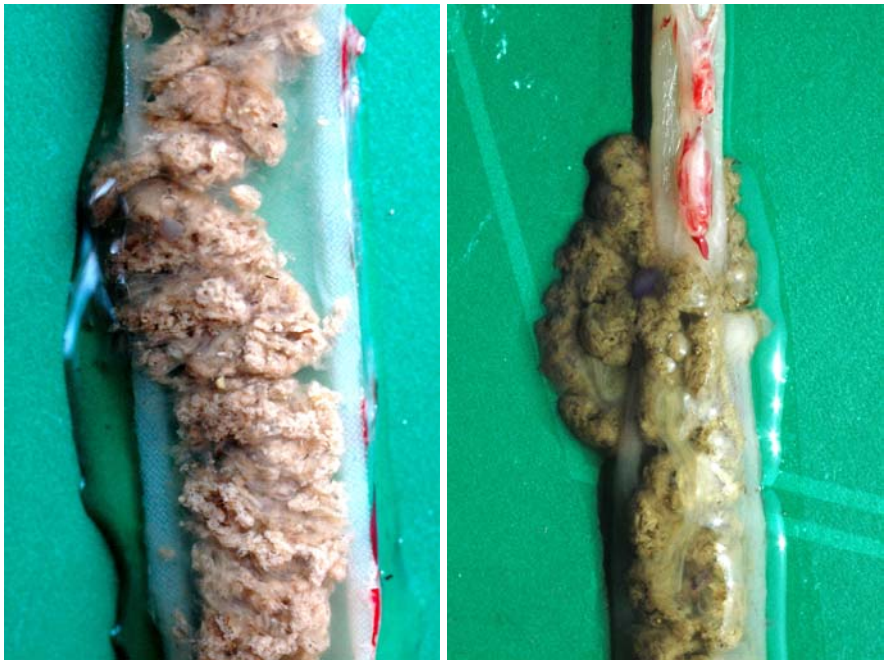
Common lesions observed in field cases of RSS.



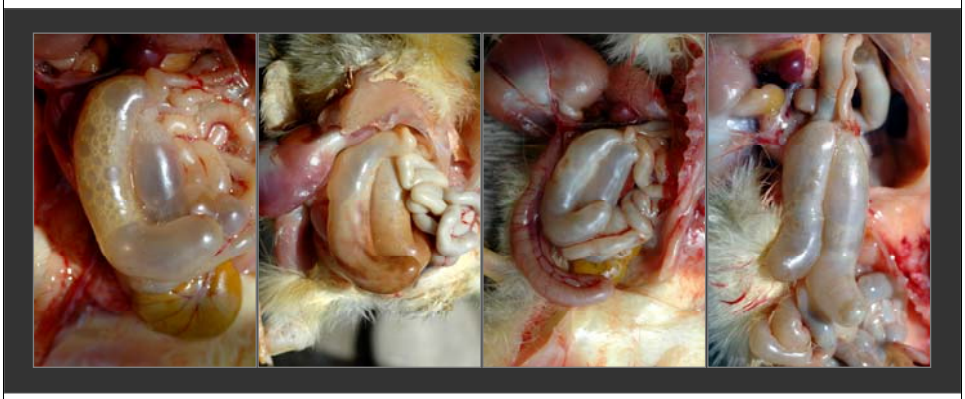
Common lesions observed in field cases of RSS.



Common lesions observed in field cases of RSS. Note the thin-walled, translucent small intestines.

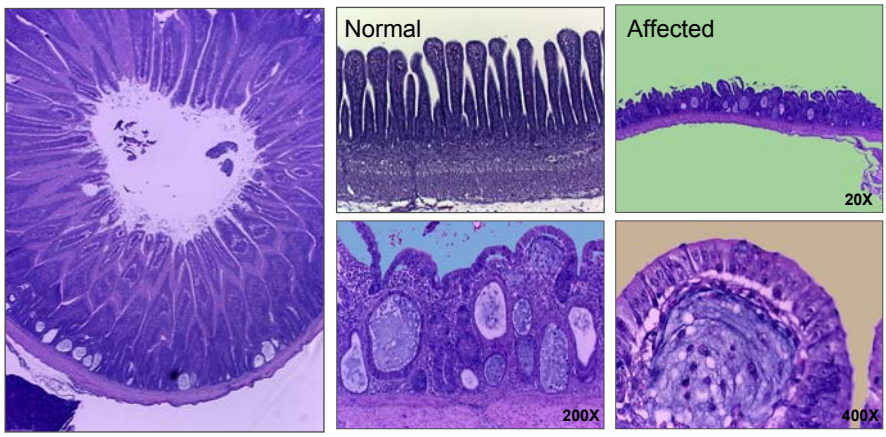


Common lesions observed in field cases of RSS. Abundant fluid and poorly digested feed are seen in the intestinal lumen.



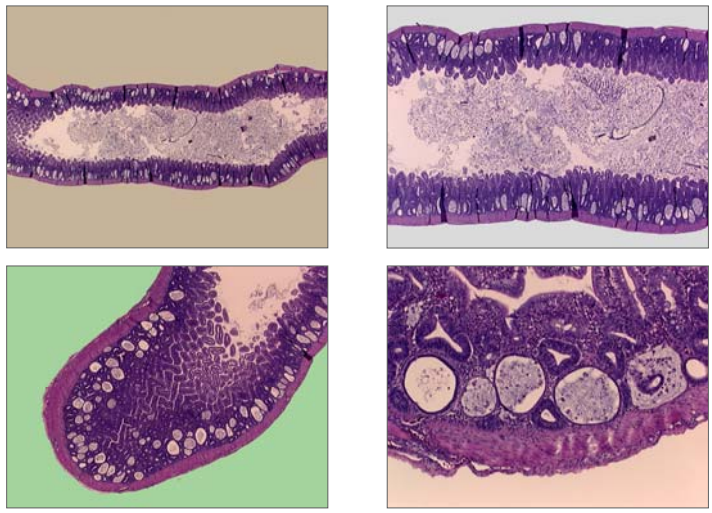
On occasion, gas-filled ceca can be seen in affected broilers, albeit this change is not present in every affected flock.

Cystic Enteritis – Field Samples



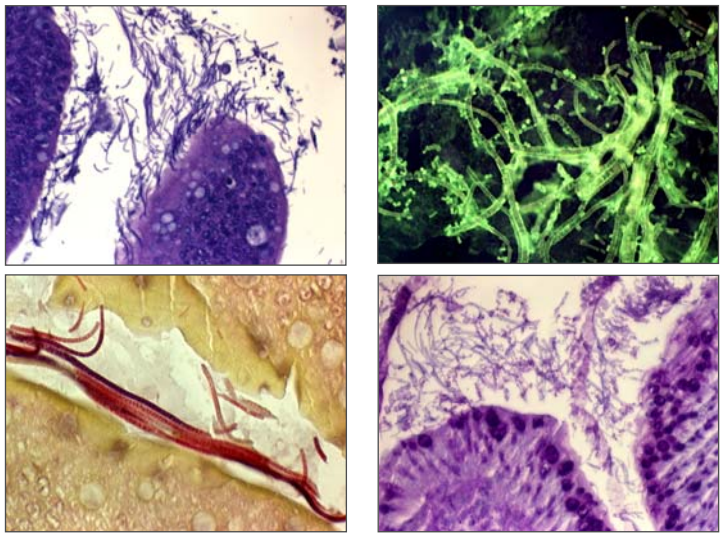
The hallmark microscopic lesion is “Multicystic enteropathy or enteritis”. Although this lesion is not exclusively seen in RSS, it is one of the most constant lesions in the syndrome. These lesions correspond to field cases.

Cystic Enteritis – Experimental



The hallmark microscopic lesion is “Multicystic enteropathy or enteritis”. Although this lesion is not exclusively seen in RSS, it is one of the most constant lesions in the syndrome. These lesions were reproduced expeimentally.

LSFO + Bacterial Rods



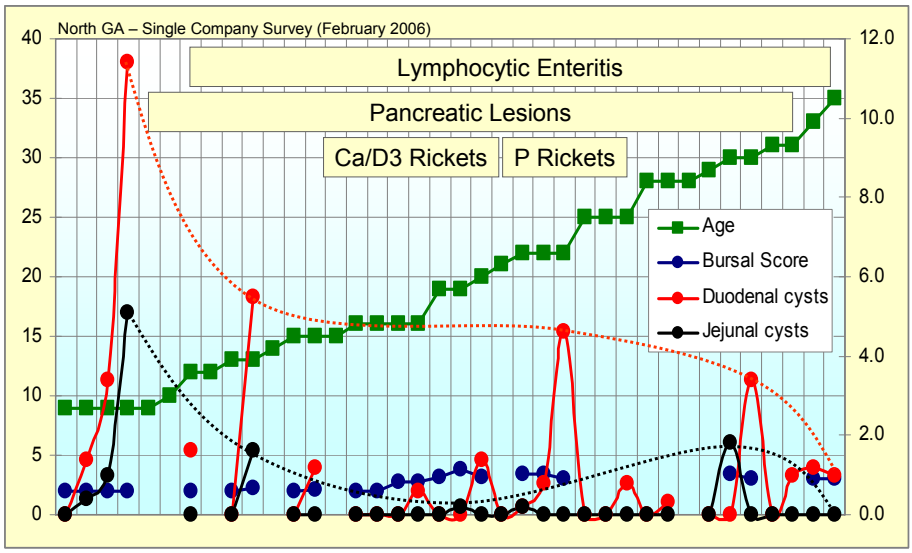
Long segmented filamentous organisms (LSFO) can be seen in association with RSS, but they are not present in every case of RSS.

Chronology of Lesions - Field

- Peak intestinal lesions = 7-12 days
- Cystic enteropathy/enteritis = 9-35 days
- Duodenal lesions > Jejunal lesions
- Lymphocytic enteritis = ≥12 Days
- Pancreatic lesions = ≥9 Days
  - Vacuolar degeneration (exocrine pancreas)
  - Fibroplasia (exocrine pancreas)
  - Lymphocytic infiltration

The peak of gross and microscopic lesions occurs between 7 and 12 days, albeit RSS-related lesions can be seen before or well after this age range.

Chronology of Field Lesions



The peak of gross and microscopic lesions occurs between 7 and 12 days, albeit RSS-related lesions can be seen before or well after this age range. This graph illustrates the average number of cystic or degenerating crypts in the duodenum (red dots), and jejunum (black dots) as age progresses. This company was experiencing simultaneous problems with rickets in some flocks.

In vivo Experimental Models

- Gavage inoculations
  - Broiler chicks
  - SPF chicks
  - Mature breeders
- RSS exposure (contaminated litter)
  - Broiler chicks

Most RSS-reproduction attempts have been made by gavage inoculation of intestinal contents or intestinal homogenates, or by rearing susceptible broilers on contaminated litter.

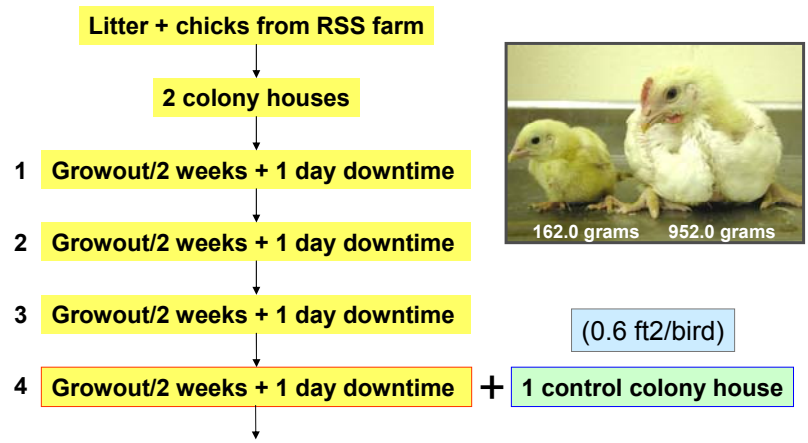
RSS Exposure in a Colony House Model

- Objective:
  - A continuous source of RSS
  - Reproducible model
    - Pathogenesis
    - Etiologies
    - Intervention strategies

One experimental model used in Dr. Guillermo Zavala's model at the University of Georgia used contaminated litter obtained from broiler houses where RSS had been diagnosed in several consecutive flocks.

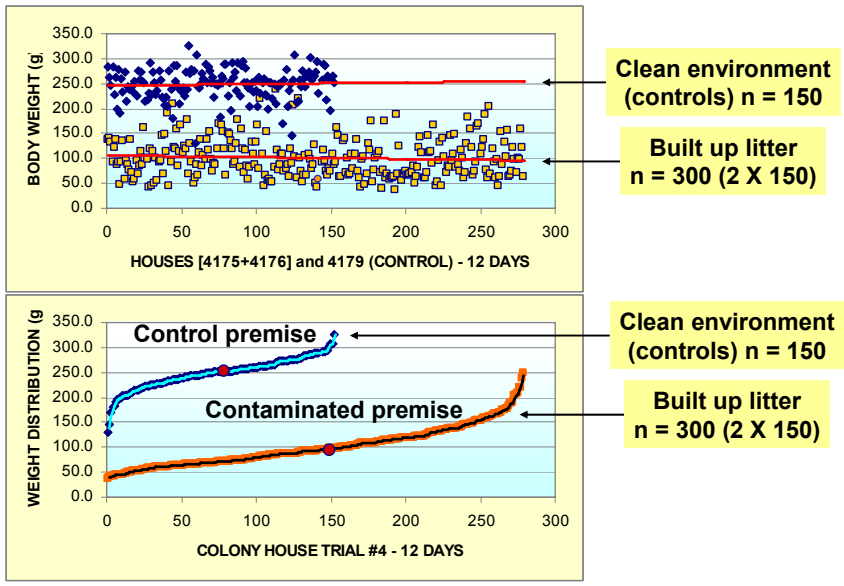
RSS in a Colony House Model

- Protocol:



In addition to using contaminated litter, the existing affected broilers in one flock were used to “seed” the contaminated litter. Two of the original “donor” broilers are seen in this slide. After approximately 2 weeks, the donor broilers were removed and immediately replaced with susceptible broiler chicks. This procedure was repeated twice before initiating any controlled experiments.

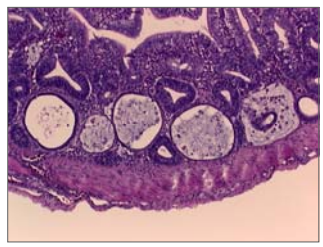
Impact of RSS on Early Body Weight



These graphs illustrate the individual body weights (at 12 days of age) of the control chicks (blue) reared on clean litter, and the RSS-exposed chicks (orange) reared on contaminated litter. No direct inoculations were done (just simple exposure).

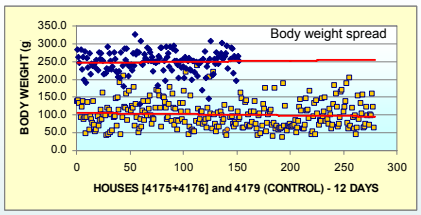
Experimental Reproduction of RSS by Exposure to Contaminated Litter

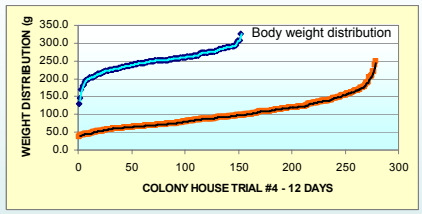
- Clinical findings
  - Stunting + low uniformity
  - Soft bones (w/o rickets)
- Gross and microscopic pathology
  - Thin-watery intestines
  - Poorly digested feed
  - Cystic enteropathy

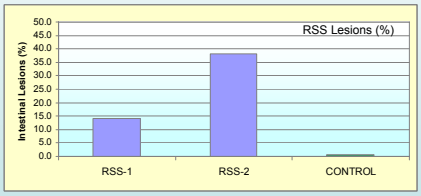


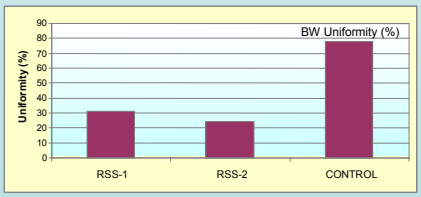
The gross and microscopic lesions were easily reproduced in broilers reared on contaminated litter.

Experimental Reproduction of RSS by Exposure to Contaminated Litter



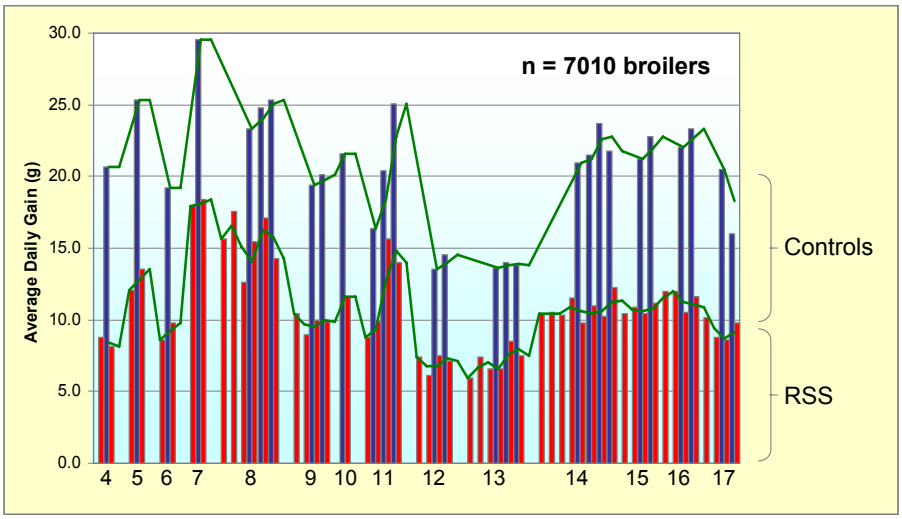






The gross and microscopic lesions were easily reproduced in broilers reared on contaminated litter. In addition, body weight and uniformity were severely affected.

A Reproducible Model For RSS



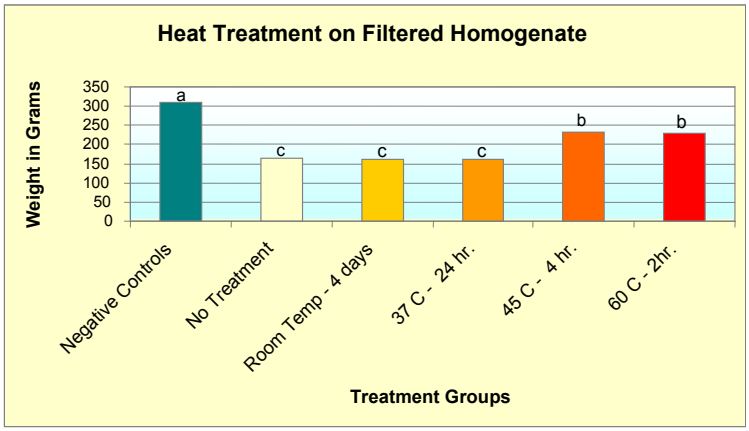
Multiple similar experiments were done following the same model. Blue bars represent the average body weight of control chickens, while the red bars represent the average body weights of groups of broilers exposed to RSS-contaminated litter. It can be observed that there was a consistent body weight depression of approximately 40-60% in comparison with the controls.

Thermal Susceptibility of RSS Agents

- RSS agents are heat-sensitive
- Thermal treatment of litter mitigated slightly effects of RSS
- Potential application: Litter heating or composting might reduce RSS

Intestinal contents from affected broilers were heat-treated and then administered by gavage to susceptible chickens. It was found that the higher the temperature and the longer the heat treatment, the lower the impact on body weight and uniformity.

RSS Agents are Heat Sensitive



H. S. Sellers et al., unpublished (2005).

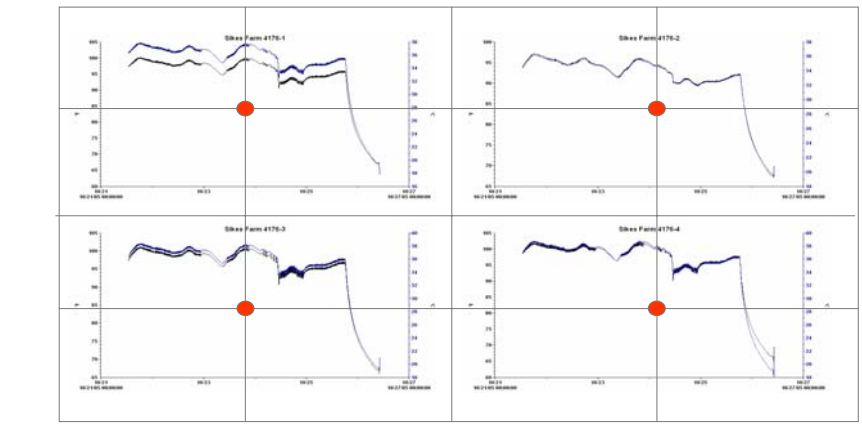
Intestinal contents from affected broilers were heat-treated and then administered by gavage to susceptible chickens. It was found that the higher the temperature and the longer the heat treatment, the lower the impact on body weight and uniformity.

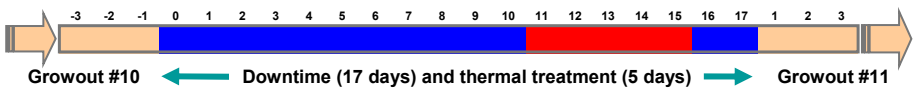
Impact of Downtime and Heat Treatment

- 17 days downtime (built up litter)
- Heat treatment (≥90°F or ≥30°C/100 hours)
- Previous flock:
  - BW = 50.41% of BW in controls (@ 10 days of age)
  - Uniformity = 36.3% vs. 73.12% in controls

To apply the concept of thermal sensitivity of RSS-related microorganisms, the contaminated litter was heat-treated at 100 degrees Fahrenheit for approximately 100 hours before placing susceptible broilers on it. An untreated RSS-contaminated pen and a control pen were used for comparison.

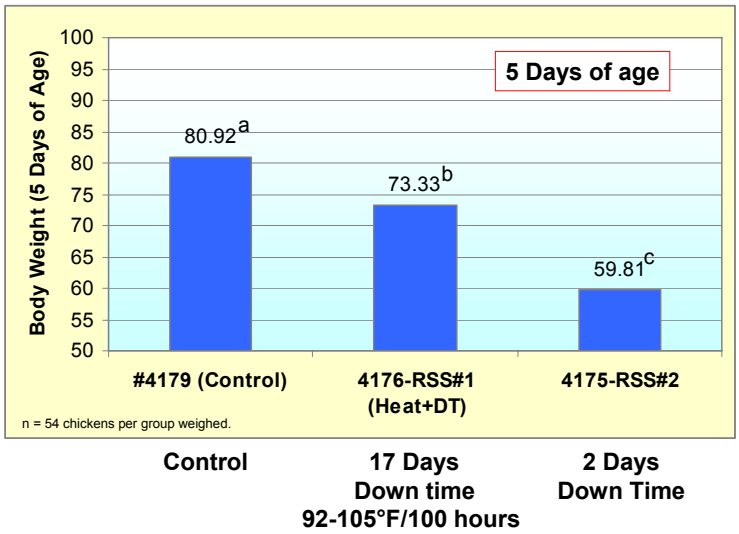
Impact of Downtime and Heat Treatment





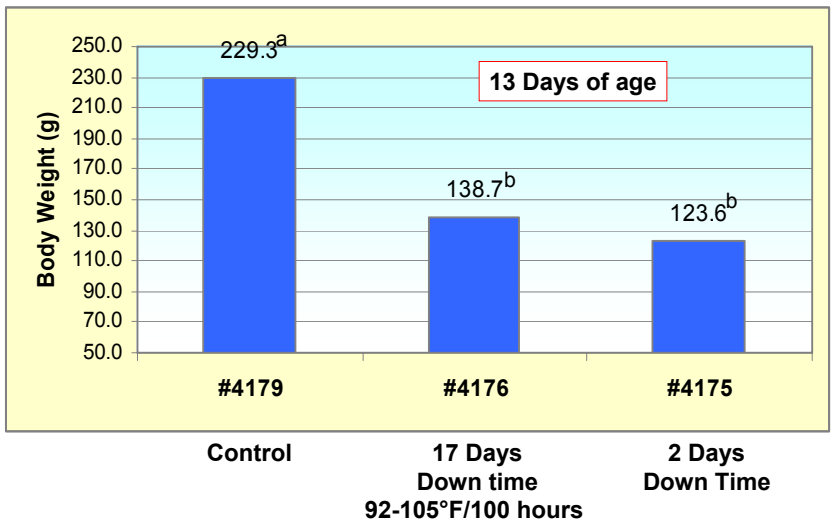
To insure effective and even heat treatment of the litter, the litter temperature in the experimental pen was measured continuously for 5 days in four quadrants of the pen. The temperatures achieved ranged between 92 and 105F.

Impact of Downtime and Heat Treatment



Then cntrol group weighed an average of 80 grams; the heat-treated RSS-contaminated pen weighed 73 grams; and the untreated RSS-contaminated pen weighed only 59 grams at 5 days of age.

Impact of Downtime and Heat Treatment



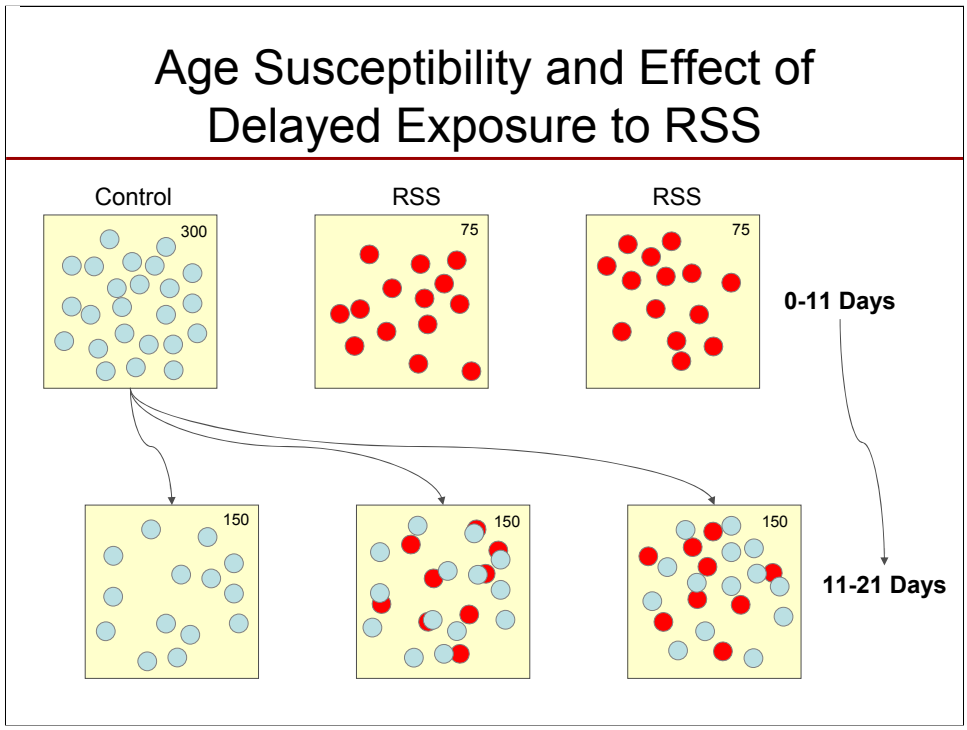
n = All surviving chickens weighed.

Although the heat-treated pen was heavier than the untreated pen at 13 days of age, thedifference was not significant. However, these results suggest that heat may decrease the detrimental impact of RSS both in vitro and in vivo.

Age Susceptibility and Effect of Delayed Exposure to RSS

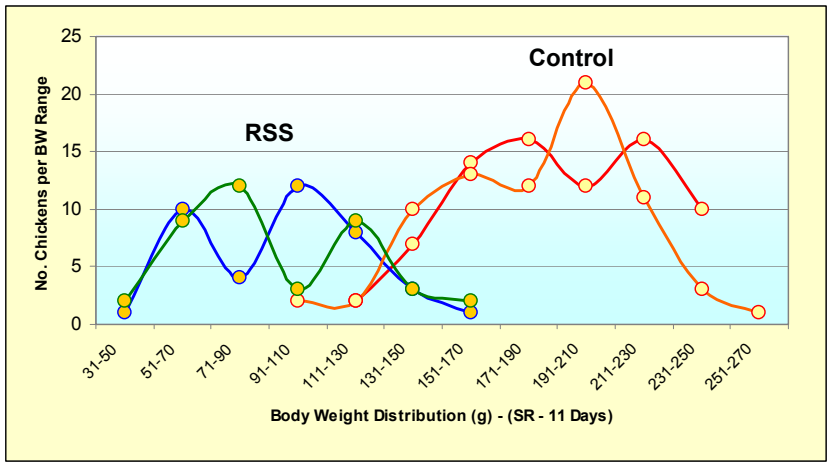
- Highest susceptibility = <11 days
- Delayed exposure (≥11 days) to RSS significantly mitigated detrimental effect of RSS on ADG, uniformity and lesions
- Potential application: C&D + top dressing with fresh litter in brood chamber might reduce RSS

Chickens are most susceptible to RSS during the first 10 days of age. To test this hypothesis, suscpetibel chicks were reared on clean litter and then transferred to a copntaminated pen at 11 days of age.

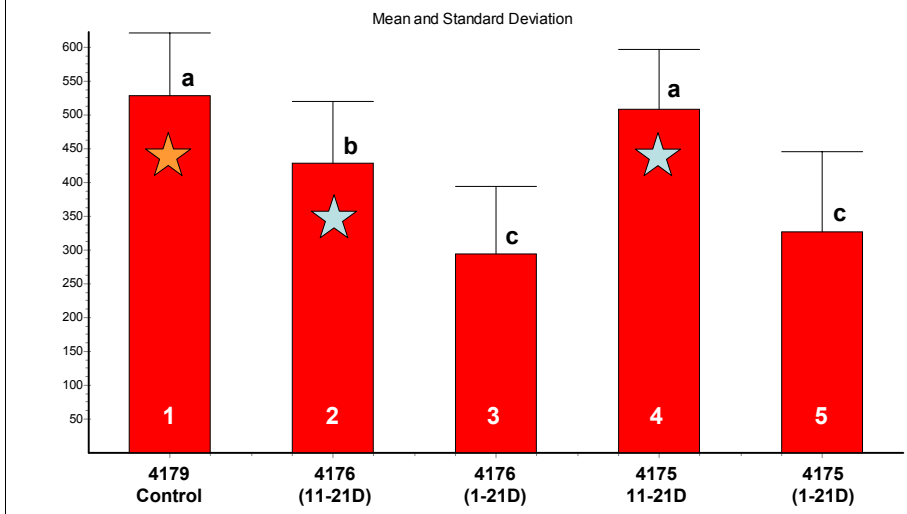


At 11 days of age, the broilers reared on clean litter wer commingled with broilers that had been reared on contaminated litter from one day of age.

Age Susceptibility (11 Day Weights)

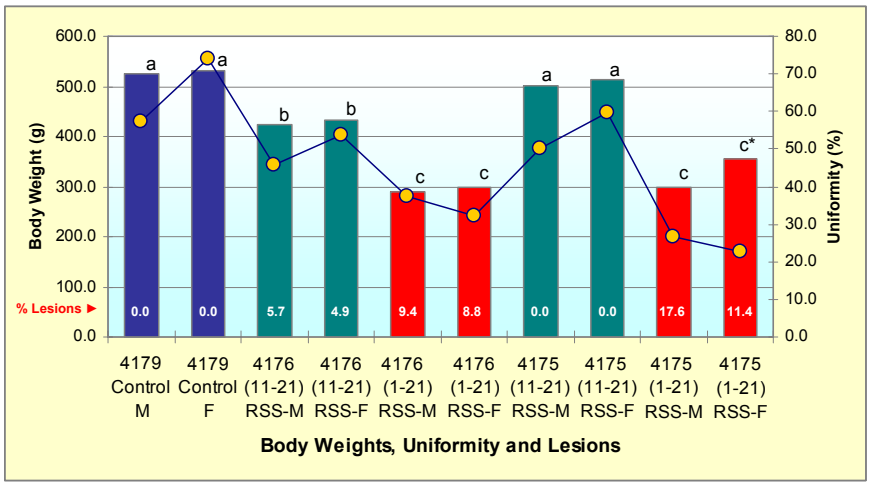


Age Susceptibility (21 Day BW)



At 21 days of age, the broilers reared for 11 days on clean litter and then on contaminated litter weighed significantly more than broilers reared on contaminated litter from one day of age. In addition, microscopic lesions (multicystic enteropathy) were only seen in the broilers exposed to RSS-contaminated litter from day 1, but not in the broilers reared on clean litter and transferred to a contaminated pen at 11 days of age. The bars with a blue star represent the groups transferred at 11 days of age. The orange bar is the average body weight of the controls. The bars without bars represent the average body weights of the groups exposed to RSS-contaminated litter from their first day of age.

Age Susceptibility (21 Day BW)



This graph represents the same data as the previous slide, except that the body weights of males are depicted separate from the body weights of the females. This graph demonstrates that males may be more susceptible than the females.

Effect of Cold Brooding

- BW at 7 and 14 days was higher when brooding was at 95°F vs. 84°F
- Uniformity at 7 and 14 days was higher when brooding was at 95°F vs. 84°F
- Potential application: Proper brooding temperature might mitigate RSS

Cold temperatures during brroding aggravate the effects of RSS.

Role of Breed and Generation

- All breeds or breed crosses tested (4) proved susceptible
- Higher yielding breeds appeared to be more susceptible
- BC and BB byproduct are susceptible
- Potential application: Optimize brooding conditions in higher yielding breeds

All breeds or breed crosses tested are susceptible, although there were some differences in the severity of lesions and body weight depression, with the higher yielding breeds being more susceptible.

Role of Breed

Breed Comparison (19 Days)

	Average Daily Gain (grams @ 19 Days)		
Breed cross	Control	Exposed #1	Exposed #2
A	443.4[a]	296.1[b]	293.7[b]
B	471.5[a]	335.2[c]	324.1[c]
C	480.6[a]	239.5[d]	272.6[d]

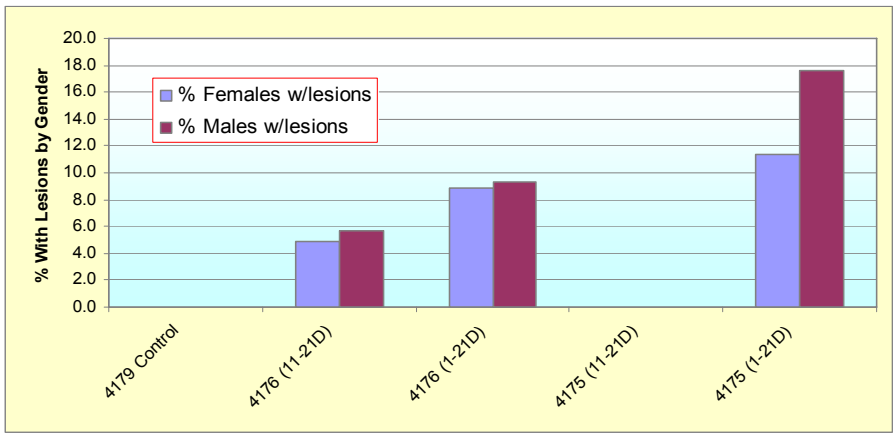
Breed “C” was consistently more severely affceted than two other popular breeds.

Role of Gender in RSS Lesions and Mortality

- Males consistently more susceptible
- Higher mortality in males with or without RSS-associated lesions

Role of Gender in RSS Lesions (Expt. #12)

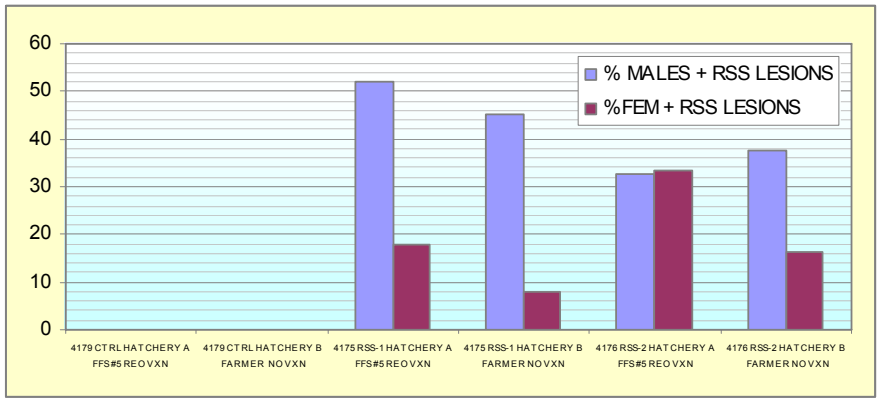
Delayed RSS Exposure – Males vs. Females



Experiment #12

In this separate experiment, it was corroborated that males are more severely affected than females.

Role of Gender in RSS Lesions (Expt. #15)
Role of Reovirus Maternal Antibody



In yet another experiment, males expressed more lesions than females.

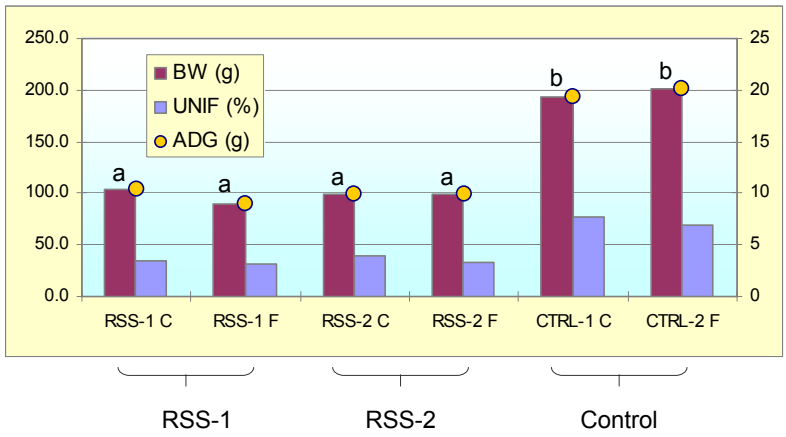
Role of Maternal Antibody (CAV, REO, IBD)

- No correlation between day old maternal antibody titer (ELISA) and body weight at 10 days
- Slight advantage when REO titers were high
- Methods:
  - Progeny from 2 integrators
    - CAV vaccination (Company “C”)
    - More robust REO+IBDV vaccination (Company “F”)
  - Identical breeder age
  - MAT at hatch (CAV, IBDV, REO)
  - Body weight, ADG, uniformity at 10 days of age
  - Histopathology
    - Duodenum, jejunum, pancreas (~35 chickens ea/group)

In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no correlation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age.

Role of Maternal Antibody (CAV, REO, IBD)

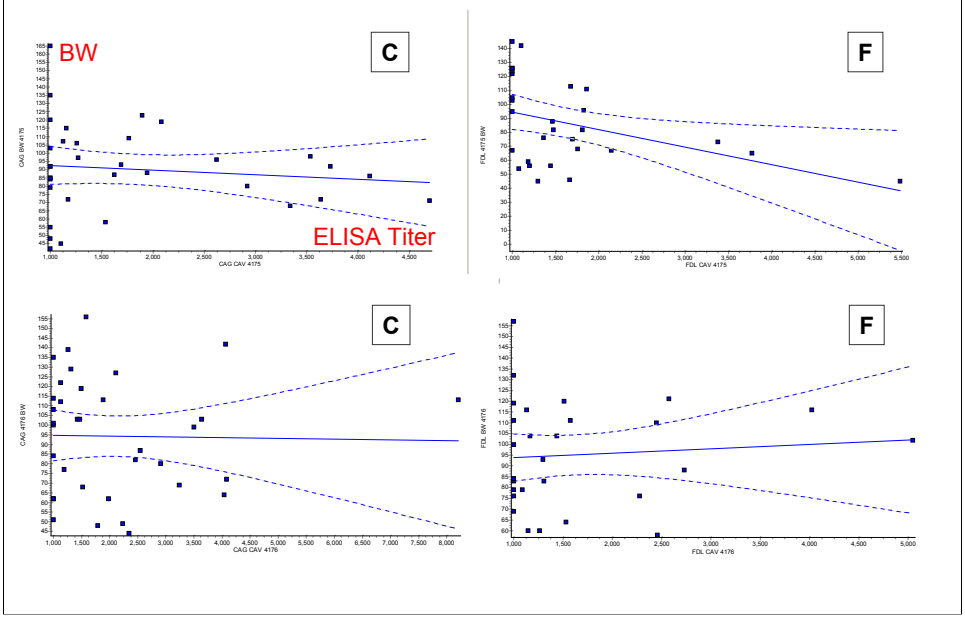
10 Day BW Comparison



P value = <0.0001 (ANOVA – Tukey Kramer multiple comparisons test)

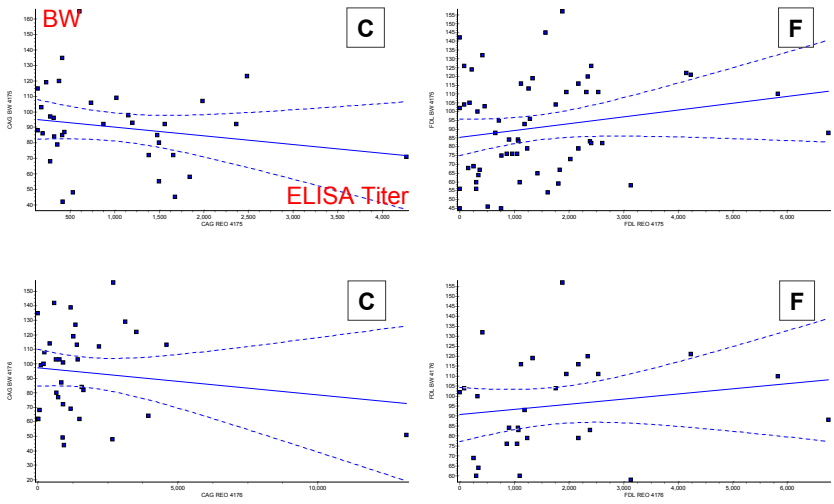
In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no corelation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age. This graph demonstrates that the RSS model was working properly for this experiment to be valid.

CAV Maternal Antibody vs. 10-Day Body Weight



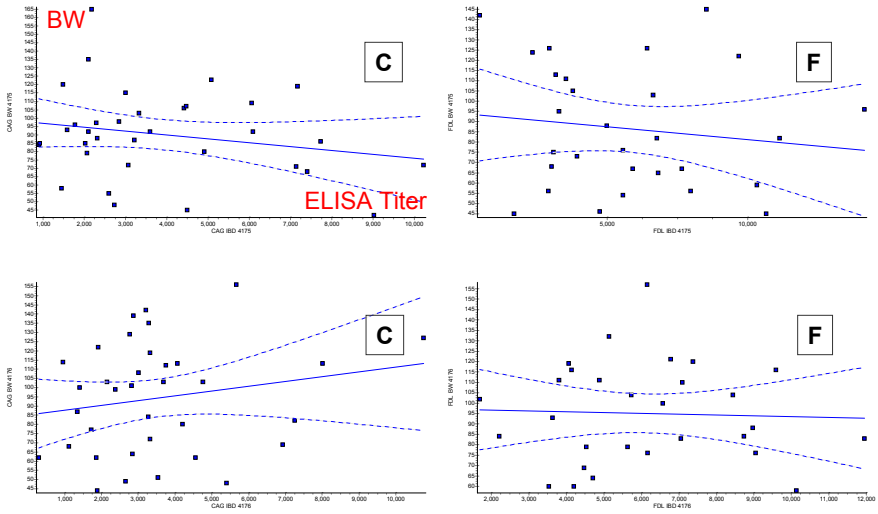
In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no correlation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age.

REO Maternal Antibody vs. 10-Day Body Weight



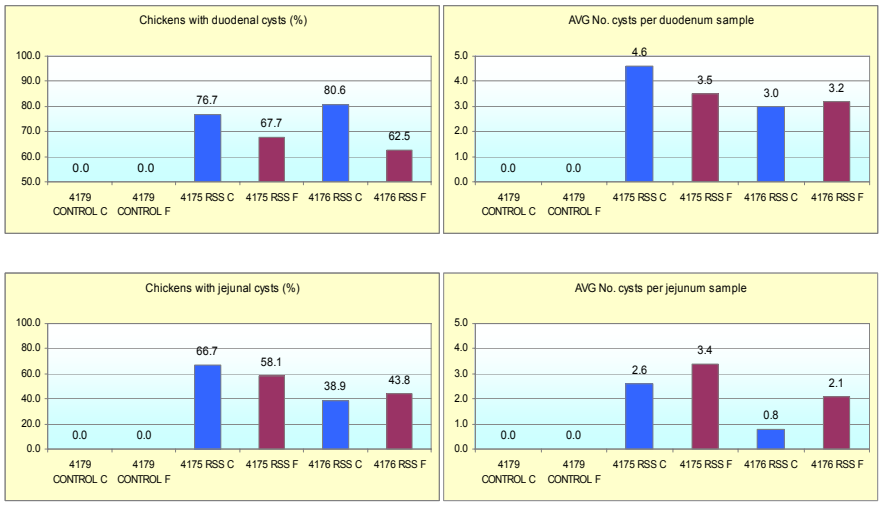
In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no correlation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age. This graph depicts the body weights and linear regression for 10-day-old body weights and day-old reovirus maternal antibody titers for 4 groups of broilers.

IBD Maternal Antibody vs. 10-Day Body Weight



In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no correlation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age. This graph depicts the body weights and linear regression for 10-day-old body weights and day-old IBDV maternal antibody titers for 4 groups of broilers.

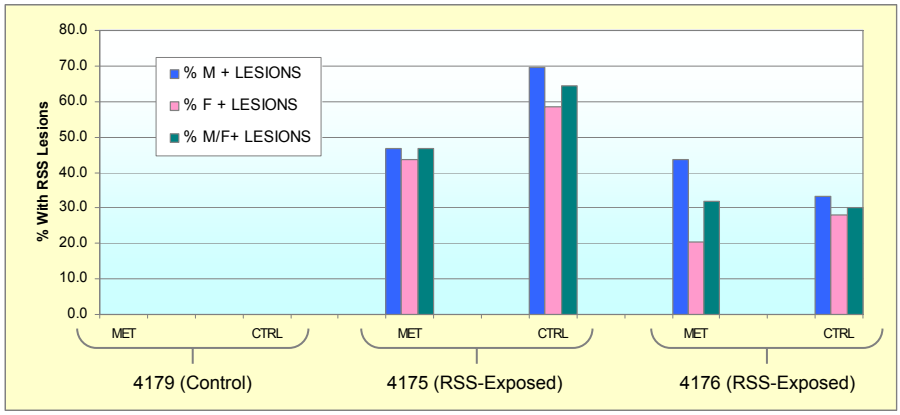
Role of Maternal Antibody (CAV, REO, IBD) on Cystic Enteropathy



In this experiment, the maternal antibody titers against reovirus, CIAV and IBDV were determined individually for broilers placed on RSS-contaminated litter. There was no correlation between the 1-day-old maternal antibody titer and the body weights attained at 10 days of age. These graphs depict differences in the progeny from 2 different companies (blue vs. purple).

Role of Gender in RSS Lesions (Expt. #13)

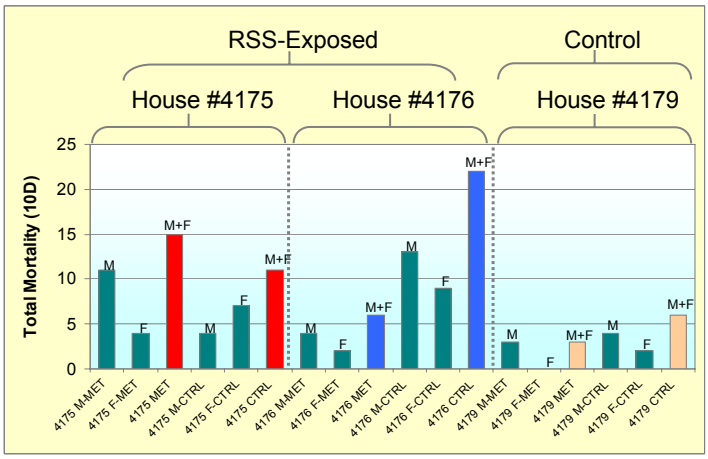
Effect of Metronidazole on RSS Expression



It has been suggested that Clostridium may play a role in RSS pathogenesis. In this experiment, RSS-exposed broilers were treated daily with metronidazole experimentally without any significant advantage over untreated chickens.

Role of Gender in RSS Mortality (Expt. #13)

Effect of Metronidazole on RSS Mortality



This graph illustrates the same data as the previous slide, except that the male body weights are depicted separately from the female body weights.

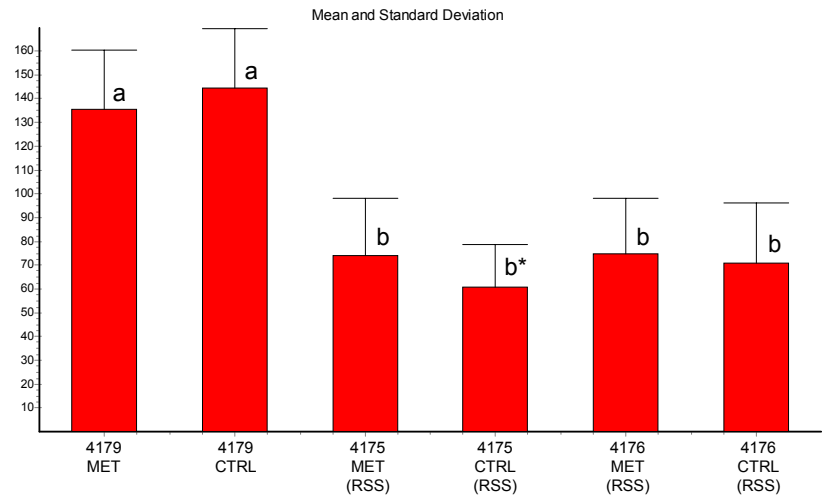
Effect of Metronidazole on RSS Expression

- No mitigation of RSS by metronidazole
- Higher frequency of RSS lesions in males
- Lower uniformity in males with RSS
- Higher mortality in males with RSS
- Protocol:
  - 50% of chicks medicated + 50% not medicated
  - Dose: 60 mg/kg LBW by gavage for 7 days (0,1,2,3,4,5,7)
  - Rearing until 10 days of age
- Application: Indirect diagnosis by response to therapeutic treatment (Metronidazole)

Summary of observations on the role of metronidazole in mitigation of RSS-related lesions or impact.

Effect of Metronidazole on RSS Expression

10 Day Old Body Weights



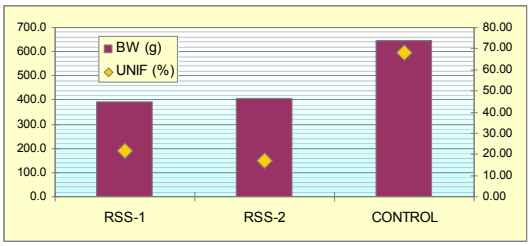
The use of metronidazole did not reduce the detrimental effects of RSS in treated (MET) broilers.

Effect of RSS on GP Byproduct

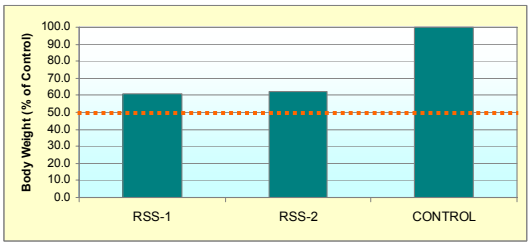
- GP byproduct was susceptible to RSS
- Body weight and uniformity significantly reduced
- Body length significantly reduced
- Potential application: PS is likely to be as susceptible as broilers

The susceptibility to RSS has been tested using not only broiler chicks but also higher generations of chickens.

Effect of RSS on GP Byproduct (22 Days)



Body weight and uniformity



Relative body weights

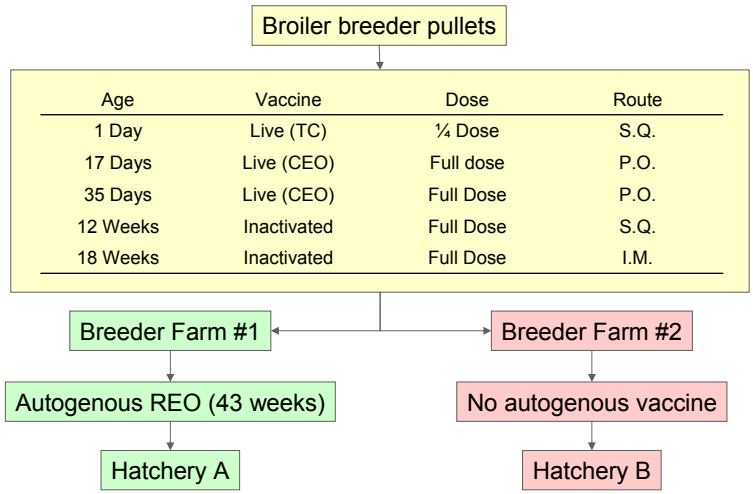
Grandparent byproduct was susceptible to RSS.

Effect of an Autogenous Reovirus Vaccine vs. RSS

- Objectives
  - Examine impact of autogenous REO vaccine
  - Rectal temperatures in RSS-affected chicks
- Potential application: Partial mitigation of RSS with autogenous REO vaccines

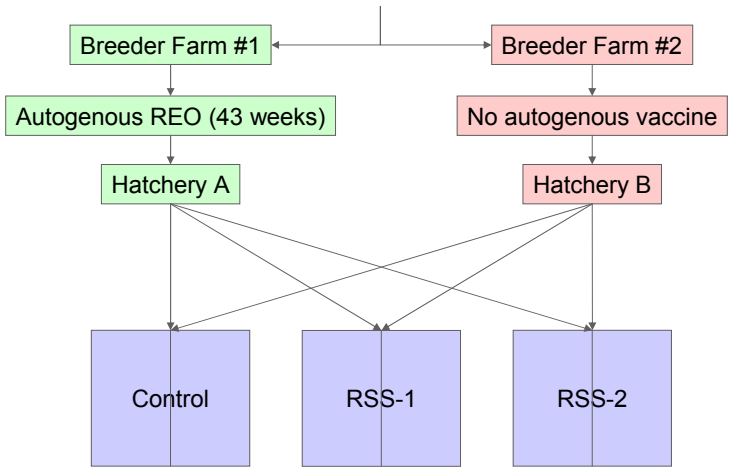
Reoviruses isolated from RSS-affected broiler flocks were used as an autogenous vaccine in broiler breeders from affected companies.

Effect of an Autogenous REO Vaccine



Experimental design to evaluate the impact of an autogenous (inactivated) reovirus vaccine. The integrated company used routinely 3 live reovirus vaccines, and 2 inactivated reovirus vaccines.

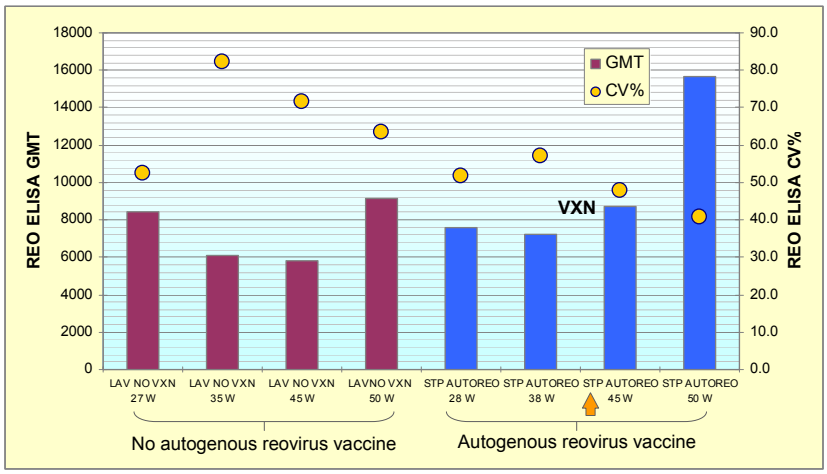
Effect of an Autogenous REO Vaccine



Experimental design to evaluate the impact of an autogenous (inactivated) reovirus vaccine. The integrated company used routinely 3 live reovirus vaccines, and 2 inactivated reovirus vaccines. The progeny broilers of the breeders vaccinated with an autogenous reovirus vaccine were placed in a clean pen and also in RSS-contaminated pens, commingled with progeny broilers produced by breeders that did not receive the autogenous reovirus vaccine.

Effect of an Autogenous REO Vaccine

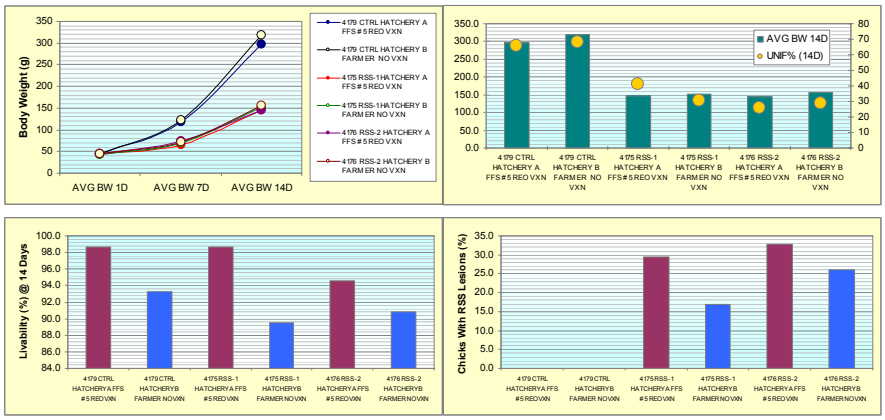
REO ELISA Serology



No differences were observed between the body weights of progeny from autogenous-reovirus-vaccinated breeders and the progeny from breeders that did not receive the autogenous vaccine.

Effect of an Autogenous REO Vaccine

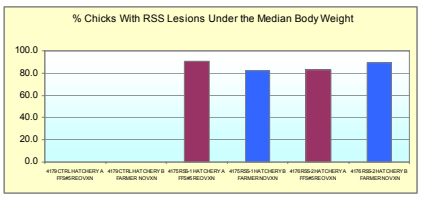
Hatchery Source and Immunity to Reovirus

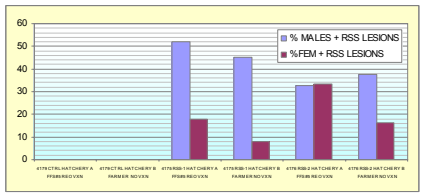


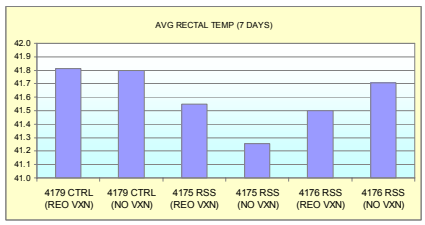
No differences were observed between the body weights of progeny from autogenous-reovirus-vaccinated breeders and the progeny from breeders that did not receive the autogenous vaccine.

Effect of an Autogenous REO Vaccine

Lesions and Rectal Temperatures

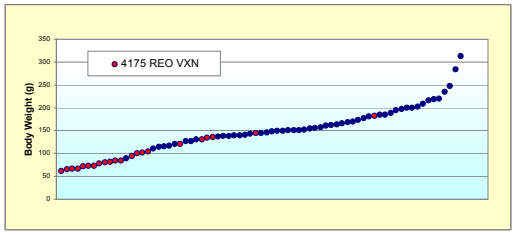




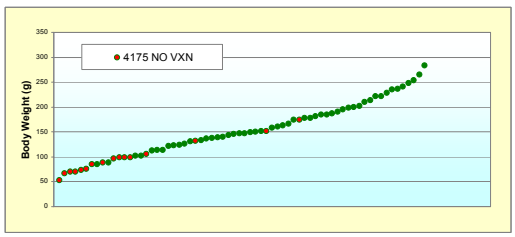


No differences were observed between the body weights of progeny from autogenous-reovirus-vaccinated breeders and the progeny from breeders that did not receive the autogenous vaccine.

Effect of an Autogenous REO Vaccine
Body Weight vs. RSS-Related Lesions



- Hatchery A
- Autogenous REO vaccine
- AVG weight = 146.3 g
- Uniformity = 41.3%
- Livability = 98.7



- Hatchery B
- No autogenous REO vaccine
- AVG weight = 152.4 g
- Uniformity = 30.9%
- Livability = 89.5

No differences were observed between the body weights of progeny from autogenous-reovirus-vaccinated breeders and the progeny from breeders that did not receive the autogenous vaccine.

Agents Detected

- Electron microscopy:
  - Small round viruses
  - Reoviridae
  - Rotavirus
- Molecular detection*:
  - Rotavirus
  - Chicken astrovirus (CAstV)
  - Avian nephritis virus (ANV)

*Dr. M. Pantin-Jackwood (SEPRL/USDA/ARS)

Some of the agents detected in affected broilers.

Summary

- Key components of RSS: Thin pale watery guts, cystic enteropathy, pancreatitis, stunting and low uniformity
- House heating may mitigate RSS
- C&D and fresh litter may delay and mitigate RSS
- ↑ RSS in cold brooding, short downtime and built up litter
- Proper brooding temperatures may mitigate RSS
- Higher yielding breed crosses and males more susceptible
- Higher susceptibility at under 11 days of age
- No correlation with CAV, IBDV, REO maternal antibodies
- Anti-protozoal and anti-anaerobic drugs ineffective
- Vertical transmission?

Summary observations on RSS.