EVALUATION OF THE EFFECTS OF COMMERCIAL YEAST CELL WALL PREBIOTICS AND BACILLUS PROBIOTICS ON BROILER PERFORMANCE WHILE SUBJECTED TO GASTROINTESTINAL STRESS

A Thesis

by

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MASTER OF SCIENCE

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ABSTRACT

The purpose of this study was to compare the effects of four prebiotic and probiotic treatments on Cobb 500 broilers under gastrointestinal (GI) stress induced by a double coccidiosis vaccination at Day (D) 1 and again on D 7, with a mild heat stress beginning on D 22 of the trial. This study used a Negative Control (NC) treatment (TRT) to test for a positive response to the GI stress challenge and a Positive Control (PC) basal diet with 5% DDGS, to represent current industry type diets more closely, and serve as the control for the 4 antibiotic alternative treatments. The study was conducted using a 4-phase feeding program over 42 D with approximately 3000 broilers housed within 90 pens (3’x 6’) of a single tunnel ventilated rearing barn located at the TAMU Poultry Science Research Farm. The hypothesis was to determine if prebiotics and/or probiotics can be effective alternatives to the use of antibiotics as growth promoters in broilers.

Dependent variables analyzed on D 21 for cumulative weight gain, and cumulative feed to gain showed MicroSaf as the best performing. For cumulative weight gain ratios to NC on D 21 the Safmannan was the best, with similar results from Microsaf, and the PC. For the FCR to NC ratio Microsaf performed better, but similarly to the Calsporin, Safmannan, and the PC on D 21. On D 42 with the feed to gain ratio Safmannan, Calsporin, and Microsaf performed significantly better than Celmanax. With the FCR on D 42 Safmannan performed significantly better than Celmanax, but similarly to the other treatments. Based on this data Safmannan, Calsporin, and Microsaf are all viable alternatives to the use of antibiotics for growth promotion.

DEDICATION

This Thesis is first dedicated to the three main influences in my life. Those are my wife, Marites, my daughter Candace, and God. It took a great deal of patience and understanding from all three to allow me to return to school, and pursue this program, for their undying devotion, and love, I am forever in their debt.

Second, I also dedicate this thesis to my small army of supporters who helped me get back up to date in modern science. The support professors, and peers, including Dr. Bailey, and Connor Padgett have been invaluable over these past two years.

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Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my wife and daughter for their infinite patience and assistance in helping me through this program. Their help as well as that of my friends has made this experience much easier than it otherwise could have been.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Christopher Bailey of the Department of Poultry Science and Dr. Rosemary Walzem of the Department of Poultry Science, and Dr. Stephen Smith of the Department of Animal Science.

All other work conducted for the thesis (or) dissertation was completed by the student independently. The data analyzed within this thesis was conducted by the student, with the assistance of Dr. Bailey and Connor Padgett.

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This graduate study was supported by funding from Phileo Lesaffre Animal Care.

NOMENCLATURE

B/CS	Bryan/College Station
CFU	Colony Forming Unit
D	Day
DDGS	Dried Distillers Grain
FCR	Feed Consumption Ratio
FSIS	Food Safety Inspection Service
GI	Gastrointestinal
HSUS	Humane Society of the United States
HACCP	Hazardous Analysis Critical Control Point
MOS	Mannan-Oligosaccharide
NC	Negative Control
PC	Positive Control
SIG	Significant Difference
TDA	Texas Department of Agriculture
TRT	Treatment
TxDOT	Texas Department of Transportation
USDA	United States Department of Agriculture
YCW	Yeast cell wall

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF GRAPHS

Page

CHAPTER I

INTRODUCTION

Antibiotics Alternatives

Feed and vaccination expense in the poultry industry accounts for two of the highest costs for the poultry industry. As such, maintaining a minimum feed to weight ratio in poultry is vital for cost reductions for producers. Traditionally, antibiotics have been used for both growth, and disease prevention (Gadde *et al.,* 2018). As far back as 2003 the poultry industry sought to replace antibiotics using probiotics, or prebiotics such as mannan oligosaccharides from *Saccharomyces cerevisiae* (See Figure 1.5). This was to find an effective replacement for antibiotics as nations began to restrict prophylactic use of antibiotics in animals (Fritts and Waldroup, 2003).

The World Health Organization (WHO) provides the most basic definition of a probiotic as “a probiotic is a live microorganism which when administered in adequate amounts confer a health benefit on the host,” (WHO, 2002). While as far back at the 1990’s the definition, “a probiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon of the intestine, and thus improves host health” has also been used (Gibson and Roberfroid, 1995).

The prebiotic, while not alive, also improves the growth of the birds.
One definition of prebiotics that was proposed in 1995 says it is “a non-digestible food ingredient that beneficially affects the host by selectively

stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Davani-Davari, 2019).

Within the last decade, additional legislation has been introduced in the United States and countries within Europe to remove the use of supplemental antibiotics for use as a growth promoter (Penaloza-Vazquez *et al.*, 2019). The European Food Safety Authority introduced restrictions on the use of supplemental antibiotics for weight gain as early as 2006 and the United States Veterinarian Feed Directive passed in 2017 to ensure accountability for antibiotic use in animal production.

While antibiotic use has been substantially reduced from its more historically prominent role, the increasing dependency of both probiotics and prebiotics has continued to rise, both within the United States of America and elsewhere in the world. For example, in 2019 the poultry prebiotic ingredient market managed to exceed $83 million despite continual challenges such as standardization, and technological limitations that have not yet been overcome. This dollar figure is expected to continue to rise at a rate of approximately 5.5% over the following seven years, to an expected $120 million, with 7% of that total being in the bacillus segment alone (Ahuja and Mamtani, 2021).

Yeast Cell Wall

The use of a fungal cell's Yeast cell walls (YCWs) as antibiotic alternative has been ongoing for decades. These alternatives such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), or mannan-oligosaccharides (MOS), generally follow the three main requirements to be prebiotic , "(1) the prebiotic candidate must be neither hydrolyzed or absorbed in the upper part of the gastrointestinal tract (GI); (2) serve as a selective nutrient source that supports growth and/or metabolic activity of members of the GI microbial community that could be considered beneficial; and (3) induce luminal or other systemic physiological responses that benefit the host in some form or fashion" (Ricke *et al.*, 2020).

In 2003 research demonstrated the benefits of various glucan and mannan compounds within the YCWs themselves (Aguilar, 2003). *Escherichia coli* (*E. coli*) and salmonella species can be prevented from adhering to the intestine walls by YCW that includes approximately 1/3 mannan-oligosaccharides (MOS). This in turn is thought to prevent the initial formation of pathogenic colonies, in addition to other benefits to the host animal such as increased villi heights, goblet cell density, and productivity. (Fowler *et al.*, 2015).

In addition to using the fungal cell's YCW as a prebiotic component, beta 1-3, 1-6 glucans have also been used in poultry trials to show improved responses to *Salmonella enteritidis* (S.E.) intestinal challenges in chicks, pullets, broilers and laying hens (Connor *et al.*, 2021).

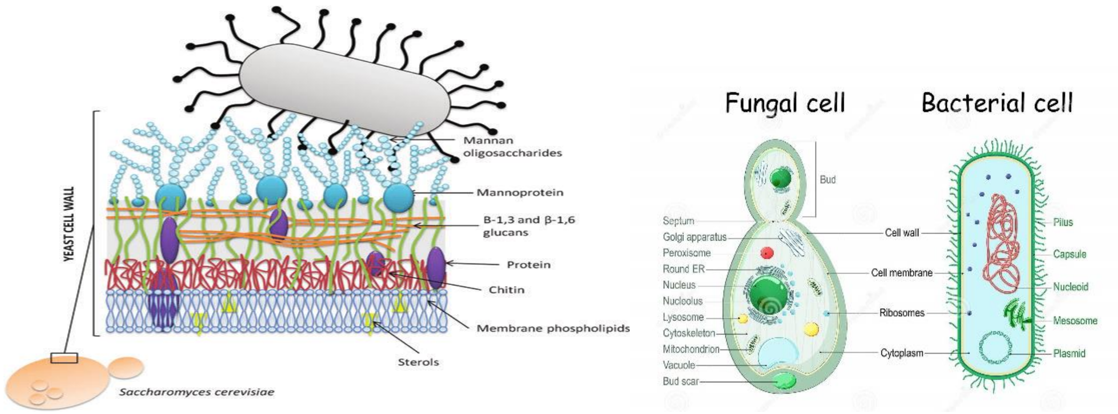

Figure 1.1 Yeast cell wall binding to a pathogen, and fungal/bacterial cell comparison, Reprinted from (Santovito *et al.*, 2018), and thumbs.dreamstime.com

Yeast cell wall prebiotic addition to feeds have been reported (Santin *et al.*, 2006) to improve the feed conversion ratio of birds regardless of whether or not the birds have been given feed contaminated with either aflatoxins or ochratoxins. This is beneficial to the poultry industry because those toxic substances can contaminate ingredients used to formulate the bird's feed (Santin *et al.*, 2006).

There has been a theory, or concept of using prebiotic YCW in conjunction with a probiotic that has been tested with promising results. In a 2020 Veterinary World Journal article, YCW supplements were shown to reduce levels of *Salmonella* in the ceca in a flock of Hy-Line Brown pullets

(Price *et al.*, 2020). Through this trial, the evidence of the viability of using both probiotic (*B. amyloliquefaciens*), and YCW treatments was validated as both treatments reduced the number of positive Salmonella samples by15%, (Price *et al.,* 2020)

The value of how the YCW can bind to pathogens like *Salmonella* can be seen in Figure 1.1. The mannoproteins on the surface of *Saccharomyces cerevisiae* binds to the type 1 fimbriae surrounding *Salmonella enterica*. This form of agglutination denies the pathogen the ability to bond to the gastrointestinal tract (GI), or the ability to multiply, and thus allows for reduced pathogen loading in birds. (Santovito *et al.*, 2018).

The type 1 fimbriae of the pathogen bind to the mannan oligosaccharide portion of mannoproteins. The Beta-1, 3 and the Beta-1,6 glucans serve as the "bait" to initiate mannoprotein pathogen binding (Santovito *et al.*, 2018).

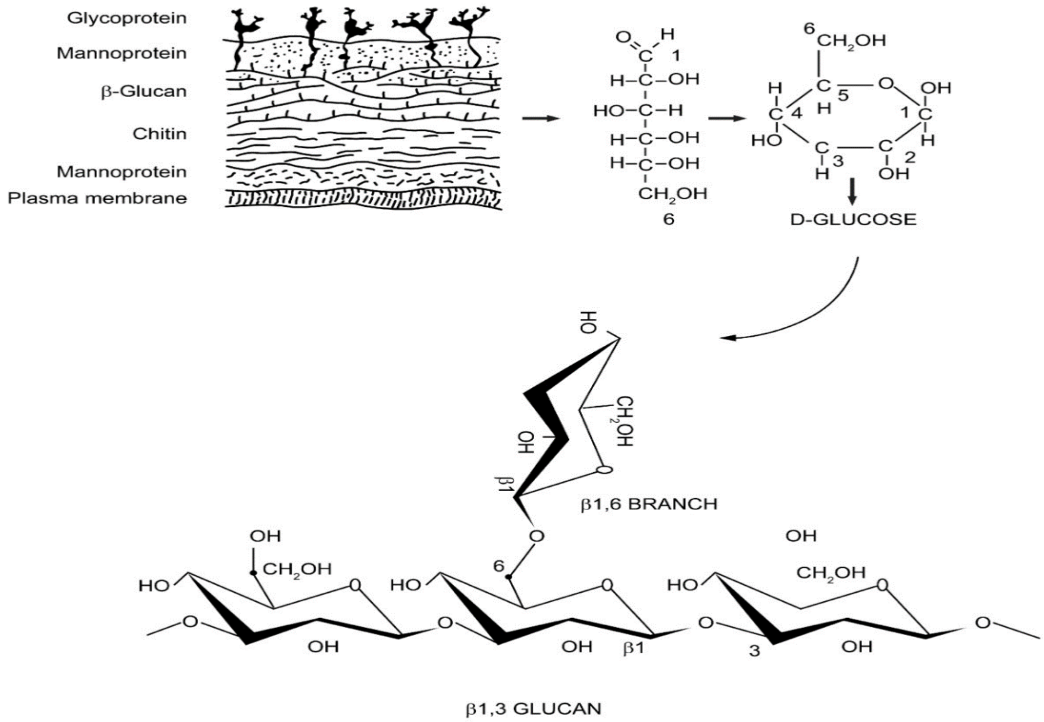

Figure 1.2 Diagram of Yeast Beta-glucan structure and Cell wall

Reprinted from (Santovito *et al*, 2018)

The two commercial prebiotic yeast cell wall products in the trial were Celmanax™, and Safmannan™. Both products report mannan oligosaccharide as their active ingredient (Hofacre, 2018). The other two commercial probiotic products tested in this trial wereMicroSaf™ and Calsporin™, will utilize live bacillus strains of bacteria in the intestines to promote healthier growth.

Safmannan is a yeast product from the Phileo Lesaffre. It utilizes both mannan-oligosaccharides and beta-glucans (1,3 and 1,6) premium yeast fraction, rich in mannan-oligosaccharides and beta-glucans (Phileo, 2020). This product has been tested in previous trials where it showed promise in increasing the bodyweight gains compared to the control. It did not, however show a significant change in the feed consumption, nor in the feed consumption ratio against the control group at that time (Benites, 2008).

Celmanax is an enzymatically hydrolyzed *Saccharomyces cerevisiae* yeast cell wall product that contains a combination of mannan-oligosaccharides, mannose, beta glucans, and other functional carbohydrates (Hofacre, 2018).

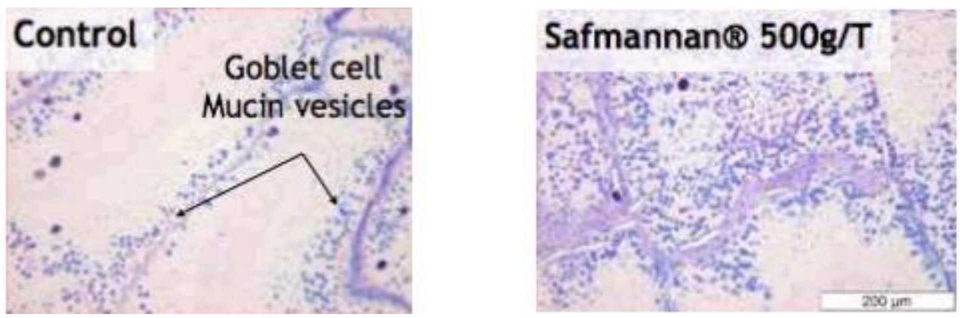

Figure 1.3 Safmannan effect of increasing mucin vessels and goblet cells

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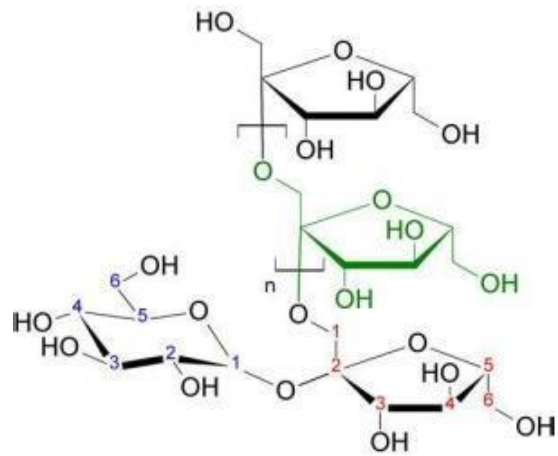

Figure 1.4 Mannan-oligosaccharides

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Probiotics

Examples of products that can deliver probiotics include fermented foods, supplements, drugs, and medical foods (Reid, 2016). This is contrast to a prebiotic which while also a non-digestible food ingredient for stimulating growth to improve health, they are not alive (Davani-Davari *et al*., 2019). Generally, the probiotic can use direct competitive inhibition to prevent or reduce binding of pathogens to the mucosal surface of the GI tract (Posadas *et al.*, 2017).

Probiotic use has continually expanded over the last few years as an alternative to antibiotics formally used as growth promoters. For example, a live *Saccharomyces cerevisiae* yeast (Figure 1.3) product was shown to increase intestinal absorption capability by increasing villus height, mucus thickness and number of goblet cells in

Ross 308 broiler chickens. (Morales-Lopez *et al.*, 2010). In this study it was found that the use of probiotics led to not only the increase of villi height, but also weight gain (Morales-Lopez *et al.*, 2010). The increase in the villi height may be attributed to the probiotics activating cell mitosis and gut epithelial-cell proliferation after probiotic supplementation (Samanya and Yamauchi, 2002).

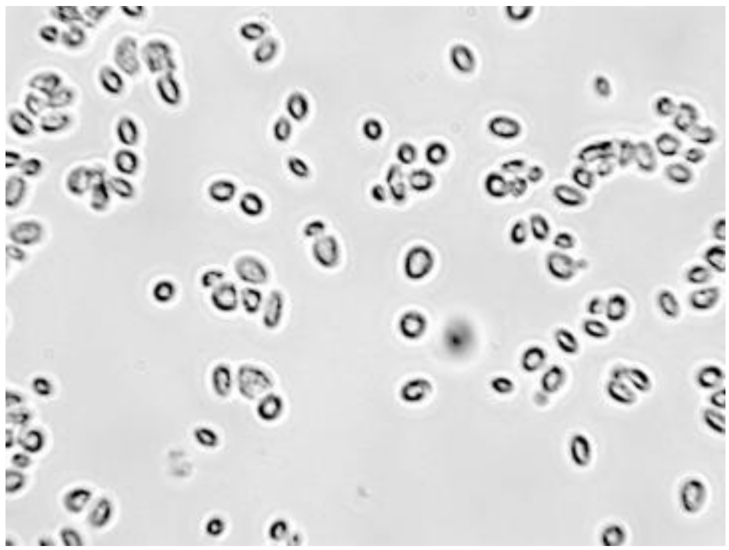

Figure 1.5 *Saccharomyces cerevisiae*

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The commercial product MicroSaf®, (Phileo by Lesaffre) has a unique association of Bacillus species, *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus pumilus*. MicroSaf® is a spore forming bacteria probiotic with a high resistance to feed processes, such as high temperatures during pelleting or the heat treatment of mash feed that is said to "help mitigate the negative effects on nutritional challenge or necrotic enteritis caused by Clostridium perfringens". (https://en.engormix.com/phileo-lesaffre-animal-care/MicroSaf-new-generation-bacteria-probiotic-poultry-sh1405_pr35695.htm).

Calsporin® is also a spore forming probiotic sold by Asahi Calpis Wellness Co., Ltd. that utilizes spores of Bacillus subtilis C-3102 as a probiotic. It has been used for increased meat yield and egg production in a variety of poultry species including chickens, turkeys, geese, and many others, though for laying hens it did not improve the quality of the eggs, only increasing the size (Bampids *et al.*, 2015). According to a 2019 study the use of Calsporin was useful for increasing weight gain, both in chickens and turkeys. It was also shown to be safe alternative for both humans, and the environment (Bampids *et al.*, 2019).

Heat Factors

As shown in Figure 1.5 birds will begin to experience heat stress at 25° C (77° F) (Shakeri *et al.,* 2020). Injuries from wet litter often associated with heat stress can also reduce growth rates in the birds leading to smaller growth yields, while dry litter can reduce the risk of the injuries and reduce the loss of growth rate (Martland, 1985).

In the poultry industry, the use of properly treated litter is being considered as a pathogen control and has been a commonly used Hazardous Analysis Critical Control Points (HACCP) (Payne *et al.*, 2002). During the course of this trial, new unused litter was used.

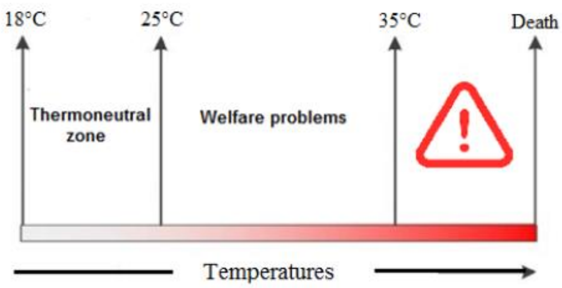

Figure 1.6 Temperature range for poultry health

Reprinted from Shakeri *et al.*, 2020

Hypothesis

To determine if prebiotics and/or probiotics can be effective alternatives to the use of antibiotics as growth promoters in broilers.

Objective

The objective of this experiment is to use a “Phase Stress Model” to determine the most efficient alternative to antibiotics for growth and pathogen reduction using 4 commercial prebiotic and probiotic products.

The birds were introduced to possible gastrointestinal stress utilizing 5% dietary distillers dried grains in their diets. An immune stress will be introduced via multiple live coccidiosis vaccinations from the Advent product line of the Huvepharma company. Finally, on D 22 the birds were introduced to an environmental challenge as increased heat and reduced ventilation during the remaining portion of the 42 D rearing period.

CHAPTER II

METHODOLOGY

Birds and Housing

A total of 3,000 replacement one-day-old Cobb 500 male broilers were supplied by a local commercial hatchery facility. These birds were then divided into 6 treatment groups of 480 birds each (2880 birds total after sorting out replacements) and housed at the Texas A&M Poultry Science Farm facility rearing barn #1211 in ninety 3'X 6' floor pens. The pens were designed using steel 3′ tall walls made of steel wire, with litter covered concrete floors. A total of 32 birds were placed inside each pen, equipped with hanging feeders and nipple drinkers. Basal diets were formulated following Cobb's 2018 nutritional recommendations for Cobb 500 broilers (Cobb, 2018). The remaining 120 birds were placed into 2 pens (3'X 12') and used to replace any mortalities that occurred during the first five days of the experiment. Surplus birds were fed NC and PC feed.

The birds were randomly placed into the 90 pens in a randomized block order according to treatment. Fifteen Blocks, containing all 6 treatments were set up among 3 rows from the West (exhaust fans) to the East (cooling fans) end of the rearing facility. The building #1211 heat and ventilation were maintained by a programmable Rotem® Environmental Controller (QC Supply, Schuyler, Nebraska). Previous experiments conducted in rearing barn #1211 have shown ambient temperatures to be a higher on the West end of the building and cooler on the East side where the exhaust fans pull in outside air through evaporative cooling pads. This is called "tunnel ventilation" and is a very common in today's commercial poultry production farms. An entrance walkway separates the building into both East and West halves and the air flow is from East to West down the length of the building.

Barn: 1211

Large Fan

WEST

Pen	TRT	Block	Pen	TRT	Block	Pen	TRT
1	1	block 1	31	2	block 1	61	6
2	4		32	5		62	3
3	3	block 2	33	1	block 2	63	2
4	5		34	6		64	4
5	6	block 3	35	4	block 3	65	5
6	2		36	3		66	1
7	5	block 4	37	1	block 4	67	2
8	3		38	4		68	6
9	5	block 5	39	3	block 5	69	2
10	4		40	6		70	1
11	6	block 6	41	2	block 6	71	3
12	1		42	5		72	4
13	2	block 7	43	3	block 7	73	5
14	6		44	4		74	1
15	5	block 8	45	6	block 8	75	2
16	3		46	1		76	4
17	1	block 9	47	2	block 9	77	6
18	4		48	5		78	3
Door		Walkway				Rotem	
19	3	block 10	49	2	block 10	79	5
20	6		50	4		80	1
21	4	block 11	51	1	block 11	81	2
22	5		52	3		82	6
23	1	block 12	53	5	block 12	83	3
24	2		54	6		84	4
25	5	block 13	55	1	block 13	85	2
26	4		56	3		86	6
27	1	block 14	57	4	block 14	87	3
28	2		58	6		88	5
29	6	block 15	59	5	block 15	89	4
30	3		60	2		90	1

SOUTH (Fan, Cool Cell) — NORTH (Fan, Cool Cell)

EAST

T1	NC
T2	PC
T3	Safmannan
T4	Celmanax
T5	MicroSaf
T6	Calsporin
Aisles	
Fans	
Cool Cells	
Block	

Airflow is East to West

Figure 2.1 Pen block layout, randomized treatment arrangement by block

Due to the small size of the chicks, and the design of the pens, there was a chance of crossovers from one cage to another. To mitigate that risk the birds were marked using liquid chalk on their heads in a different color (green, blue, red, and one with no color) matching the color assigned to the pens on the blocking chart. Using that pattern going down the pens any chick crossing over could be quickly identified and replaced. The cages were also marked with index cards identifying the treatment used and marking color for the chicks as a final precaution.

Experimental Treatments

The experiment involved 6 different treatments over the 42 D rearing period as shown in Table 2.1.

Table 2.1 – Independent Variables assigned as treatment groups.

Treatment	Challenge	Reps	Birds/Rep	Total Birds
T1 - Negative Control (NC)	No vaccine, Corn/Soy diet	15	32	480
T2 - Positive Control (PC)	Cocci Vaccine + 5% DDGS	15	32	480
T3 - PC + Safmannan®	Cocci Vaccine + 5% DDGS	15	32	480
T4 - PC + Celmanax®	Cocci Vaccine + 5% DDGS	15	32	480
T5 - PC + MicroSaf®	Cocci Vaccine + 5% DDGS	15	32	480
T6 - PC + Calsporin®	Cocci Vaccine + 5% DDGS	15	32	480

- Treatment 1 served as a negative control consist of a corn/soy diet that did not undergo any of the GI stressors other that mild heat stress beginning at D22. It was included to represent birds raised with minimal GI stress other than the mild heat stress.
- Treatment 2 was the positive control treatment formulated with 5% DDGS which underwent GI stress from a double live coccidiostat vaccination at D 1 and D 7. It represents the appropriate control group for the remaining 4 probiotic and prebiotic treatments.
- Treatment 3 was supplemented with a proprietary yeast product (Safmannan®, Phileo® by Lesaffre®, Milwaukee, WI) at 1 lb/T.
- Treatment 4 was supplemented with a proprietary yeast product (Celmanax®, Arm & Hammer™, York, PA) at 1 lb/T.
- Treatment 5 was supplemented with a proprietary bacillus probiotic (MicroSaf®, Phileo® by Lesaffre®) at 1 lb/T.
- Treatment 6 was supplemented with a proprietary bacillus product (Calsporin®, Quality Technology International™, Inc, Elgin, IL) at 0.02 lb/T.

Products

All the products used were used in accordance with the product labeling. During the diet mixing process, the PC diet was separated into a PC diet, and the 4 treatment diets. The 4 treatment diets were then mixed again, with the appropriate additive concentration according to the product label instructions.

The T1 Negative Control treatment was statistically compared to the T2 Positive Control treatment to validate the broilers receiving the antibiotic alternative treatments were in fact challenged by the dietary addition of DDGS and live coccidiosis vaccination. These types of "challenge designs" are common "today" because previous research has shown little positive effects of prebiotics and/or probiotics given ideal rearing conditions with minimal GI stress.

Experimental Diets

The diets were provided over a four-phase feeding program (see Table 2.2) in accord with breeder recommended diets for Cobb 500 broilers (Cobb, 2018). The starter diet was used until day 10 and was based on a corn and soybean meal basal diet and did not receive the vaccine, nor DDGS, while the positive control, and the four treatments used a basal diet containing distillers dried grains (DDGS) as a gastrointestinal (GI) stressor. Prebiotics, and probiotics were added to the DDG containing basal diet. The grower, finisher 1 and finisher 2 diets for test and negative control groups were similarly reformulated compared to the positive control group diets.

The starter diet was used until D 10, after which the grower diet was used until D 21 when the Finisher 1 diet formulation was then introduced. At D 21, the pen populations were reduced to 26 birds each to achieve the desired 13 birds/m². On D 35 the Finisher 2 diets was introduced and used until termination of the birds on D 42. All birds were provided water *ad libitum* throughout the experiment.

Table 2.2 – Feed in pounds per phase

Feed in Pounds				
Type	**Starter**	**Grower**	**Finisher 1**	**Finisher 2**
Negative	550	1200	2550	1550
Positive	2750	6000	12750	7750
Total	3300	7200	15300	9300

Diet composition and mixing

The feed was mixed on site at the Poultry Science farm a week prior to each feeding phase. Manufactured feed was stored in barrels and placed in the house with birds until used. The feed was weighed using the same floor scale as was used with the birds prior to being placed in the pens on D 1. On subsequent weighing days, remaining feed was weighed before being discarded, and the new feed added.

Table 2.3 Diets for treatments and phases. All treatments except for the NC group are included in the PC diets

Diets in Percentages								
Type	Starter D 0-9 NC	Starter D 0-9 PC	Grower D 10-20 NC	Grower D 10-20 PC	Finisher 1 D 21-34 NC	Finisher 1 D 21-34 PC	Finisher 2 D 35-42 NC	Finisher 2 D 35-42 PC
Corn	61.598	57.375	64.159	60.927	69.106	64.341	70.035	65.269
SBM 48%	33.302	31.642	30.779	28.213	25.605	24.492	23.978	22.865
Soybean Oil	.703	1.659	1.038	1.857	1.462	2.481	2.168	3.188
Vitamin[1]	.250	.25	.25	.250	.25	.25	.25	.25
Trace Minerals[2]	.05	.050	.050	.050	.05	.05	.05	.05
Limestone	1.524	1.498	1.433	1.41	1.306	1.288	1.319	1.292
Mono-Dical	1.546	1.540	1.417	1.416	1.257	1.248	1.270	1.261
Salt	.337	.265	.339	.267	.224	.224	.181	.181
NaHCO3	0.0	0.0	0.0	0.0	.165	.064	.226	.124
L-Lysine	.227	.259	.179	.239	.214	.229	.203	.218
DL-Methionine	.227	.310	.278	.278	.273	.261	.249	.237
L-Threonine	.317	.148	.079	.093	.078	.072	.071	.066
DDGS	0.0	5.00	0.0	5.00	0.0	5.00	0.0	5.00

[1]Vitamin Premix provided by DSM Animal Nutrition and Health. [2]Per pound of premix; Cu: Copper minimum 1.40%, I: Iodine minimum 800.0ppm, FE: Iron minimum 12.00%, Mn: Manganese minimum 12.00%, Zn: Zinc minimum 12.00%, [2]Per pound of premix; Vitamin A: 4,000,000 IU, Vitamin D3: 1,400,000 IU, Vitamin E: 16,666 IU, Vitamin B12: 6mg, Riboflavin (B2): 2166mg, Niacin (B3): 16,666mg, d-pantothenic acid (B5): 7334mg, Choline: 47383mg, Menadione: 534mg, Folic acid (B9):634mg, Pyridoxine (B6): 2,600mg, Thiamine (B1): 1,066mg, d-Biotin (B7): 200mg

Dried Distiller's Grains (DDGS) were utilized for the PC, as well as for the diets supplemented with probiotics and prebiotics, but was not utilized for the NC. The NC was included to allow for an additional nutritional comparison between the PC and the 4 supplements which were undergoing the coccidiosis vaccine, and DDG nutrition stress, and the NC which was only undergoing a ventilation and heat stress. The DDGS did not restrict nutritional access to the birds but changed the nutritional composition of the diet and still allowed for bird growth (Alizedeh *et al.*, 2016).

Table 2.4 Dried Distillers Grain Composition

Nutrients	Actual (%)
Dry Matter	93.00
ME (kcal/kg)	2480
Crude Fiber	9.1
Protein	27.40
Methionine	0.60
Lysine	0.75
Calcium	0.17
Phosphorus	0.72
Non-Phytate Phosphorus	0.39
Sodium	0.48

Table 2.5 Nutrient concentrations per diet

Calculated Nutrient Content								
Diet	Starter NC	Starter PC	Grower NC	Grower PC	Finisher I NC	Finisher I PC	Finisher II NC	Finisher II PC
ME Kcal/Kg	2975	2975	3025	3025	3100	3100	3150	3150
Protein %	22.02	22.03	20.88	20.78	18.82	19.22	18.07	18.47
Calcium %	0.9	0.9	0.84	0.84	0.76	0.76	0.76	0.76
Phosphorus, Available %	0.45	0.45	0.42	0.42	0.38	0.38	0.38	0.38
Sodium %	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride %	0.28	0.25	0.27	0.25	0.21	0.22	0.18	0.19
Methionine %	0.62	0.65	0.6	0.6	0.567	0.567	0.53	0.53
Lysine %	1.35	1.35	1.24	1.24	1.12	1.13	1.07	1.08

Vaccination Stress

Broilers assigned to treatments 2-6 were exposed to a 2-part coccidiosis vaccination on D 1 and D 7 (Advent®, Sofia, Bulgaria) to apply an additional immune stress for the experiment. This vaccine contains strains of *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* and was administered through spray form onto the bird feed after they had been placed into their pens (vials were in 10,000 dose form and diluted into 2,500 ml water and sprayed onto feed).

Heat Stress

The day-old chicks were kept warm, over 90ºF until after D 10. Then the temperature was lowered to an average of 80ºF. A mild heat stress, 88° F, was applied on D 22. This temperature does not result in increased risk for heat related mortalities (See Figure 2.1) and should be considered as mild since it was only a 6° F average increase from the pre-heat stress phase. The data from the initial 21 days (pre-heat stress) were compared with the results of the data from the D 1-42 days which is a very common rearing period for commercial broilers.

Timeline

Table 2.6 – Schedule for activities by day

Schedule for Activities by Day	
Day	**Activity**
D 1	Body Weight / Feed/Sprayed vaccine
D 7	Body Weight / Feed/Sprayed vaccine
D 10	Body Weight / Feed / Change to Grower
D 14	Body Weight / Feed
D 21	Body Weight / Feed / Necropsy / Start Finisher 1
D 22	Started Heat Stress
D 28	Body Weight / Feed
D 35	Body Weight / Feed / Start Finisher 2
D 42	Body Weight / Feed / Necropsy /Terminate

Birds were weighed by pen using a floor scale. On D 1 the birds were brought in from the trucks and sorted by average weights to ensure the pen weights were within a between 1316 g and 1348 g range. Birds were weighed by placement in one of a set of 4 baskets that were pre-weighed and tared to the same weight. Any discrepancies were compensated for by adding strips of duct tape to the lighter baskets to equal the weight of the heaviest one.

The D 7, 10, 14, and 21 weights were also weighed by placing the birds into one basket per pen and then weighing them on a single floor scale. By D 28 the birds were too large to be weighed together, so the remaining birds in each pen were separated into 2 equal sized groups, weighed and group weights added together to provide a total pen weight.

Statistics

All data were analyzed as a one-way ANOVA using IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY:IBM Corp. This thesis focus is on 21 and 42 D cumulative production results (weight gain and feed conversion ratios) prior to heat-stress (D 0-21) and post heat stress (D 0-42). All data were first evaluated for outliers based on the work of John Tukey (Technote 1479545, NY:IBM Corp.) Outliers are defined as cases lying more than 1.5 box-lengths outside of the box, and extremes are cases lying more than 3 box-lengths distant. The D 21 Feed to Gain production data from Pen 37, Block 4 Treatment 1 NC was found to be greater than 10 box-lengths distant and therefore this pen was discarded for all further statistical analysis.

The data were first analyzed as a 2x6 factorial arrangement to look for treatment interactions between the cool pad (East) side of the house and the fan side (West) side. This was important to determine to check if the treatments responded differently relative to each other depending on relative pen location.

Data were next evaluated for successful GI stress by comparing the Treatment 1 NC group against the Treatment 2 PC group at D 21 prior to heat stress. This was important to ascertain our vaccination and dietary DDGS stressors significantly reduced 21 D cumulative weight gains versus the non-stress PC treatment. This comparison was also made for the 42 D post heat-stress data.

One-way ANOVA was next performed for treatments 2-6, NC and 4 antibiotic alternative products. Upon a significant F statistic ($p<0.05$) the treatment means were separated using the Duncan's multiple means comparative procedure also at ($p<0.05$). Production data were next transformed by creating ratios of each of the 4 product means to the Treatment 1 NC mean to help identify how each treatment related to the PC group which did not undergo the GI stress, just the heat-stress.

To evaluate production performance over time average pen replicates for weight gain and feed conversion were plotted on Days 7, 10, 14, 21, 28, 35 and 42 of the experiment (See Appendix).

CHAPTER III

RESULTS AND DISCUSSION

Evaluation of West versus East Location Interactions

Previous experiments within rearing barn #1211 have shown that birds usually perform better with respect to weight gain and feed efficiency if located on the East side of the barn versus the west side next to the exhaust fans. For this reason, it was important to evaluate if pen location affected how each treatment performed relative to each other. If the treatments responded differently on one side versus the other a factorial analysis accounting for this East West interaction would be important to consider regarding our statistical analysis.

Therefore, all treatments 1-6 were first evaluated as a 2x6 arrangement of treatments with East or West location representing the 2 main effects and response to the 6 treatments representing the other main effects variable. This interaction was not found to be significant at ether D 21 or D 42. While there were significant treatment effects on for both the East and West pens, the treatments all responded similarly relative to each other. For this reason, the data for this experiment was analyzed as a one-way ANOVA.

Evaluation of GI stress

A stress model was used in this experiment to better gauge the effects of the 4 alternatives to traditional antibiotic growth promoters to give us a better chance of seeing a potential improvement in productive performance versus the PC group. We wanted to evaluate each antibiotic alternative to the PC group to evaluate their effectiveness under GI stress. Ideally, a truly effective antibiotic alternative would perform at a similar level as the NC group which only received a mild heat stress beginning at D 21.

To evaluate the effectiveness of our GI stress model we compared the NC treatment 1 to the PC treatment 2 at D 21 and D 42. All the birds receiving the NC treatment received simple corn soy diets formulated to meet the Cobb 500 feeding guidelines over the 42 days of this experiment. The PC treatment 2 was similar in nutrient content as the NC diet but contained 5% DDG’s to provide a possible GI stress over and above the plain corn soy diets fed to the NC group. The remaining treatments 3 – 6 were compared with the PC control group for all future analysis. Groups T2 – T4 also received a double dose of attenuated live coccidiosis virus using a common commercial vaccine (Advent® from Huvepharma). This set of birds was vaccinated on both D 1 and D 7. Groups T3 -T6 received the 2 pre and 2 probiotics evaluated as antibiotic alternatives with respect to productive performance.

The results indicate we did achieve a GI stress in the 21 D birds as indicated by Tables 3.1. However, by D 42 after both NC and PC groups had been exposed to mild heat stress there was not a significant difference between the 2 control groups, as indicated by Table 3.2. The heat stress reduced performance similarly. This result is not unexpected as both groups were under the same relative heat stress from D 22 – 42.

Table 3.1 Evaluation of Cumulative Weight Gain per Bird (g) of the Negative (1) and Positive (2) Control Treatments at D 21

Between-Subjects Factors

		Value Label	N
Treatment	1	Negative Control	14
	2	Positive Control	15

Descriptive Statistics

Dependent Variable: Cumulative Weight Gain per Bird (g)

Treatment	Mean	Std. Deviation	N
Negative Control	1642.2629	239.51514	14
Positive Control	1611.4747	185.60109	15
Total	1626.3379	210.00939	29

Tests of Between-Subjects Effects

Dependent Variable: Cumulative Weight Gain per Bird (g)

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	10861.361[a]	1	10861.361	14.123	.001
Intercept	21390738.698	1	21390738.698	27814.165	.000
TRT	10861.361	1	10861.361	14.123	.001
Error	20764.597	27	769.059		
Total	21414561.188	29			
Corrected Total	31625.958	28			

a. R Squared = .343 (Adjusted R Squared = .319)

Table 3.2 Evaluation of Cumulative Weight Gain per Bird (g) of the Negative (1) and Positive (2) Control Treatments at D 42

Between-Subjects Factors

		Value Label	N
Treatment	1	Negative Control	14
	2	Positive Control	15

Descriptive Statistics

Dependent Variable: Cumulative Weight Gain per Bird

Treatment	Mean	Std. Deviation	N
Negative Control	1642.2629	239.51514	14
Positive Control	1611.4747	185.60109	15
Total	1626.3379	210.00939	29

Tests of Between-Subjects Effects

Dependent Variable: Cumulative Weight Gain per Bird

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	6864.195[a]	1	6864.195	.151	.701
Intercept	76663091.500	1	76663091.500	1685.526	.000
TRT	6864.195	1	6864.195	.151	.701
Error	1228046.234	27	45483.194		
Total	77939187.341	29			
Corrected Total	1234910.429	28			

a. R Squared = .006 (Adjusted R Squared = -.031)

Evaluation of the 4 Antibiotic Alternatives

Weight Gain

To determine if any of the 4 antibiotic alternatives could potentially be effective alternatives to traditional antibiotics each altenative was compared as individual groups with the PC group by ANOVA. Treatment means were then seperated using Duncan's mean seperation procedure. In Table 3.3, which depics D 21 resutls, MicroSaf, Safmannan and the PC were significantly higher than Celemax which had the lowest cumulative weight gain. Overall, the MicroSaf treatment had the highest cumulative weight gain of all the treatments at D 21.

Table 3.3 Evaluation of Cumulative Weight Gain per Bird (g) of 4 antibiotic alternatives at D 21

Descriptive Statistics

Dependent Variable: Cumulative Weight Gain per Bird (g)

Treatment	Mean	Std. Deviation	N
Positive Control	839.9900	28.51686	15
Safmannan	845.0667	37.14503	15
Celmanax	807.1360	19.52547	15
MicroSaf	852.0013	28.23960	15
Calsporin	823.3513	33.97525	15
Total	833.5091	33.51239	75

Tests of Between-Subjects Effects

Dependent Variable: Cumulative Weight Gain per Bird (g)

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	19743.941[a]	4	4935.985	5.453	.001
Intercept	52105302.316	1	52105302.316	57562.184	.000
TRT	19743.941	4	4935.985	5.453	.001
Error	63364.016	70	905.200		
Total	52188410.273	75			
Corrected Total	83107.957	74			

a. R Squared = .238 (Adjusted R Squared = .194)

Cumulative Weight Gain per Bird (g)

Treatment	N	Subset		
		1	2	3
Celmanax	15	807.1360		
Calsporin	15	823.3513	823.3513	
Positive Control	15		839.9900	839.9900
Safmannan	15		845.0667	845.0667
MicroSaf	15			852.0013
SIG		.144	.065	.308

By D 42 the cumulative weight gains the prebiotic Safmannan and Celmanax had the highest means, but also had the highest standard deviations. This showed less consistency with the results, but a higher overall yield. There was no significant difference regarding this analysis.

Table 3.4 Evaluation of Cumulative Weight Gain per Bird (g) of 4 antibiotic alternatives at D 42

Descriptive Statistics

Dependent Variable: Cumulative Weight Gain per Bird (g)

Treatment	Mean	Std. Deviation	N
Positive Control	1611.4747	185.60109	15
Safmannan	1653.2127	182.65152	15
Celmanax	1630.8533	183.44639	15
MicroSaf	1613.0453	179.55883	15
Calsporin	1623.4627	212.92286	15
Total	1626.4097	184.67574	75

Tests of Between-Subjects Effects
Dependent Variable: Cumulative Weight Gain per Bird (g)

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	17227.371[a]	4	4306.843	.120	.975
Intercept	198390646.551	1	198390646.551	5540.417	.000
TRT	17227.371	4	4306.843	.120	.975
Error	2506552.130	70	35807.888		
Total	200914426.052	75			
Corrected Total	2523779.501	74			

a. R Squared = .007 (Adjusted R Squared = -.050)

The data was then analyzed for a pre heat weight gain (D 1-21) in Table 3.3. From D 1-21 MicroSaf had the highest cumulative growth rate of any of the other treatments, however there was no significant differences by D 42 (Table 3.4).

Feed to Gain Ratio

The D 21 analysis of the feed to gain ratio of the treatments showed MicroSaf as having the significantly better feed to gain ratio when compared against the Celmanax treatment (which was the lowest performing treatment) and the PC by D 21 (Table 3.5). These results did not remain consistent when compared to D 42.

At D 42 the MicroSaf, Calsporin, and Safmannan were all significantly better performing than the Celmanax treatment (Table 3.6).

Table 3.5 D 21 feed to gain ratio of treatments

Descriptive Statistics

Dependent Variable: Cumulative Feed to Gain Ratio

Treatment	Mean	Std. Deviation	N
PC	1.2980	.04632	15
MicroSaf	1.2853	.03758	15
Celmanax	1.3627	.02658	15
MicroSaf	1.2600	.04326	15
Calsporin	1.2873	.04949	15
Total	1.2987	.05310	75

Tests of Between-Subjects Effects

Dependent Variable: Cumulative Feed to Gain Ratio

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	.088[a]	4	.022	12.880	.000
Intercept	126.490	1	126.490	73663.139	.000
TRT	.088	4	.022	12.880	.000
Error	.120	70	.002		
Total	126.699	75			
Corrected Total	.209	74			

a. R Squared = .424 (Adjusted R Squared = .391)

Cumulative Feed to Gain Ratio

Treatment	N	Subset 1	2	3
MicroSaf	15	1.2600		
Safmannan	15	1.2853	1.2853	
Calsporin	15	1.2873	1.2873	
PC	15		1.2980	
Celmanax	15			1.3627
SIG		.092	.436	1.000

Table 3.6 D 42 cumulative feed to gain ratio of treatments

Descriptive Statistics

Dependent Variable: Cumulative Feed to Gain Ratio

Treatment	Mean	Std. Deviation	N
PC	1.8080	.08326	15
Safmannan	1.7587	.08879	15
Celmanax	1.8527	.04590	15
MicroSaf	1.7687	.04103	15
Calsporin	1.7660	.03334	15
Total	1.7908	.07071	75

Tests of Between-Subjects Effects

Dependent Variable: Cumulative Feed to Gain Ratio

Source	Type III Sum of Squares	Df	Mean Square	F	SIG
Corrected Model	.094[a]	4	.023	5.954	.000
Intercept	240.522	1	240.522	60993.205	.000
TRT	.094	4	.023	5.954	.000
Error	.276	70	.004		
Total	240.892	75			
Corrected Total	.370	74			

a. R Squared = .254 (Adjusted R Squared = .211)

Cumulative Feed to Gain Ratio

		Subset	
Treatment	N	1	2
Safmannan	15	1.7587	
Calsporin	15	1.7660	
MicroSaf	15	1.7687	
PC	15	1.8080	1.8080
Celmanax	15		1.8527
SIG		.052	.055

Ratio of Treatments to Negative Control

When reviewing the cumulative weight gain to NC ratio for D 21 the MicroSaf and Safmannan had the highest cumulative weight gain ratios when compared to the NC. The Celmanax maintained a significantly lower cumulative weight gain ratio through this period.

On D 42 there was no significant differences between the treatments (See Table 3.8).

Table 3.7 Cumulative weight gain ratios to NC at D 21

Descriptive Statistics

Dependent Variable: cumulative weight gain ratio to NC

Treatment	Mean	Std. Deviation	N
PC	.95773	.048479	15
MicroSaf	.96329	.051302	15
Celmanax	.92011	.037282	15
Safmannan	.97092	.036042	15
Calsporin	.93870	.051729	15
Total	.95015	.047969	75

Tests of Between-Subjects Effects

Dependent Variable: Cumulative weight gain ratio to NC

Source	Type III Sum of Squares	Df	Mean Square	F	SIG
Corrected Model	.025[a]	4	.006	3.071	.022
Intercept	67.709	1	67.709	32719.418	.000
TRT	.025	4	.006	3.071	.022
Error	.145	70	.002		
Total	67.879	75			
Corrected Total	.170	74			

a. R Squared = .149 (Adjusted R Squared = .101)

Cumulative weight gain ratios to NC

Treatment	N	Subset 1	Subset 2
Celmanax	15	.92011	
Calsporin	15	.93870	.93870
PC	15		.95773
MicroSaf	15		.96329
Safmannan	15		.97092
SIG		.267	.080

Table 3.8 Cumulative weight gain ratios to NC at D 42

Descriptive Statistics

Dependent Variable: Cumulative weight gain ratios to NC

Treatment	Mean	Std. Deviation	N
PC	.99417	.080843	15
Safmannan	1.01911	.067381	15
Celmanax	1.00512	.062501	15
MicroSaf	.99403	.060876	15
Calsporin	.99847	.063078	15
Total	1.00218	.066162	75

Tests of Between-Subjects Effects

Dependent Variable: Cumulative weight gain ratio to NC

Source	Type III Sum of Squares	Df	Mean Square	F	SIG
Corrected Model	.007[a]	4	.002	.364	.834
Intercept	75.328	1	75.328	16616.185	.000
TRT	.007	4	.002	.364	.834
Error	.317	70	.005		
Total	75.651	75			
Corrected Total	.324	74			

a. R Squared = .020 (Adjusted R Squared = -.036)

Cumulative weight gain ratios to NC

Treatment	N	Subset 1
MicroSaf	15	.99403
PC	15	.99417
Calsporin	15	.99847
Celmanax	15	1.00512
MicroSaf	15	1.01911
SIG		.373

Regarding FCR, all the treatments were significantly better performing than the Celmanax treatment (Table 3.9).

Table 3.9 FCR to NC Ratios at D 21

Descriptive Statistics

Dependent Variable: Cumulative FCR to NC Ratios

Treatment	Mean	Std. Deviation	N
PC	1.02081	.049569	14
Safmannan	1.02015	.032049	14
Celmanax	1.07621	.038306	14
MicroSaf	.99560	.034987	14
Calsporin	1.01990	.047739	14
Total	1.02653	.048079	70

Tests of Between-Subjects Effects

Dependent Variable: Cumulative FCR to NC Ratios

Source	Type III Sum of Squares	Df	Mean Square	F	SIG
Corrected Model	.050[a]	4	.012	7.331	.000
Intercept	73.764	1	73.764	43623.370	.000
TRT	.050	4	.012	7.331	.000
Error	.110	65	.002		
Total	73.923	70			
Corrected Total	.159	69			

a. R Squared = .311 (Adjusted R Squared = .268)

Cumulative FCR to NC Ratios at D 21

Treatment	N	Subset 1	Subset 2
MicroSaf	14	.99560	
Calsporin	14	1.01990	
Safmannan	14	1.02015	
PC	14	1.02081	
Celmanax	14		1.07621
SIG		.144	1.000

By D 42 the FCR to NC ratio for Safmannan was better than for Celmanax (P=0.140). Duncan's means separation depicted this difference as significant.

Table 3.10 FCR to NC Ratios at 42 D

Descriptive Statistics

Dependent Variable: FCR Ratio to NC

Treatment	Mean	Std. Deviation	N
Positive Control	1.03126	.089360	14
Safmannan	1.00171	.039967	14
Celmanax	1.05173	.054211	14
MicroSaf	1.00980	.045208	14
Calsporin	1.00674	.049542	14
Total	1.02025	.059676	70

Tests of Between-Subjects Effects

Dependent Variable: FCR Ratio to NC

Source	Type III Sum of Squares	df	Mean Square	F	SIG
Corrected Model	.024[a]	4	.006	1.797	.140
Intercept	72.863	1	72.863	21405.535	.000
TRT	.024	4	.006	1.797	.140
Error	.221	65	.003		
Total	73.109	70			
Corrected Total	.246	69			

a. R Squared = .100 (Adjusted R Squared = .044)

FCR Ratio to NC

Treatment	N	Subset	
		1	2
Safmannan	14	1.00171	
Calsporin	14	1.00674	1.00674
MicroSaf	14	1.00980	1.00980
Positive Control	14	1.03126	1.03126
Celmanax	14		1.05173
SIG		.229	.066

CHAPTER IV

SUMMARY AND CONCLUSIONS

The hypothesis, and goal of this experiment was to determine if prebiotics and/or probiotics can be effective alternatives to the use of antibiotics as growth promoters in broilers. The reason was to find an efficient replacement that accommodates the regulations and laws have been put in place to further remove the use of supplemental antibiotics for use as growth promoters (Penaloza-Vazquez *et al.*, 2019).

First, all treatments 1-6 were evaluated as a 2x6 arrangement of treatments with East or West location representing the 2 main effects and response to the 6 treatments representing the other main effects variable. The interaction was not found to be significant at ether D 21 or D 42. There were significant treatment effects on for both the East and West pens, but the treatments all responded similarly relative to each other. Therefore, the data for this experiment was analyzed as a one-way ANOVA.

A stress model was used in this experiment to better gauge the effects of the 4 alternatives to traditional antibiotic growth promoters. The NC which did not undergo the vaccination or DDGS challenges, performed significantly better than the PC which undergo these challenges. This validated the use of the experimental model.

Cumulative weight gain was significantly better at D 21 for Microsaf versus Calsporin and Celmanax. At D 42 there was no significant differences in cumulative weight gain.

Cumulative feed to gain ratios were significantly better at D 21 for Microsaf compared to all the other treatments. Celmanax was the poorest of all the treatments on D 21. On D 42 Safmannan, Calsporin, and Microsaf performed significantly better than Celmanax.

For cumulative weight gain ratios to NC on D 21 the Safmannan was the best, with similar results from Microsaf, and the PC. Celmanax performed worse than the than all treatments except Calsporin. There was no significant difference on D 42.

For the FCR to NC ratio Microsaf performed better, but similarly to the Calsporin, Safmannan, and the PC on D 21. Celmanax was significantly poorer than the other treatments. On D 42 Safmannan performed significantly better than Celmanax, but similarly to the other treatments.

Based on this data Safmannan, Calsporin, and Microsaf are all viable alternatives to the use of antibiotics for growth promotion.

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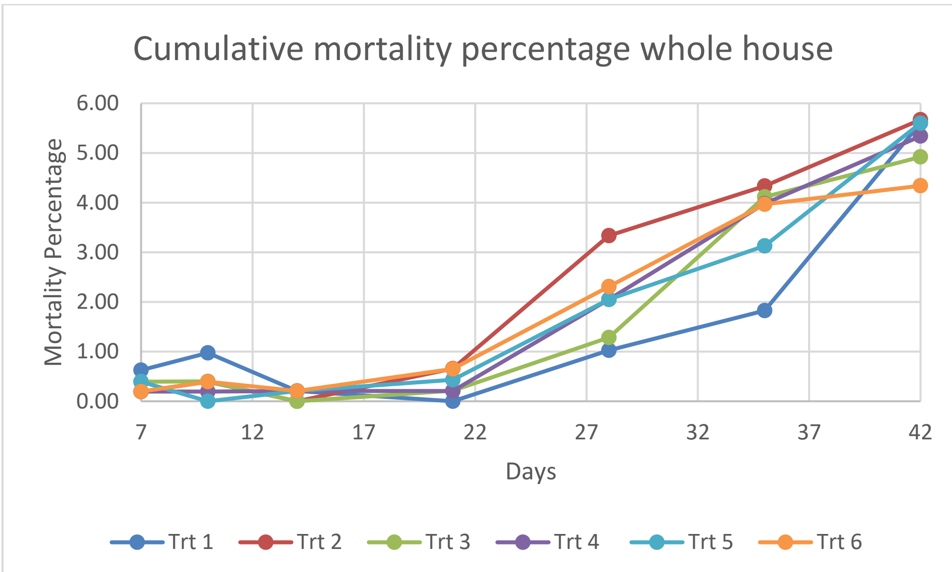
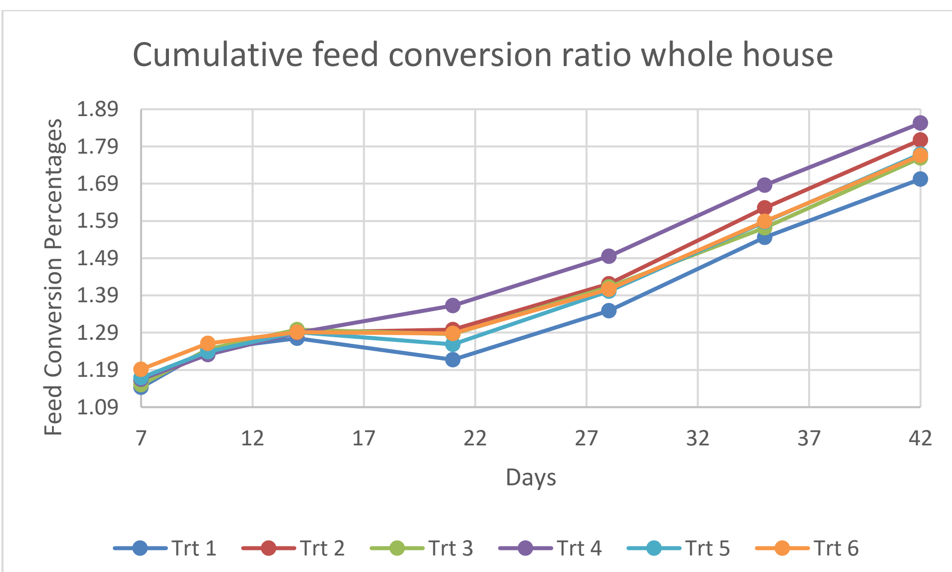
APPENDIX A

ADDITIONAL SUPPORTING TABLES AND GRAPHS

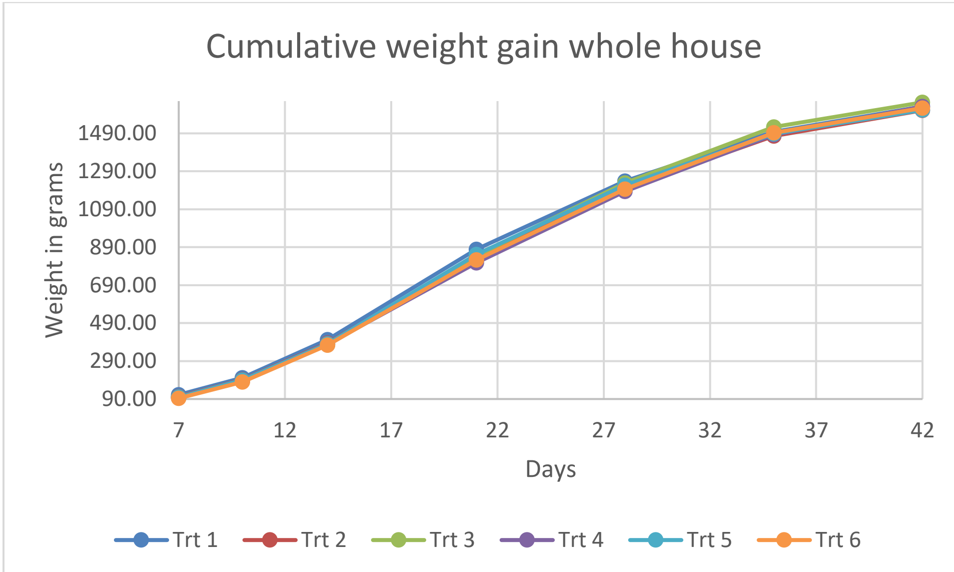
Graph A.1 Cumulative mortality percentage of the whole house



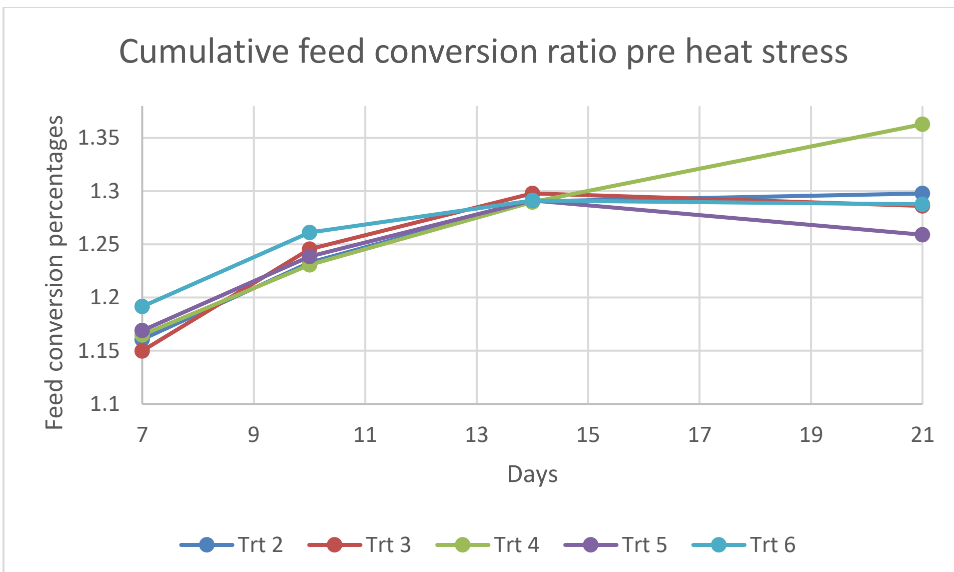
Graph A.2 Cumulative feed conversion ratio for the whole house



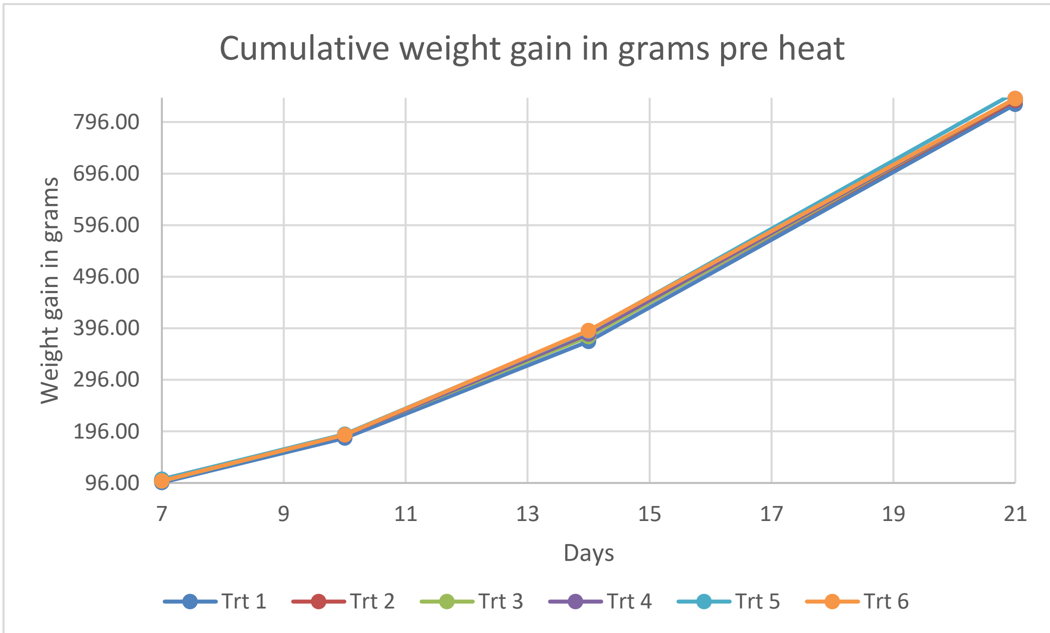
Graph A.3 Cumulative weight gain for the whole house



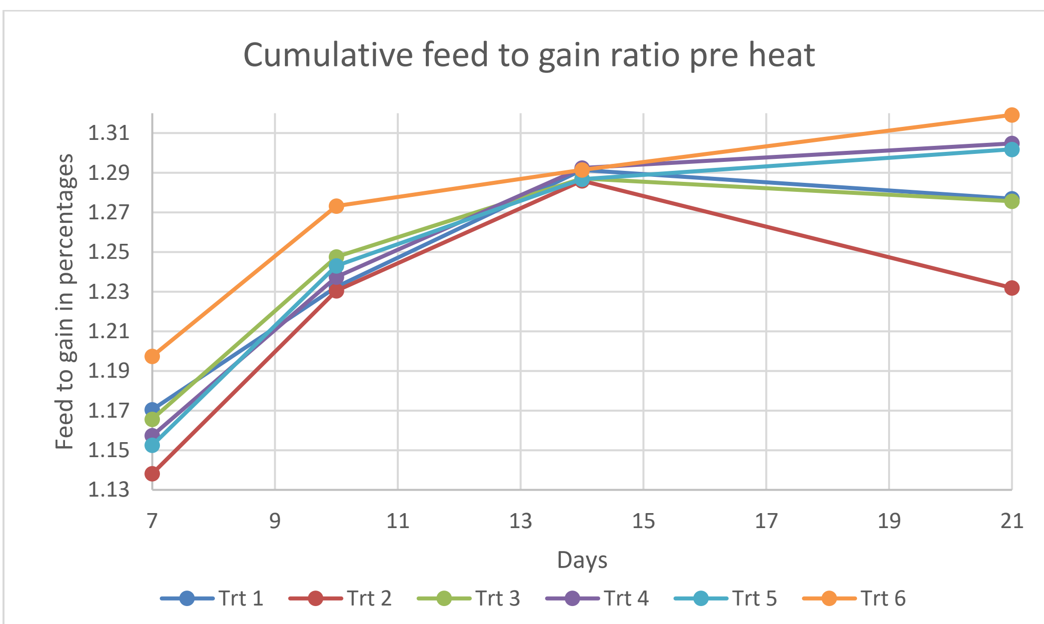
Graph A.4 Cumulative feed conversion ratio pre heat stress



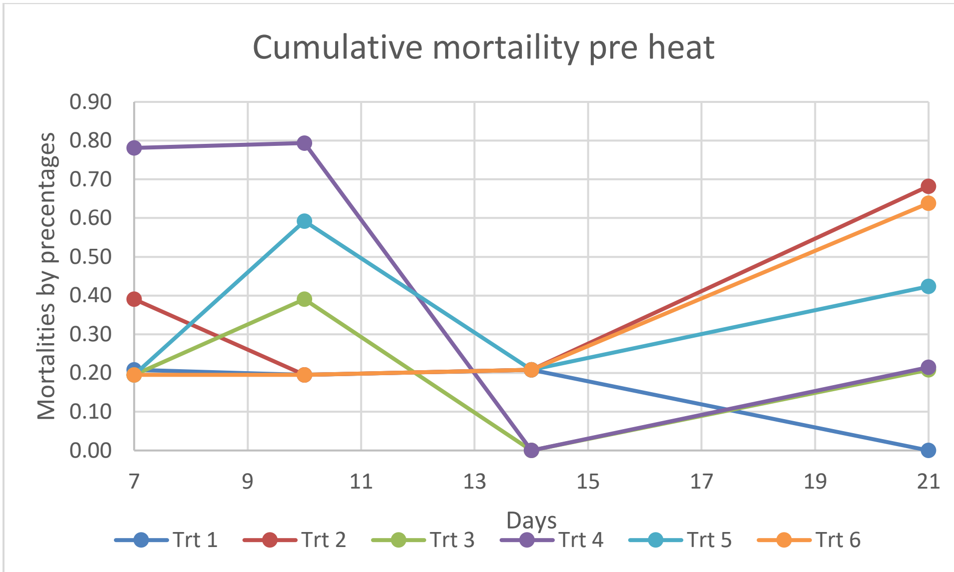
Graph A.5 Cumulative weight gain in grams pre heat



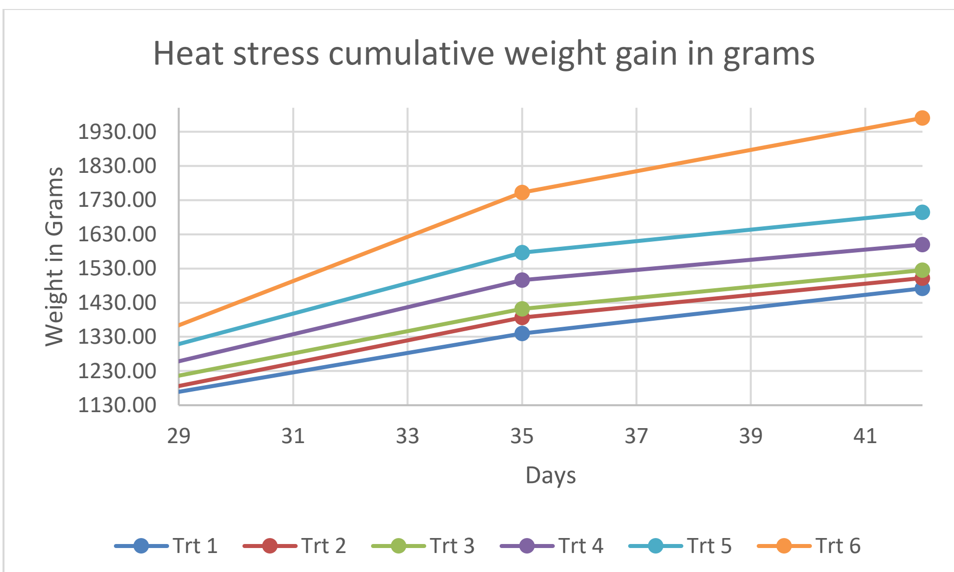
Graph A.6 Cumulative feed to gain ratio pre heat



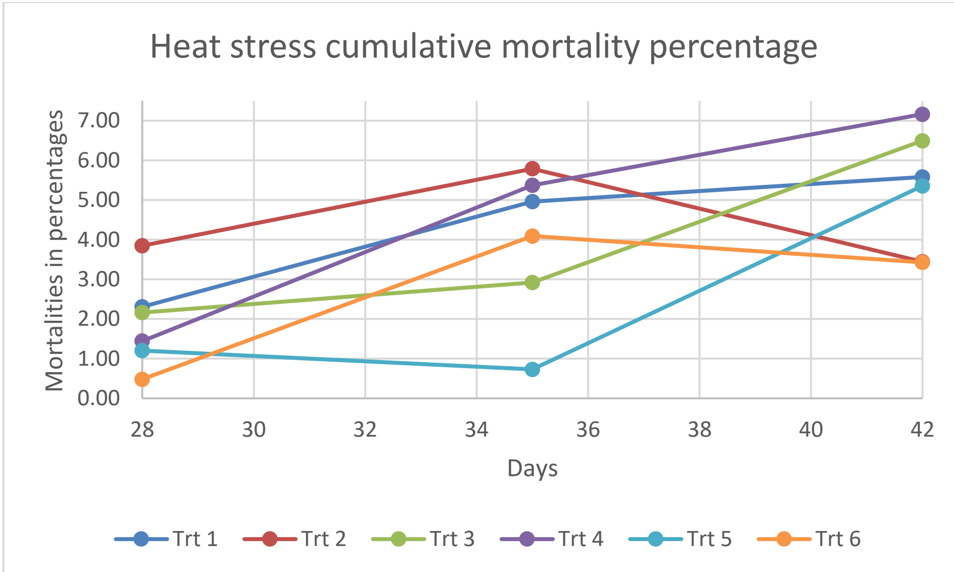
Graph A.7 Cumulative mortality pre heat



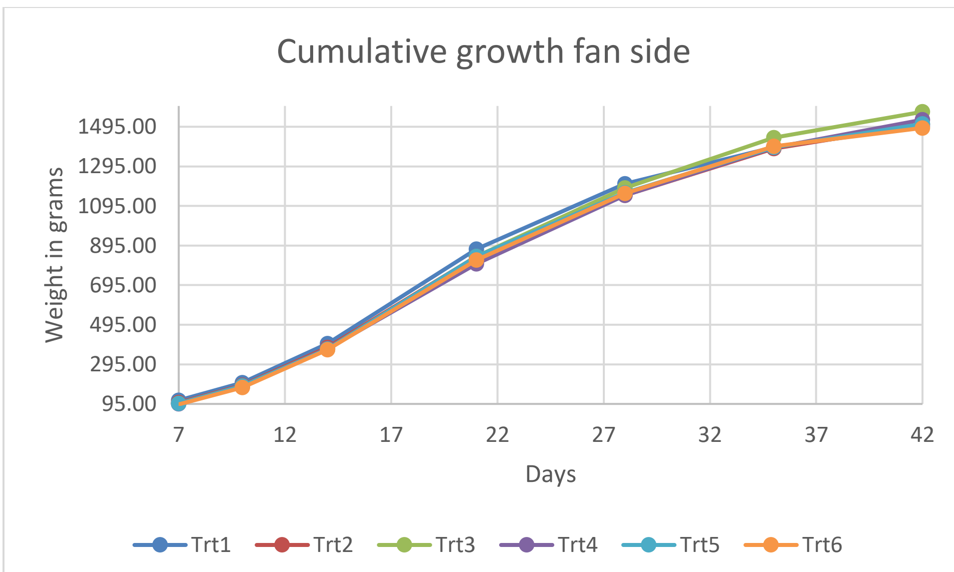
Graph A.8 Heat stress cumulative weight gain in grams



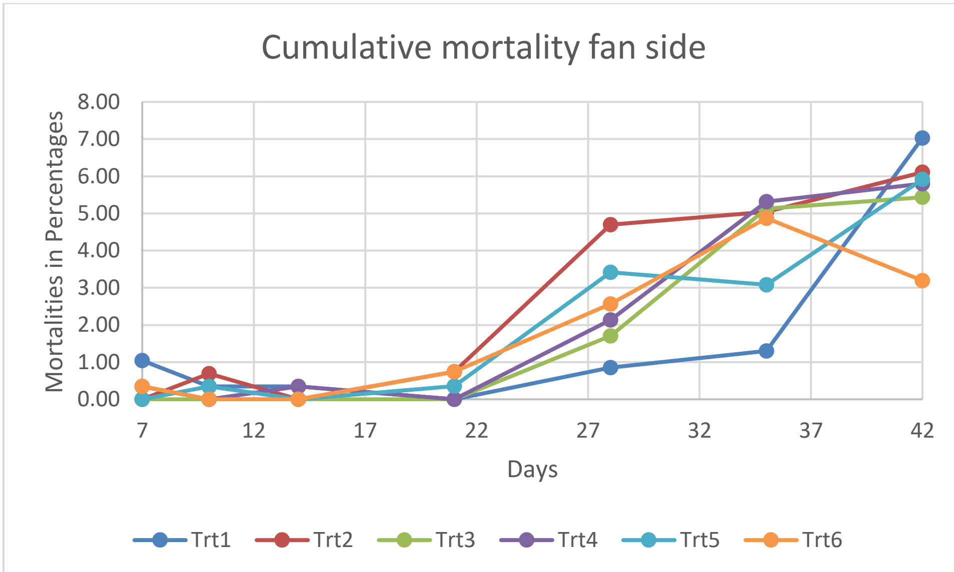
Graph A.9 Heat stress cumulative mortality percentage



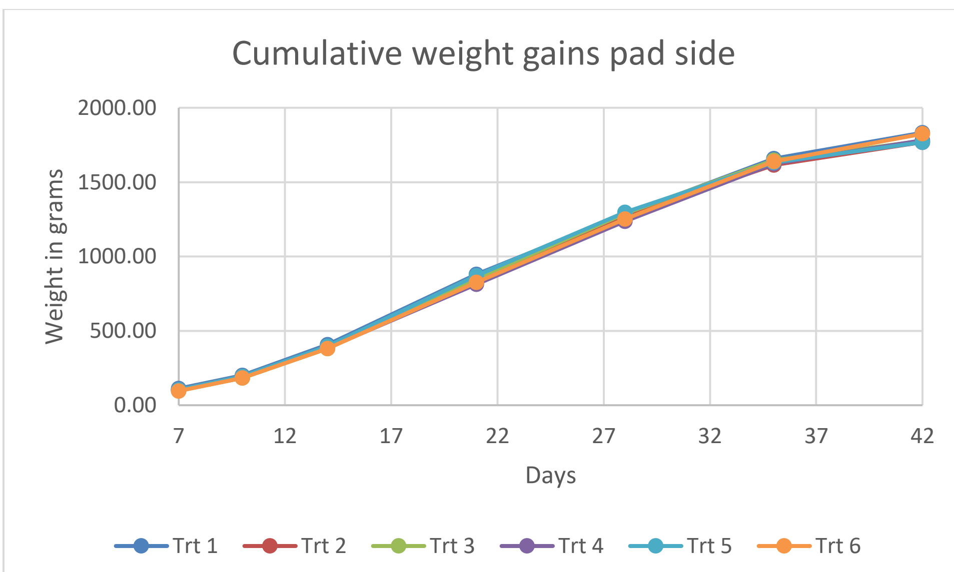
Graph A.10 Cumulative growth fan side



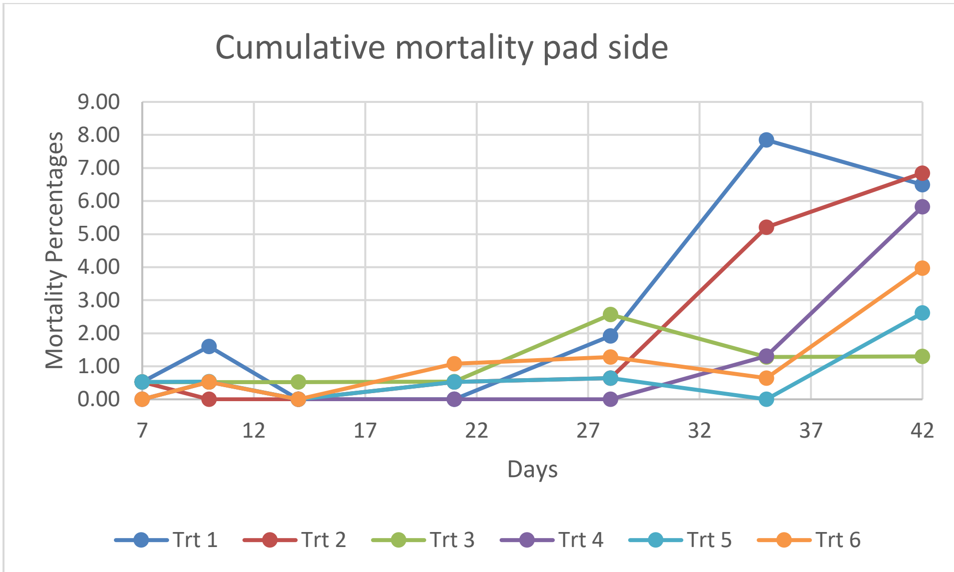
Graph A.11 Cumulative mortality fan side



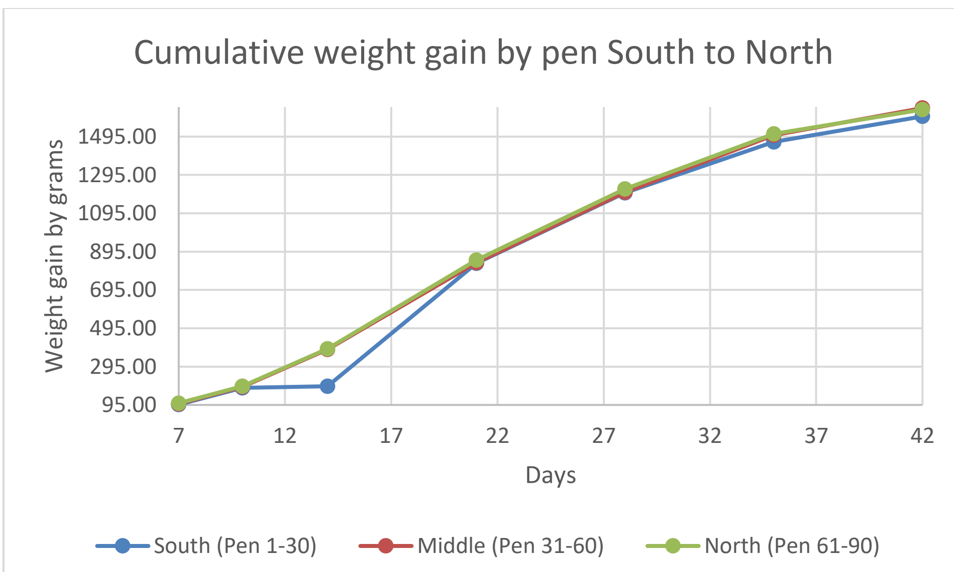
Graph A.12 Cumulative weight gains pad side



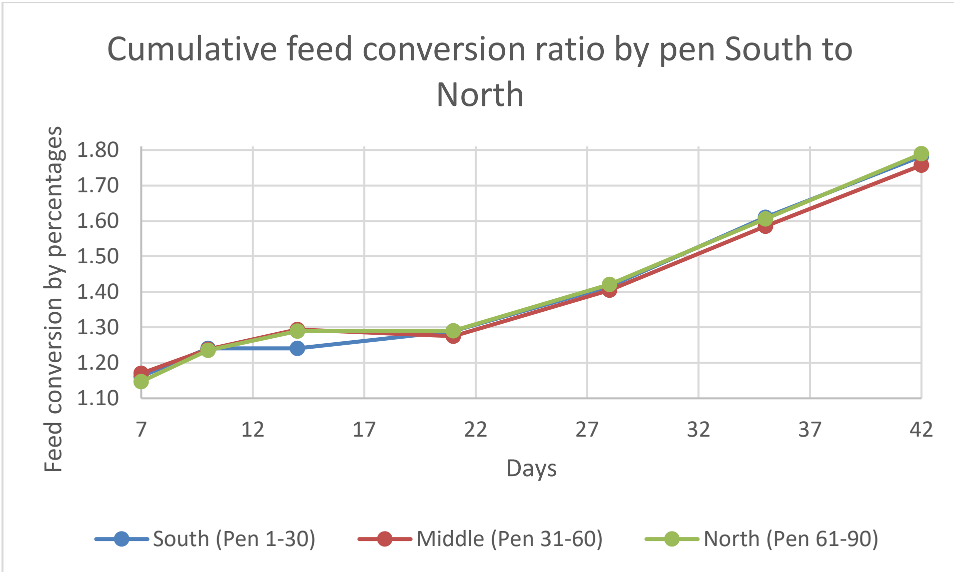
Graph A.13 Cumulative mortality pad side



Graph A.14 Cumulative weight gain by pen South to North



Graph A.15 Cumulative feed conversion ratio by pen South to North



Graph A.16 Cumulative mortality percentage by pen South to North