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Potential Value of Brazzein-Yeast as a Broiler Chicken Feed Additive

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POTENTIAL VALUE OF BRAZZEIN-YEAST AS A BROILER CHICKEN FEED ADDITIVE

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43 Pages

A variety of flavors, including sweeteners, have been used in animal feeds to improve feed palatability and increase feed intake by livestock (Chen et al., 2020). While many of these sweeteners are found in the form of carbohydrates, brazzein is a sweet-tasting protein. This study was conducted to identify the potential of brazzein to influence feed intake by livestock, using poultry as a model.

The preliminary study's purpose was to develop methods appropriate for the subsequent feeding study. In the preliminary study, eight pens of five birds were fed one of four treatment diets: Control or the Control plus one of three yeast preparations (baker’s yeast, dry yeast, and wet yeast; included at a rate of 1.5%). Feed and water intake were measured daily; breast circumference, pelvis width, and back length of each bird were also measured daily. Following euthanasia on day 12, liver weight, heart weight, and breast weight were recorded from each bird.

The Control group had greater average daily feed intake (ADFI; $p < 0.05$), suggesting the control diet was a more palatable than those containing yeasts. Breast circumference and breast weight were the highest in the Control group ($p < 0.05$, $p < 0.04$, respectively); this group also had the highest feed intake. Other parameters measured (pelvis width, back length, heart weight, or liver weight) were not affected by treatment diet, suggesting that feed intake has less of an effect on those physiological measurements compared to breast circumference and weight.

In the second trial, nine groups of three birds were fed one of three treatment diets: CON, YEA, or BRA. The CON diet was a commercially available poultry starter feed; the YEA diet was the control diet plus a preparation of saccharomyces cerevisiae included at a rate of 1.5%. In the third diet (BRA), saccharomyces cerevisiae yeast was modified to include the brazzein gene. Yeast was then grown, dried, and incorporated into the control diet at a rate of 1.5%.

Feed and water were offered ad libitum; intake of each was measured daily. Breast circumference, pelvis width, and back length of each bird were also measured daily. Following euthanasia on day 21, liver weight, heart weight, breast weight, crop weight, and small intestine weight and length were recorded from each bird. No differences were identified between any of the diets for feed intake, water intake, breast circumference, pelvic width, back length, heart weight, breast weight, liver weight, small intestine length, or small intestine weight. Crop weight was significantly less for the birds on BRA ($p < 0.02$), compared to those on CON or YEA. Results from this research suggest feeding yeast containing brazzein to growing broilers does not affect feed intake or growth performance. This is the first study in which brazzein-containing yeast was fed to livestock. Additional research investigating the impact of brazzein-yeast inclusion rates on palatability, intake, and performance is warranted.

KEYWORDS: palatability; sweetener; protein; feed intake; breast weight; body measurements

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L. R. N.

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CHAPTER I: INTRODUCTION

A variety of flavors, including sweeteners, have been used in animal feeds as a means of improving feed palatability and increasing feed intake by livestock (Chen et al., 2020). This practice has been useful in stimulating feed intake during critical periods (post-weaning or when the animal is not feeling well) or when less-palatable ingredients have been included in the diet. Several livestock species show a moderate to strong preference for sweet flavors. Due to the relatively low cost of high-intensity sweeteners (saccharin, sucralose, aspartame, neotame, etc.), research has investigated the potential of these compounds to improve palatability and feed intake. Inconsistent results across species suggest more research is needed to identify appropriate sweetening agents and inclusion rates. Some research suggests these sweeteners may have additional beneficial effects on intestinal development and inflammation-modulation, as well as gut microbiota. Therefore, there is interest in developing alternative sweetening compounds that could be used in human food or animal feeds.

Brazzein is a small protein found in the West African plant *Pentadiplandra brazzeana*. For centuries, it has been used by Indigenous people as a sweetener for food and drink. This small protein contains only 54 amino acid residues and is one of approximately six sweet-tasting proteins. Brazzein is one of the sweetest of these proteins, being approximately 500-2000 times sweeter than sucrose (Assadi-Porter et al., 2000). Research has demonstrated that brazzein is especially heat stable and tolerant of a pH range of 2.5 to 8, making it of interest in human foods and animal feed applications (Ming and Hellekant, 1994). Moreover, modification of the amino acid composition (by cloning methodology) of brazzein may further enhance the sweetness of the small protein; genetic researchers are currently working to identify the ‘sweetest’ version of brazzein.

In addition to genetically modifying brazzein to increase its sweetness, molecular biologists have been able to incorporate the brazzein gene into a rapidly reproducing yeast. Rather than incorporating the *Pentadiplandra brazzeana* plant in human food or livestock feeds, the brazzein-containing yeast, which can be grown quickly and processed efficiently, can be included in food/feeds.

The objectives of the studies reported in the following pages were to 1) identify appropriate methodology and ideal yeast strain for use in a broiler feeding trial and 2) evaluate the effect of brazzein-containing yeast on feed intake and broiler chicken performance.

Due to its novelty, little information exists on the effect of incorporating brazzein in livestock diets or its potential to improve feed palatability. Feed intake, influenced by palatability, has been identified as a critical factor in determining growth rate of several livestock species. Therefore, identifying the effect of brazzein-yeast, using poultry as a model, is an important initial step in understanding the potential value of brazzein-yeast in the livestock industry.

CHAPTER II: LITERATURE REVIEW

Feed additives are often defined as non-nutritive substances that are used in livestock diets with the purpose of improving production performance and maintenance of good health (Pond and Church, 2005). This may include improvements in feed intake, growth rates, feed efficiency, diet digestibility, or gut health or performance parameters such as milk, egg, or meat production. As a result of their beneficial impact on feed palatability and feed intake, high-intensity sweeteners have been used as feed additives for several years to improve animal health, efficiency, and performance.

Sweeteners

Taste plays a more important role in livestock animals than humans (Lohmann, 2000), increasing possibility of influencing the taste of the feedstuffs positively is using sweeteners. Sucrose, a sugar found in some plants, has been used widely in the livestock industry to improve feed palatability and drive feed consumption. Because of its relatively high cost, several high intensity sweeteners (HIS) have been developed, including acesulfame potassium, advantage, aspartame, neotame, saccharin, and sucralose (FDA, 1999). Additionally, there are two natural sweeteners (steviol clycoside and neohesperidin) allowed in livestock feeds.

These sweeteners are several hundred to several thousand times sweeter than sucrose, thus only small amounts of HIS additives are required to be added to the diet, allowing more space in the diet for other essential nutrients. These sweeteners are approved for use within the European Union or have received “GRAS” status in the United States.

Results from studies evaluating the effect of these sweeteners in animal feed intake and performance are variable; several studies supplementing dietary sweeteners have demonstrated an increased feed intake and performance in pigs, cattle, and goats. Sterk et al., 2008 reported

that the inclusion of dietary sweeteners prevented the depression of feed intake at approximately one-week post-weaning in piglets. Researchers noted that at 12 d post-weaning, pigs fed the dietary sweetener had an increased percentage of feed visits that included feed intake, suggesting that the piglets were more interested in feed consumption rather than performing exploratory behaviors.

In a series of studies, Zhang et al., 2020 found that weaned pigs preferred a diet supplemented with sucralose. When sucralose was fed at a rate of 0, 75, 150, 225, and 300 mg/kg sucralose, pigs receiving 150 mg/kg had a higher ADG and ADFI relative to the remaining treatments. The observed decrease in ADG and ADFI associated with the 225 and 300 mg/kg treatments was attributed to the decreased palatability associated with the higher level of dietary sweetener.

Han et al., 2019 evaluated the effects of stevioside on feed intake of goats. Supplementation with this natural sweetener at a rate of 400 mg/kg and 800 mg/kg increased feed intake of both the forage component and total diet. Initial research in cattle showed an increase in dry-matter intake (DMI) when stressed receiving calves were supplemented with 194 mg Sucram/kg, a similar HIS (Brown et al., 2004). However, subsequent studies have shown inconsistent results. McMeniman et al., 2006 was not able to detect a difference in DMI when receiving calves were fed a diet with or without 200 mg Sucram/kg. When the calves moved to the finishing phase there was only a tendency for Sucram-supplemented cattle to have great DMI.

Currently available HIS show variable results, suggesting they can be useful in specific situations. However, researchers are continuing to look for other sweetening alternatives that yield consistently beneficial results.

Brazzein

While carbohydrates are typically used as sweetening agents, several sweet-tasting proteins have also been identified. These sweet tasting proteins include miraculin (1968), monellin (1972), thaumatin (1972), mabinlin (1983), curculin (1995), and brazzein (1994). The sweetness of each varies, but ranges from 500 to 100,000 times sweeter than sucrose and is dependent on temperature and pH (Gibbs, 1996).

Brazzein is the smallest and one of the sweetest of the six sweet proteins that have been identified thus far (Hellekant and Danilova, 2005). This protein is isolated from fruit of the *Pentadiplandra brazzen* ballion, found in West Africa (Chung et al., 2018). It was commonly used to sweeten human foods in local communities.

Brazzein composed of a single chain of 54 amino acids. Of the six sweet proteins, brazzein is the most stable at high temperatures and over a wide range of pH, making it more useful in food and feed production applications. It has been reported to be 500 – 2000 times sweeter than sucrose (Assadi-Porter et al., 2000)

In addition to being sweet tasting, Brazzein has antioxidant, anti-inflammatory, and anti-allergic activities (Chung et al., 2018), leading to further benefits as a feed additive in poultry diets. Brazzein can be considered a natural high-intensity sweetener, high-intensity sweeteners have intense sweetness at low doses with little to no calories (Chen et al., 2020). High-intensity sweeteners have been previously used in livestock feed; Chen et al., 2020, discovered that high-intensity sweeteners in broiler diets act more as an immunomodulator rather than a sweetener.

Yeast

Yeast and its derived products are potential feed additives because of their beneficial impacts on poultry, such as acting as probiotics and prebiotics, modulates growth performance

and enhances gut development (Bilal et al., 2021). Saccharomyces cerevisiae, one of the most widely commercialized types of yeast, has long been fed to animals (Zhang et al., 2005). The addition of yeast in broiler chicken diets varies in form and includes granules, yeast cell walls, yeast extract, whole yeast, live yeast, dried, etc. The addition of yeast to broiler chicken diets tends to vary upon the study, potential growth improvements and carcass characteristics are not the same across research. Kim et al. (2002) reported that the supplementation of live yeast cells did not affect overall growth parameters or carcass meat yield. Varied results suggest that more research should be conducted.

Poultry

Early studies identified a lack of taste buds in the chicken. Twenty-four tastebuds were identified in early work. However, in more recent work, Liu et al. (2018) identified 240-360 taste buds in the chicken, with broilers having higher number of taste buds compared to layer-type breeds. Kudo et al., 2008 showed the number of tastebuds remains stable from one day to 140 d of age. While this is fewer tastebuds than other livestock and companion species (25,000 in cattle, 19,000 in swine, and 1,700 in dogs) it provides evidence that palatability may affect feed intake by birds. Therefore, improved palatability may improve bird health and performance. Taste-related genes have been detected in 21-day boilers at different expression levels in the palate, tongue, ventriculus, duodenum, jejunum, ileum, cecum, and colon (Cheled-Shoval, et al. 2015).

In poultry, olfactory, gustatory, and visual cues are among the most important senses utilized in the acquisition of feeds (Roura et al., 2013). Several studies have indicated poultry can identify differences in feed flavor (Balog and Millar, 1989; Sawamura et al, 2015, Yoshida et al., 2015). It is proposed that chickens have taste receptors for sweet, umami, bitter, salty, sour, and fatty acid flavors (Yoshida et al., 2022). Studies specifically investigating the influence of sweet

flavor on intake and performance have yielded varying results. In a preference test comparing glucose solution, sucrose solution, and plain water, chickens showed no preference (Urata et al., 1992; Cheled-Shoval et al., 2017). Similarly, work by Shi and Zhang (2006) showed a lack of T1R2 receptors (receptors that are responsible for sensing sweet tasting substances). In other work, chickens were able to discriminate between sweet stimuli and water (Ganchrow et al, 1990) and had a preference for moderate concentrations of glucose (Brindley et al., 1965) and significantly rejected solutions containing a high sucrose concentration (Cheled-Shoval et al., 2017) This data suggests the ability to sense sweetness may be present in chickens.

The presence of umami receptors has been well documented. Baldwin et al. (2014) has suggested that ancestral umami receptor has been repurposed in hummingbirds to function as a carbohydrate receptor. This may help explain their difference in ability to detect sweetness in other avian species.

Other Roles of Feed Additive (decreased use of antibiotics)

Several studies suggest that sweeteners not only induce the sense of sweet taste but may have other biological functions in the gastrointestinal tract such as gut motility and hormonal effects (Brown and Rother, 2012 and Meyer-Gerspach et al., 2018).

Recent prohibition of in-feed antibiotics in the United States due to concerns of antibiotic resistance has led to an increase in gut diseases and chicken mortality (Zhen et al., 2023) As the use of antibiotics has been reduced, research has focused on identifying alternative prevention and treatment options (Al-Khalaifah, 2018). Chung et al. (2018) has shown that brazzein has strong antioxidant, anti-inflammatory, and anti-allergic activity, suggesting brazzein-containing feed may serve as an effective and valuable alternative to antibiotic use in the poultry industry.

Importance of Improving Performance b/c of Industry/Consumer Demands

In recent years, the number of livestock operations has fallen, and production has shifted to larger and more specialized operations. These structural changes have been accompanied by a movement towards cost-saving production technologies and practices (USDA, 2023). The U.S. poultry industry is the world's largest producers of and second largest exporter of poultry meat. The United States poultry products hold leading positions in both international and U.S. meat commodity markets. Over 46 million pounds of poultry meat was produced by the broiler chicken sector in 2022 (USDA, 2023). Poultry meat consumption has trended up and displaced a substantial amount of red meat consumption in recent decades (USDA, 2023). Chickens contribute 90 percent of world poultry meat production (FAO, n.d.)

To meet the growing consumer demand for chicken meat, the poultry industry has selected broiler chickens for increased efficiency and breast yield (Torrey et al., 2021). At six weeks of age layer type chicks were 3.9 – 4.6-fold lower, compared to broilers. Between hatching and slaughter, broiler breeds increase their weight by fifty to sixty-fold, and on day 42 their body weight is five times as high as in layer hens. Buzala et al. (2015) continue to elaborate that daily feed intake and feed consumption rate is 2 to 3-fold higher in broilers than in layers starting at day two of age. Difference in weight and carcass quality begins in day-old chicks; Murawska and Bochno (2006) reported that broiler chicks weighed 7 grams heavier than layers of the same age. Optimal feed intake is key to supporting the rapid growth rates achieved in the broiler industry.

Growth Measurements and Sampling

Latshaw and Bishop (2001) demonstrated that the composition of a chicken's body could be estimated from models using noninvasive measurements. They defined several noninvasive

measurements including back length (from nadir of the curve of the neck to the base of the tail), body circumference (using cloth tape placed under the wings and anterior to the legs), keel length (while chicken was held on its back), width of the pelvis (measuring outer edges of thighs), and breast width (at anterior end of keel bone).

Euthanasia methods for poultry include gas inhalation, manually applied blunt force trauma, cervical dislocation, decapitation, electrocution, gunshot, captive bolt, and injectable agents. (AVMA 2020) In addition to humane outcome, an important consideration in the choice of method for euthanasia of laboratory animals is the research objectives for the animals being euthanized (AVMA). Koechner Euthanizing Device (KED) is a tool developed for mechanical cervical dislocation for use in separating the skull from the vertebrae. KED is a useful alternative of cervical dislocation when personnel are minimally trained, not yet skilled or capable of preforming cervical dislocation (Boyal et al., 2020)

CHAPTER III: TRIAL ONE – PRELIMINARY STUDY

Introduction

Yeast has been included in broiler chicken diets to increase average daily gain and bioactivities. Several studies have evaluated the effect of including various yeast strains in poultry diets. While Saccharomyces cerevisiae has been widely used in poultry diets, several other strains of yeast have been evaluated. Saccharomyces cerevisiae has been included into diets in various forms, including cell wall (Santin, 2001), granules (Hosseini, 2001) and mixed yeast cultures (Sun, 2018) In addition to yeast strain, several studies have evaluated specific yeast inclusion rates.

Some studies have demonstrated a beneficial effect of yeast inclusion on bird health, growth, and performance while other studies showed no differences because of yeast inclusion. Chicks fed 1.5% yeast inclusion of S. cerevisiae, had higher body weight gain, feed intake, feed conversion ratio and improved carcass characteristics (Paryad and Mahmoudi, 2008). Due to the varied results, it was important to establish baseline intake and performance values for specific yeast strains that might be used in subsequent research. Therefore, the objective of the research was to establish methodology for use in a study that evaluated the effect of brazzein-containing yeast on chicken performance.

Materials and Methods

Birds and Housing

All procedures including animals were reviewed and approved by the Institutional Animal Care and Use Committee at Illinois State University, protocol number 2023-1213. Cornish Cross Broiler chickens were purchased from a commercial hatchery and arrived unsexed. For this preliminary trial chicks were reared from 1to 15 days of age, with the first

three days serving as an acclimation period. Chicks were housed in pens that were 121.92 cm x 91.44 cm x 121.92 cm, allowing 1,019 cm^2 per bird. Pine wood chips were used for bedding. Feed was offered in one-pound galvanized chick feeders with a 15.24 cm base; water was offered in quart jars with a quail base waterer. Feeders were placed on plastic trays 20.32cm x 50.8cm x 2.54cm to allow easy collection of orts. Heat lamps were stationed on the opposite side of the food/water and one foot above the ground.

Chicks were housed under continuous heat lamps to maintain the appropriate pen temperature; overhead rooms lights were controlled to provide 23 hours of light and 1 hour of dark during the first 72 hours of trial to ensure chicks could find water. Thereafter, birds received 14 hours of light and 10 hours of dark each day for the duration of the 12-day study.

Yeast Inclusion

The yeast and growth protocols were a generous gift of Dr. Stephen Hughes (manuscript in preparation) PJ69-4 PYAC. S. cerevisiae PJ69-4 diploid was cultured in sterile YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) at 28°C, with shaking at 166 rpm, for 48 hours prior to harvesting by centrifugation. Four liters of YPD medium were prepared and incubated and grown at the same time inoculated with one of the two strains. An overnight 200 mL flask with sterile medium was inoculated using yeast colonies from sterile plates; to start the large cultures, 50 mL of the overnight culture was added sterilely to each liter of medium. Medium was sterilized using a Steris Autoclave operating at 121°C for sterilization time of 21 min and a total of 52 minutes of operation. Following inoculation of the four 1-liter flasks, yeast was cultured for 48 hours prior to harvesting and cells grown in incubation chamber with shaking. To harvest cells, a Beckman Avanti J-25I centrifuge with a JLA-10.5 rotor was used to pellet the cells from the medium (1000 rpm, 4°C for 6 min. Cells were then pooled into 50 mL

polypropylene (tared) tubes and freeze dried to remove water using a Labconco Freeze Dryer (#5).

Experimental Design

Four treatments were utilized: Control, Baker's Yeast, Wet Yeast, and Dry Yeast. Yeast strain (S. cerevisiae PJ69-4) utilized for this trial was prepared in two different ways, freeze dried and as a wet slurry. Freeze dried yeast was used for the Dry Yeast treatment and the wet slurry for the Wet Treatment. Baker's Yeast was a commercially available Saccharomyces cerevisiae.

Treatments were replicated for eight groups total. Forty chicks were allocated to each group by weight, allowing for five birds per group and two groups per treatment. Leg bands of various colors were used to identify individual chicks within a group. Purina Start and Grow Medicated Crumbles was used as the base diet; this is the diet received by the CON group. The other three treatments received the base diet with one of the three yeasts included at a rate of 1.5% of diet by weight.

Wet yeast was added as is (containing moisture) whereas dry yeast was added as a slurry. Once combined yeast was added to the base feed the crumbles were dried in a forced-oven at 55°C overnight. Crumbles were manipulated to break up clumps and create a more uniform size.

Data Collection

Individual chicks were measured daily during the 12-day study initial group measurements rotated from day to day. Body weight, hind end width, breast circumference and back length were measured. Weight was recorded utilizing a gram scale and rounded to the closest hundredth. Hind end measurements were taken using a caliper (in millimeters) and recorded to the tenth decimal place. Both breast width and back length were measured with a

tape measure and were reported in centimeters. Back length was recorded from the nape of the neck to the end of the tail feathers. Breast circumference was measured from the keel bone to the top of the back. The pelvic width was recorded in millimeters and measured with a caliper. Pelvic width measured the outer distance of the thighs while chicks were manually restrained.

Feed and water consumption were recorded as a group basis a. The prepared diets and water were offered to each pen ad libitum. Each day, the remaining feed and water were measured. Additional feed and water were added to ensure the opportunity for ad libitum consumption. Additional environmental measurements were recorded daily, including pen temperature and ambient temperature. Heat lamps were adjusted to warm or cool the pen as needed to maintain housing temperatures appropriate for their age. Mercury thermometers located in each pen were used to record pen temperature; ambient temperature was recorded using temperature sensors that were evenly distributed throughout the housing area.

Bird Harvest

Chicks were euthanized on day 12 of the trail. A Koechner Euthanasia Device (KED-S) was utilized for this process. After chicks were euthanized, the heart, liver and breast meat were removed from each chick. The weights of these tissues were recorded in grams and stored in Ziplock bags. Samples were then placed on dry ice to spare the integrity of the sample. Once in the lab samples were stored in a negative -80°C freezer for subsequent analysis.

Statistical Analysis

Statistical analyses were conducted with summary statistics using Microsoft Excel and SAS 9.4. The mixed procedure was used to determine statistical significance. The model statement included terms for the parameters of interest with treatment serving as the dependent variable. The p-diff function was used to make comparisons among all treatments.

Results and Discussion

Feed Intake

Statistical analysis of the average daily feed intake (ADFI) showed that the Control average the highest ADFI of 404.53g/day, compared to Dry Yeast (332.04g, $p>0.005$), Wet Yeast (309.22g, $p>0.0018$) and Baker's Yeast (307.60g, $p>0.0017$). Additionally, Control treatments also averaged the highest average daily water intake (ADWI) of 627.16g/day ($p>0.016$). Feed and water intake data are shown in Table 2.

Growth Parameters

Results of growth parameters measures are shown in Table 1.

Chicks in the Control group had a significantly higher final weight (515.16 g) compared to the Bakers Yeast chicks (448.37 g) and Wet Yeast chicks (449.28 g). Chicks on the Dry Yeast diet had a final weight intermediate, and not significantly different than either the Bakers Yeast and West Yeast or the Control group.

Figure 1 Weight increase over time of individual chicks on Bakers Yeast

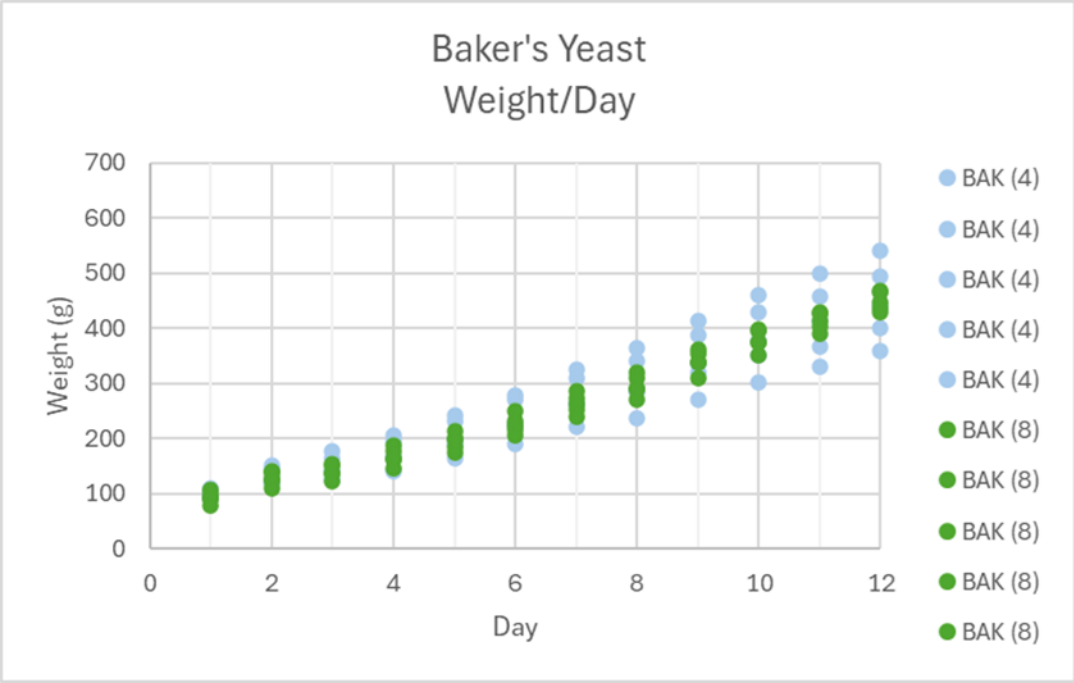


Figure 2 Weight increase over time of individual chicks on Control

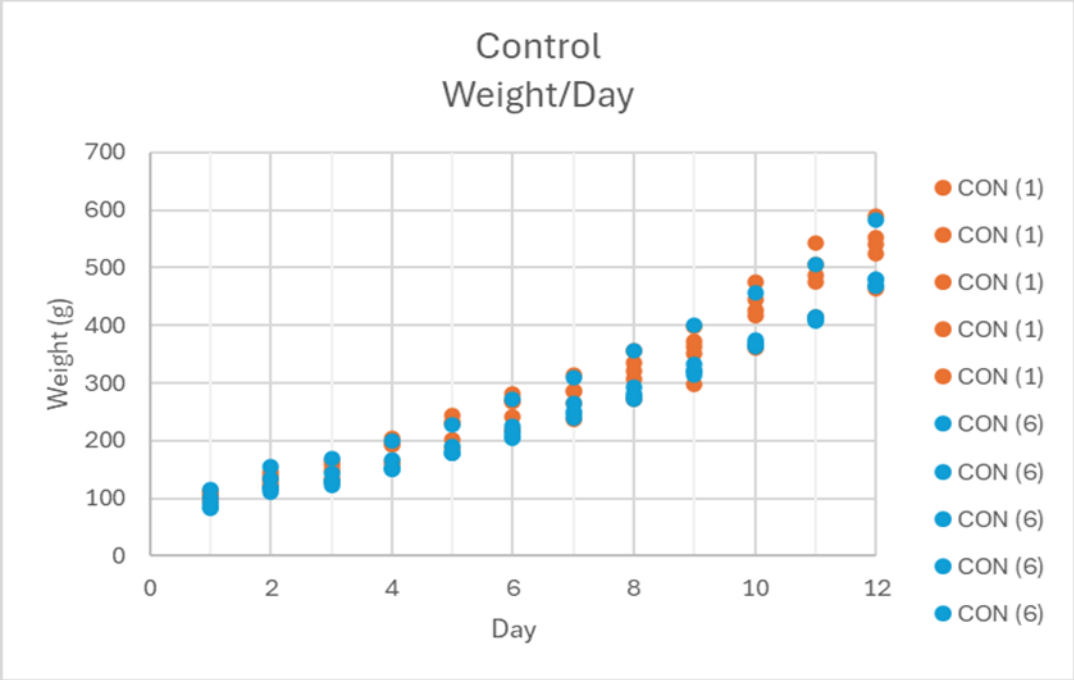


Figure 3 Weight increase over time of individual chicks on Dry Yeast

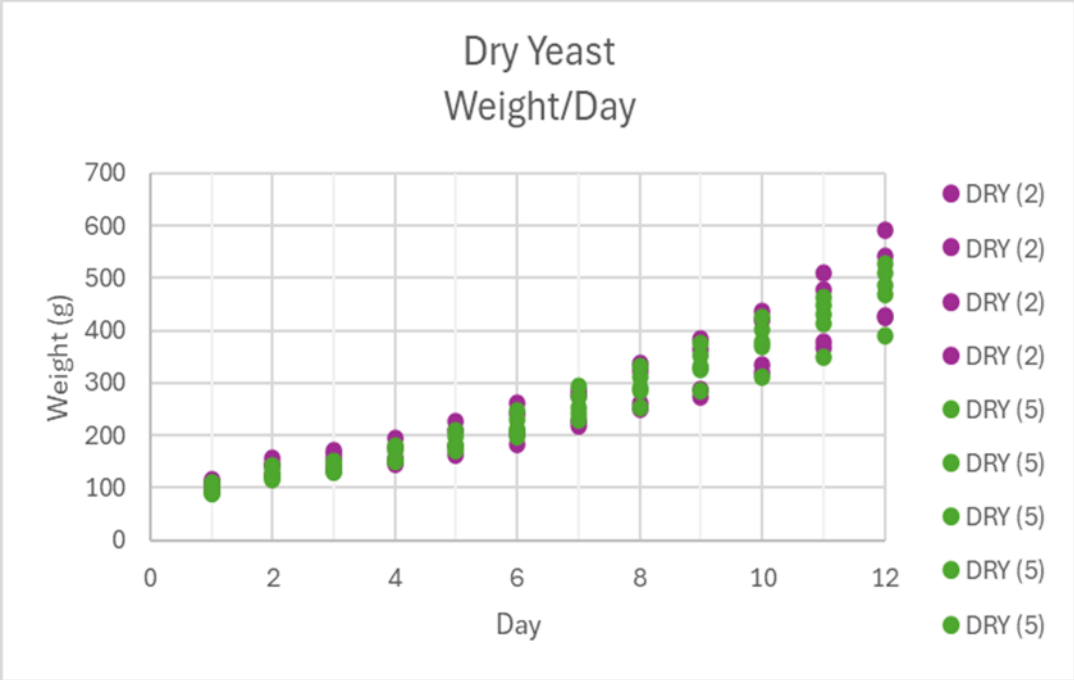
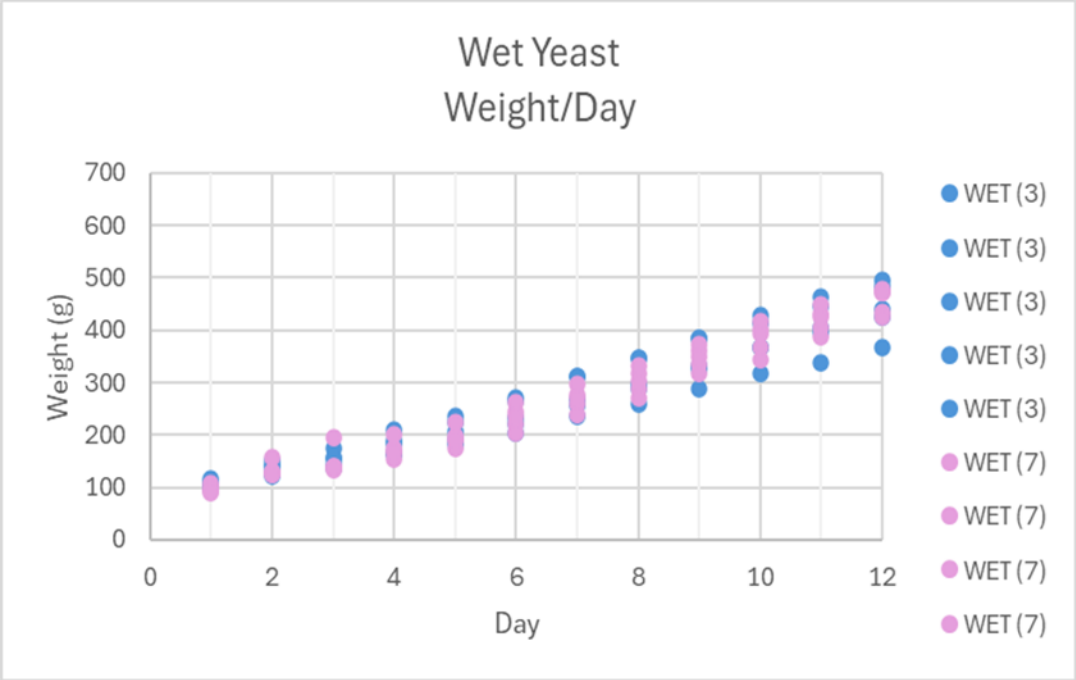


Figure 4 Weight increase over time of individual chicks on Wet Yeast



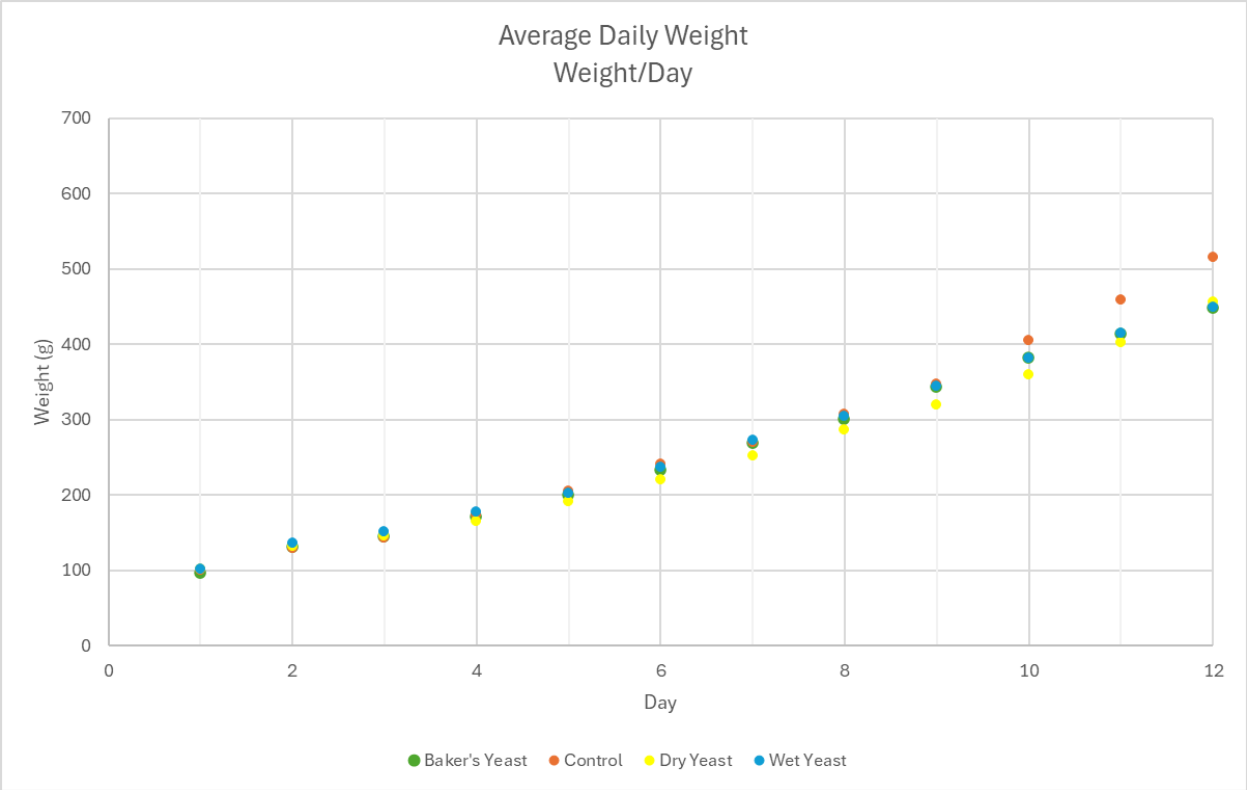
A similar relationship was seen in the ADG data; chicks in the Control group had significantly higher ADG (32.99 g/d), compared to Baker's Yeast (28.0 g/d, p=0.005), Wet Yeast (27.73 g/d, p=0.003) whereas there was not statistical difference to Dry Yeast (30.60 g/d, p=0.172). Chicks on the Dry Yeast diet had an intermediate ADG (30.6 g/d) and was not significantly different than either the Bakers Yeast and Wet Yeast or the Control group.

Control chicks also proved to have larger breast circumference (17.92 cm) than the Wet Yeast Chicks (17.23 cm; p= 0.05); Dry Yeast and Bakers Yeast were intermediate (17.53 cm and 17.28 cm, respectively).

Post-harvest measurements included liver weight, heart weight and breast weight. There were no differences in liver weight or heart weight across treatments. Breast weight was significantly greater in the Control group (106.83 g) compared to the Bakers Yeast (96.15 g), Dry Yeast (94.1 g), or Wet Yeast (93.21 g).

Treatment and pen daily weight change was plotted on scatter charts. Although some individual bird growth variations were observed, there were no statistically significant changes between treatments. Figures 1, 2, 3 and 4 show individual chicks in each treatment, treatments are denoted by the same color. A similar growth trend can be observed throughout the trial across treatments (Figure 5).

Figure 5 Weight increase over time of Treatment



Chicks in the Control treatment numerically and statistically outperformed chicks in the other three treatments (Table 1 and 2). It was observed that chicks in the Wet Yeast treatment disliked the inclusion of yeast to the base diet, although the yeast strain was the same as the Dry Yeast treatment. It was determined that the inclusion of yeast to a base diet was difficult when a wet yeast slurry was used. Overall, chicks preferred the control diet, as demonstrated by the higher ADFI exhibited by this group. It is suspected that the addition of the yeast in any form

(wet, dry, or baker's yeast) altered the taste or smell of the treatment diet and negatively impacted feed intake. A close relationship exists between water intake and dry matter intake; therefore, it was not surprising that the diet with the highest feed intake also had the highest water intake.

Breast circumference and breast weight were the highest in the Control group; this group was also the one that had the highest feed intake. Other parameters measured (pelvis width, back length, heart weight, or liver weight) were not affected by treatment diet, suggesting that feed intake has less of an effect on those physiological measurements compared to breast circumference and weight.

Despite the fact that one of the yeast-containing diets did not demonstrate improved performance capabilities, this preliminary study was critical in establishing methods for use in subsequent studies. Researchers became proficient in measuring the various body measurements.

Additionally, housing modifications were made to reduce the amount of poultry litter that contaminated the feed. This included the use of a cardboard wall to prevent the chicks from scratching litter onto the plastic tray that contained the galvanized feeder. This reduced contamination substantially and allowed for more accurate collection of orts.

Baker's yeast and dry yeast were mixed with water prior to application to the feed. The wet yeast already contained water. Despite the researcher's best effort, the application of these wet mixtures to the base feed resulted in clumping of the feed. Even after drying in a forced air oven, the feed remained clumpy. Manual manipulation was used to break up the large clumps. Due to the inconsistency of application across these yeast-including treatments, various dilutions of yeast and water were evaluated in the subsequent trial.

CHAPTER IV: TRIAL TWO – BRAZZEIN INCLUSION

Introduction

An exploratory research trial was conducted to examine the potential value of brazzein-yeast, measuring growth parameters, average daily gain, feed intake and carcass characteristics. Yeast has been included in broiler chicken diets to increase average daily gain and bioactivities. Several studies have evaluated the effect of including various yeast strains in poultry diets. Some studies have demonstrated a beneficial effect of yeast inclusion on bird health, growth, and performance while other studies showed no differences due to yeast inclusion.

In addition to supplementing diets with yeast, researchers have been researching the advantages of various high intensity sweeteners. High intensity sweeteners supplementation has been shown to increase body weight of broiler chickens during the starter stage, but also functions to reduce inflammation, improve intestinal development and gut microbiota (Chen et al., 2020). Brazzein, a sweet tasting protein, is 500-2,000-fold sweeter than sucrose, and shows properties of antioxidants, anti-inflammatories and anti-allergenics (Chung et al., 2018), making it a potentially valuable alternative to other currently available HIS. The addition of brazzein in livestock feed has not been evaluated. The purpose of this trial is to determine if the addition of brazzein-containing yeast beneficially influences growth parameters and carcass characteristics of broiler chickens.

Material and Methods

Birds and Housing

All procedures including animals were reviewed and approved by the Institutional Animal Care and Use Committee at Illinois State University, protocol number 2023-1213.

Experimental Design

This study was conducted as a completely randomized design. The treatments included: Control (CON; basal diet only), Yeast containing no Brazzein (YEA), and lastly, Yeast containing the Brazzein gene (BRA). Each treatment was run in triplicate, resulting in nine groups of three birds.

Cornish cross chicks were obtained from a commercial hatchery. Thirty straight-run chicks were purchased with the intention of using twenty-seven for this trial. The lightest three chick were not included for the trial. Initial weights were recorded and then birds were stratified to pens based on body weight. The nine pens were then assigned to one of the three treatments: CON, YEA, or BRA.

For the duration of the trial birds were housed in pens 121.92 cm x 91.44 cm with 1,019.35 cm^2 per bird and reared from one to 24 days of age, with the first three days serving as an acclimation period. Chicks were housed under continuous heat lamps to maintain the appropriate pen temperature relative to chick age; overhead rooms lights provided 23 hours of light and 1 hour of dark for the first 72 hours of trial to ensure the chicks found water and feed. Thereafter, birds receive 14 hours of light and 10 hours of dark each day. Heat lamp use was contingent on individual pen temperature and barn ambient temperature. When ambient and pen temperature was high enough without the additional heat source, heat lamps were turned off. (Colorado State University, 2018)

Yeast Preparation/Inclusion

The yeast and growth protocols were a generous gift of Dr. Stephen Hughes (manuscript in preparation). NRRL Y-1580 (control strain) and recombinant PJ69-4 PYAC. S. cerevisiae PJ69-4 diploid was cultured in sterile YPD medium (1% yeast extract, 2% peptone, and 2%

dextrose) at 28°C, with shaking at 166rpm, for 48 hours prior to harvesting by centrifugation. Four liters of YPD medium were prepared and incubated and grown at the same time inoculated with one of the two strains. An overnight 200 mL flask with sterile medium was inoculated using yeast colonies from sterile plates; to start the large cultures, 50 mL of the overnight culture was added sterilely to each liter of medium. Medium was sterilized using a Steris Autoclave operating at 121°C for sterilization time of 21 min and a total of 52 minutes of operation. Following inoculation of the 4 1-liter flasks, yeast was cultured for 48 hours prior to harvesting and cells grown in incubation chamber with shaking. To harvest cells, a Beckman Avanti J-25I centrifuge with a JLA-10.5 rotor was used to pellet the cells from the medium (1000 rpm, 4°C for 6 min. Cells were then pooled into 50 mL polypropylene (tared) tubes and freeze dried to remove water using a Labconco Freeze Dryer (#5).

Application of Yeast to Feed

To determine the best method of yeast inclusion, yeast was dissolved in various amounts of water. One gram of dry yeast was dissolved in each of the following 100, 75, 50 and 25 milliliters of water. Yeast was left to dissolve in beakers for one hour. Yeast dissolved evenly in 100mL and 75mL. After the yeast dissolved, the liquid mixture was then added to 100 grams of Purina Start and Grow Crumbles. Feed samples were dried in an oven for 48 hours at 55°C. Once the samples were removed from the oven and broken up, it was determined that when dissolved in 100mL and 75mL of water, the integrity of the feed crumbles was degraded. It was determined that dissolving yeast in water at a rate of 1 g dry yeast: 50 mL water resulted in a similar form to the base diet without alteration, therefore this ratio (1:50) will be applied to both yeast treatments (YEA and BRA).

Once the yeast was incorporated into the Purina Start and Grow Crumbles, the mixed feed was stored in individual bags to prevent any cross contamination. During preparation of the different diets, yeast types were never weighed or mixed in the same weigh boats and beakers. Table 8 shows the nutrient content of the three treatment diets.

Daily Feed Measurements

Prepared feed was stored in Ziplock bags until fed daily to chicks. Daily measurements of feed offered (g) and feed refused (g) were recorded. Each day, the amount of feed offered was added to feed refused and collected orts from the previous day. Feed refused was recorded by adding the feed that was remaining in the feeder and the orts collected. Water was also recorded daily, water offered (g) and water refused (g). Refused water was weighed and then replenished with fresh water daily. Before weighing the water offered, waterers were dried off and excess water was wiped away.

Daily Chick Measurements

Birds were measured daily. Measurements included weight, back length, breast circumference and hind end width. Data collection began every day at 8 am, although, the initial pen recorded rotated every other day.

The measurement techniques utilized throughout this trial were developed and refined during the preliminary trial. Body weight was recorded in grams and taken using a balance. The back length and breast circumference were recorded in centimeters and measured with a retractable cloth measuring tape. Back length was recorded from the nape of the neck to the end of the tail feathers. Breast circumference was measured from the keel bone to the top of the back. The pelvic width was recorded in millimeters and measured with a caliper. Pelvic width measured the outer distance of the thighs while chicks were manually restrained.

Pen feed and water intake were also measured daily. Both feed and water offered were recorded in addition to feed and water refused, weighed in grams. Individual pen temperatures were observed and recorded daily, temperature determined the height of heat lamps or if lamps were turned off. Ambient temperatures throughout the barn were recorded with temperature sensors in pens one, four, five and eight.

Bird Harvest/Sampling

Birds were euthanized on day 24 of the trial using a Kosher Euthanasia Device. Once euthanized, birds were dissected, tail feathers, heart, liver, breast meat, crop and small intestines were collected. After the sample was weighed, the hearts, livers and breast meat were frozen utilizing dry ice. The crops and small intestines were cooled in coolers full of ice until physiological measurements could be measured the following day. At harvest, feather samples were plucked from the tail and stored in a Ziplock bag until weighed in the lab. Feather sample weights (g) were recorded and then five random feathers were chosen to measure length (cm). Feather length was recorded using a metric ruler on a flat surface.

Laboratory analysis of the small intestine (gizzard to the base of the ceca) included sample weights (g), length (cm) and a sampling of the extruded contents. Small intestine (30 cm) was extracted, weighed (g) and contents of this portion were extruded into a 50mL sterile polypropylene tube. Extruded contents were weighed (g). Sterile saline (5 mL) was added and vortexed to mix well. Using saline, a series of 10-fold dilutions were made (1:10, 1:100, 1:1000, 1:10,000) and then 100uL of the final dilution was plated onto two types of media (MAC and MRS). MacConkey Agar (MAC) is reported to be selective for gram-negative bacteria (enteric). Klebsiella and Enterobacter produce mucoid colonies which appear moist, sticky, and slimy, these bacteria were not found on many of the plates. De Man-Rogosa-Sharpe agar (MRS) is

reported to be selective medium for Lactobacilli. Crops were weighed (g) then 5mL of saline was added. Crops and saline were stored in sterile polypropylene tubes; stored at 5°C cold room. Feathers from chicks were randomly selected, ten feathers per chick, total weight (g) was measured then length (cm) for 5 individual feathers. Average feather weight was calculated.

Statistical Analysis

Statistical analyses were conducted with summary statistics using Microsoft Excel and SAS 9.4. The mixed procedure was used to determine statistical significance. The model statement included terms for the parameters of interest with treatment serving as the dependent variable. The p-diff function was used to make comparisons among all treatments.

Results and Discussion

Total Feed and Water Consumed

Daily feed intake was determined by subtracting the total feed offered and feed refused. There are no statistical differences between feed and water intake across treatments, results are shown in Table 5.

Feed conversion ratio (FCR) was calculated for the duration of the trial by adding together the total feed consumed on an as pen basis and then divided by the total weight gain of the chicks (final weight minus day one weight). Pen 6 on the Control treatment diet recorded the lowest FCR 0f 1.51 however, the highest FCR Pen 8 (1.92) was also in the Control treatment. Overall, the three treatments, CON and YEA all recorded average FCRs of 1.71.

Average Daily Gain

There were no statistical differences for average daily gain, results are shown in Table 5. Treatment ADG calculations showed that BRA averaged the highest numerical ADG of 30.68 g/day. CON averaged 28.63 g/day followed by YEA with 27.54 g/day. However, ADG varied

week by week; throughout week one YEA recorded the highest ADF with 17.25 g/day, followed by 14.91 g/day (CON) and 14.91 g/day (BRA). During week two BRA chicks had and ADG of 26.21 g/day while YEA and CON had an ADG of 21.95 g/day and 21.45 g/day, respectively. During week three, CON birds had the numerically highest ADG at 36.12 g/day with BRA and YEA following at 35.42 g/day and 31.81 g/day, respectively.

Figure 6 Weight increase over time of individual chicks on Brazzein

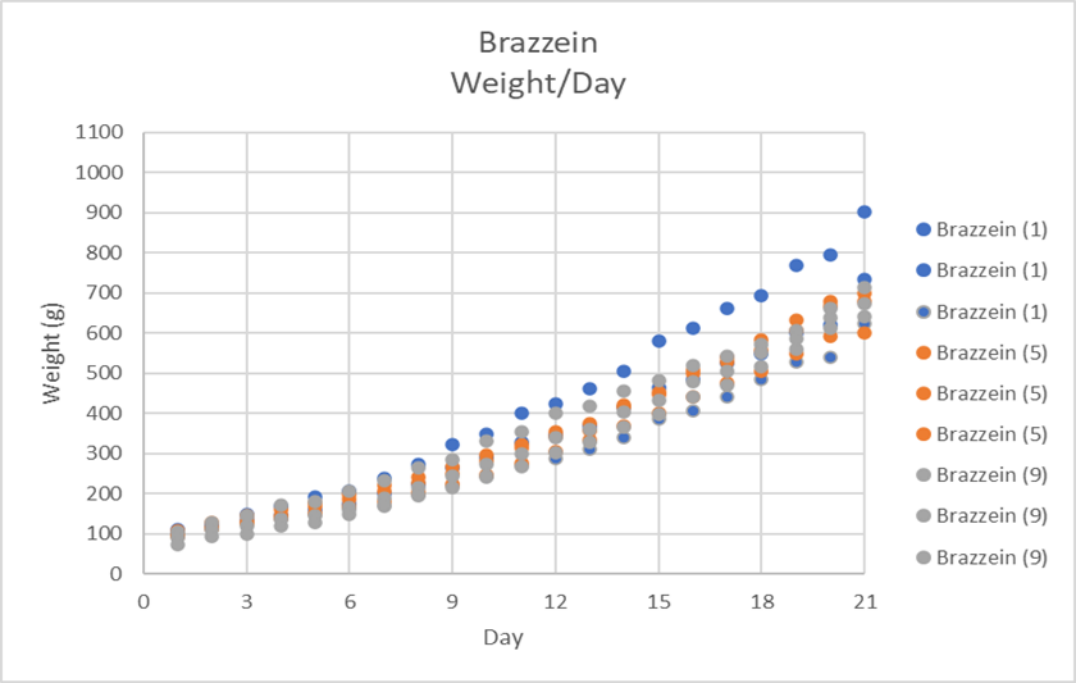


Figure 7 Weight increase over time of individual chocks on Control

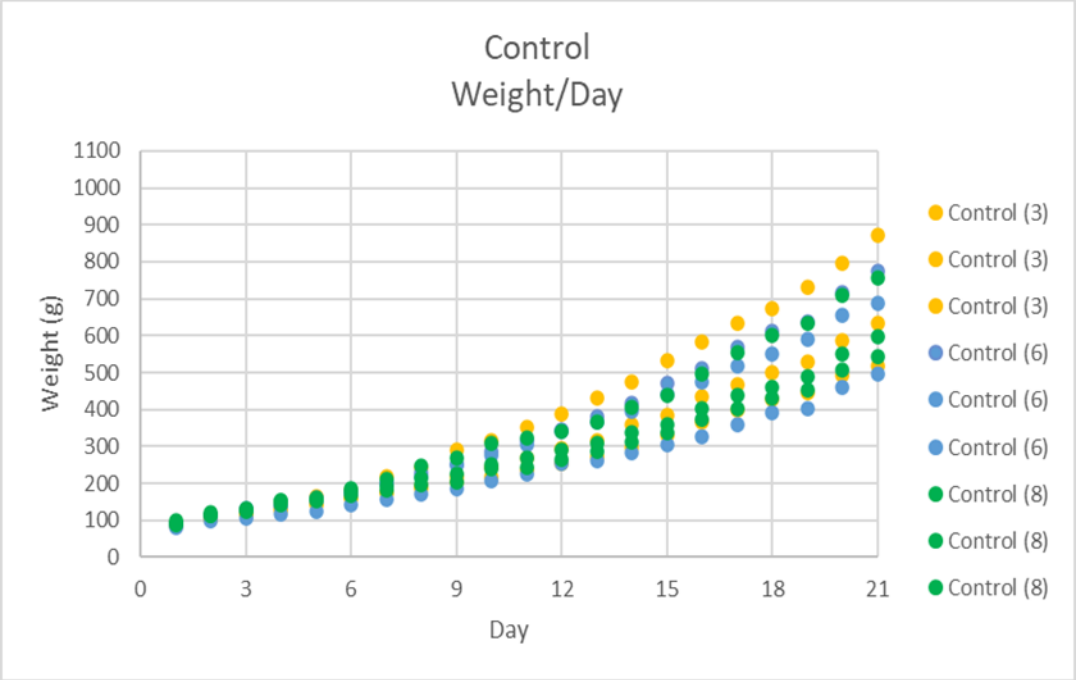


Figure 8 Weight increase over time of individual chicks on Yeast1

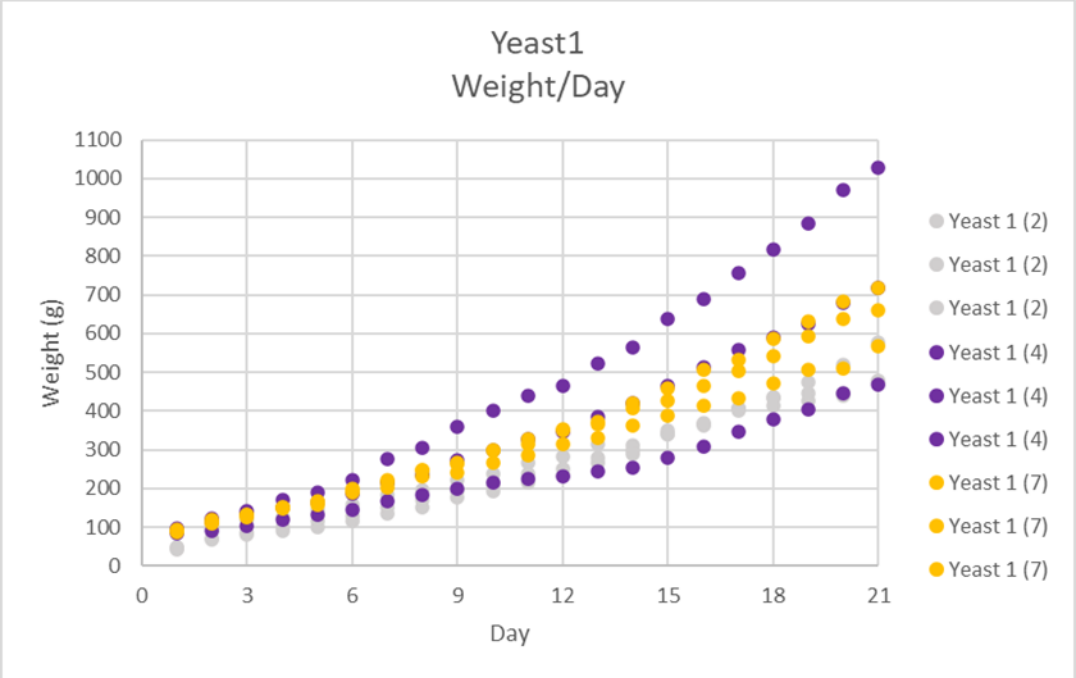
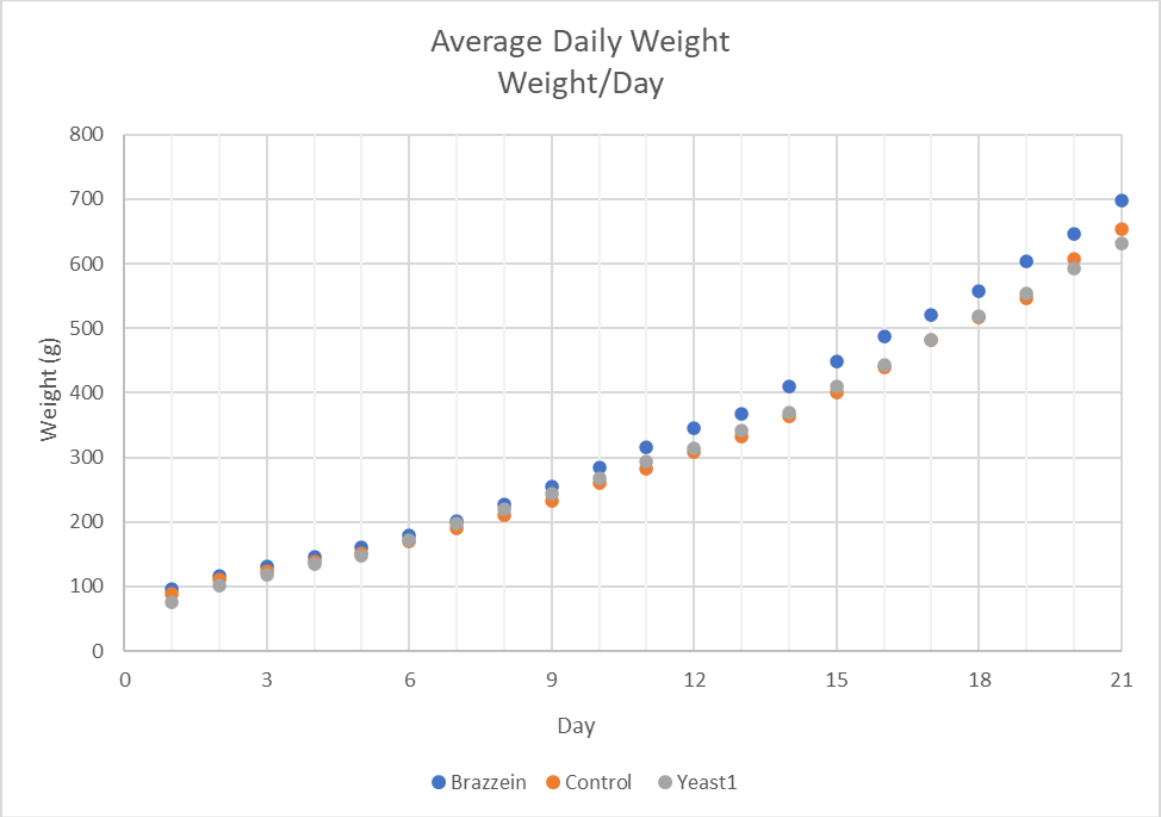


Figure 9 Weight increase over time of Treatment



Growth Parameters

No statistical differences among the treatment groups were observed in breast circumference, back length, or hind end measurements, Table 4. Overall, BRA chicks had the highest numerical increase in breast circumference and back length. On average these pens grew 0.57 cm a day for breast circumference and 0.59 cm a day for back length, compared to 0.55 cm and 0.57cm for CON and 0.55 cm and 0.56 cm for YEA. YEA chicks averaged the highest daily growth of pelvis width of 1.06 mm a day, compared to 0.99 mm for CON and 0.97 mm for BRA, while not significant. Feather length is shown in Table 7.

Organ Measurements

Weights taken of the liver, heart and breast meat were summarized; no statistical differences were identified between treatments (data shown in Table 6). Brazzein treatment

groups recorded the highest numerical heart weight (6.34 g) and breast meat weight (130.49 g), whereas the control group had the highest liver weight (21.04 g). YEA recorded the lightest weights of liver (18.22 g), heart (5.30 g) and breast meat (110.69 g). In the lab crop weights were recorded, statistical difference between BRA and CON was recorded (Table 6). Organ weights of chicks reared to five weeks on a corn-based reported liver weight 40g, heart weight 10g and small intestine weight 56g (Awad et al., 2009).

Additionally, small intestine weight (g) and length (cm) were also recorded in the lab. Mesenteries were removed before weights were recorded. Small intestine measurements and weights are shown in Table 6. Contents from the small intestine that were plated grew too many colony forming units to accurately count. Bacteria from both the MAC and MRS appeared hazy and had large colonies forming, after replating, colonies were still too subjective.

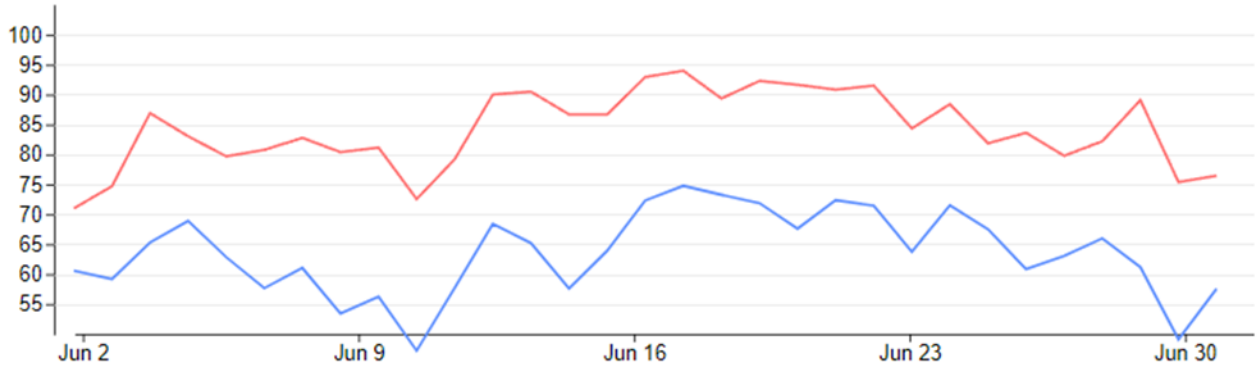
Body weight and daily growth measurements provided a baseline to determine if the addition of Brazzein-containing yeast would improve broiler chicken feed intake and performance. Growth parameters such as back length, breast circumference and pelvis width of chickens on the current trial closely resemble those of Latshaw and Bishop (2001). Latshaw and Bishop reported breast circumference of 33.6 cm, back length 19.1 cm and pelvis width of 11.1 cm (2001). Chickens from the Latshaw and Bishop study were reared to 1,200 g, 1,750 g and 2,300 g. Chickens reared during this trial showed the same trend in growth measurements throughout the study.

Chicks on trials also provided valuable data on average daily gain and feed conversion ratios. Bravo et al. reported that chickens on a base diet of corn had an average daily gain of 28.2 g/d over a 21d period; a control diet with supplemental essential oils gained an average of 32.

3g/d (2014). Chickens on this trial had similar average daily gain over a 21d period: BRA 35.4 2g/d, CON 36.12 g/d and YEA 31.81 g/d.

Temperature for June 2nd through June 30th (duration of trial) can be seen in Figure 10. Daily high and low temperatures are plotted. Temperatures from June 13th through June 25th were 5-15 degrees above the normal average (Ford, 2024), with a monthly average of 74.4° F.

Figure 10 High-low temperature of days chicks were on trial



Conclusions

Although results are not significantly different for any of the parameters measured, amongst any of the treatments, this study provided an initial understanding the effect that Brazzein-containing yeast has on broiler chicken feed intake and performance. As a novel feed additive, the purpose of this study was to determine that the addition of Brazzein-containing yeast would not negatively affect feed intake, growth parameters, average daily gain, or overall well-being of the chicks. Methodologies developed in this research can aid in determining the potential benefits of Brazzein as a livestock feed additive in future research.

Future work should gather additional data to build a more well-rounded understanding of the effect of brazzein-yeast on the gastrointestinal tract. This includes gizzard weights, large intestine weights, and microbiota of the small and large intestine. Additionally, further analysis

regarding color pigmentation of beaks and legs may prove valuable as these components are important to various ethnic groups. Lastly, protein assays on liver biopsies, blood samples and extruded content from the GIT may provide beneficial information demonstrating the value of the addition of Brazzein-containing yeast to commercial poultry diets. With additional research, the ability of brazzein-containing yeast to influencing palatability or other bioactivities will be determined.

CHAPTER V: CONCLUSIONS & IMPLICATIONS

With relatively little information available regarding the use of brazzein in livestock feed or human food, this study provides some valuable initial data concerning the potential value of brazzein-yeast as a livestock feed additive. While it was hypothesized that the brazzein-yeast yeast diet would improve feed intake and subsequent animal growth, it was found to have no effect on intake and performance. The inclusion rates used in this study (1.5% and 1.0% of final diet) were selected based on the results of a previous study showing beneficial effects when S. cerevisiae was included at similar rates. It is possible using a different brazzein-yeast inclusion rate, different method of yeast application, or a lower quality or less palatable base diet may result in beneficial results. Additional research should also include the evaluation of brazzein-containing yeast in swine diets. Sweeteners are more commonly utilized in the swine industry to improve dry matter feed consumption during the first several weeks of life.

Despite the lack of performance benefits observed from feeding brazzein-containing yeast, these two studies provide valuable, initial information regarding the potential of a brazzein-containing yeast livestock additive.

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APPENDIX A: TABLES

Table 1. Measuring Growth Parameters with Average Body Measurements

Treatment	Final Weight (g)	Final Breast Circumference (cm) [1]	Final Pelvis Width (mm) [2]	Final Back Length (cm) [3]
Baker's	448.37[a]	17.28[a,b]	56.8[b]	16.84[a]
Control	515.16[b]	19.92[b]	56.1[a,b]	17.76[a]
Dry Yeast	485.59[a,b]	17.53[a,b]	54.67[a]	17.16[a]
Wet Yeast	449.28[a]	17.23[a]	57.9[b]	17.19[a]

[1]Final breast circumference (cm) measured anterior keel bone to the back under the wings
[2]Final pelvis width (mm) measured anterior of legs while chicken was handled
[3]Final back length (cm) measured from the base of the neck to the tip of tail feathers

Table 2. Average Daily Gain and Feed/Water Intake

Treatment	Overall Daily Gain (g) [1]	Overall Daily Feed Intake (g) [2]	Overall Daily Water Intake (g) [3]
Baker's	28[a]	307.6[a]	580.87[a,b]
Control	33[b]	404.53[b]	627.16[b]
Dry Yeast	30.6[a,b]	332.04[a]	499.23[a]
Wet Yeast	27.73$_a$	309.22[a]	606.74[b]

[1]Overall daily gain (g/day)
[2]Overall daily feed intake (g/day)
[3]Overall daily water intake (g/day)

Table 3. Organ/Tissue Weights

Treatment	Liver Weight (g)	Heart Weight (g)	Breast Weight (g)
Baker's	20.36[a]	4.28[a]	96.15[a]
Control	21.42[a]	4.55[a]	106.83[b]
Dry Yeast	20.41[a]	4.19[a]	94.1[a]
Wet Yeast	19.52[a]	4.12[a]	93.21[a]

[b]Indicates significance (p>0.05) between treatments

Table 4. Measuring Growth Parameters with Average Body Measurements

Treatment	Final Weight (g)	Final Breast Circumference (cm)[1]	Final Pelvis Width (mm)[2]	Final Back Length (cm)[3]
Brazzein	697.29[a]	20.17[a]	48.44[a]	20.28[a]
Control	653.09[a]	19.67[a]	47.89[a]	19.44[a]
Yeast 1	632.13[a]	19.56[a]	47.11[a]	19.28[a]

[1]Final breast circumference (cm) measured anterior keel bone to the back under the wings
[2]Final pelvis width (mm) measured anterior of legs while chicken was handled
[3]Final back length (cm) measured from the base of the neck to the tip of tail feathers

Table 5. Average Daily Gain and Feed/Water Intake

Treatment	Overall Daily Gain (g) [1]	Overall Daily Feed Intake (g) [2]	Overall Daily Water Intake (g) [3]
Brazzein	30.68[a]	147.3[a]	311.38[a]
Control	28.63[a]	139.68[a]	285.29[a]
Yeast 1	27.54[a]	134.82[a]	311.58[a]

[1]Overall Daily gain (g/day)
[2]Overall daily feed intake (g/day)
[3]Overall daily water intake (g/day)

Table 6. Organ/Tissue Weights

Treatment	Liver Weight (g)	Breast Weight (g)	Heart Weight (g)	Crop Weight (g)	Gut Length (cm)	Gut Weight (g)
Brazzein	20.85[a]	130.49[a]	6.34[a]	6.03[b]	126.67[a]	45.97[a]
Control	21.04[a]	124.74[a]	5.82[a]	15.12[a]	135.56[a]	54.78[a]
Yeast 1	18.22[a]	110.69[a]	5.3[a]	9.51[a,b]	125.44[a]	50.38[a]

[b]Indicates significance (p>0.05) between treatments

Table 7. Feather Measurements

Treatment	Feather Weight (g)	Feather Length A (cm)[1]	Feather Length B (cm)[2]	Feather Length C (cm)[3]	Feather Length D (cm)[4]	Feather Length E (cm)[5]
Brazzein	0.009[a]	3.56[a]	3.5[a]	3.28[a]	3[a]	2.94[a]
Control	0.13[a]	4.5[a]	4.11[a]	4.39[a]	3.83[a]	2.89[a]
Yeast 1	0.009[a]	3.83[a]	2.94[a]	3.94[a]	3.22[a]	3.06[a]

[1-5]Random feather from sample set, length was measured on a flat surface post chicken harvest

Table 8. Nutrient Composition of Treatment Diets (DM basis)

Nutrients	Control	Yeast 1	Brazzein
Moisture	7.9	8.2	7.8
Crude Protein, %	22.7	23.7	23.4
ADF, %	2.2	3.4	2.3
nd, %	9.5	8.9	7.9
TDN, T	84	85	85
Calcium , %	0.91	0.91	0.77
Phosphorus, %	0.76	0.8	0.69
Magnesium, %	0.23	0.24	0.21
Potassium, %	0.99	1.06	0.93
Sodium, %	0.19	0.21	0.17
Iron, ppm	176	161	147
Zinc, ppm	96	103	96
Copper, ppm	17	16	14
Magnanese, ppm	112	123	100
Molydbenum, %	3.3	3.3	3.4

APPENDIX B: BACTERIA PLATING

From each chicken, the gut was obtained (mesenteries removed) and put into individual baggies; we weighted each gut (g) and measured the length (in cm using a ruler); we then obtained 30 cm of the small intestine end, weighted this piece, then extruded the contents into a 50 mL sterile polypropylene tube and obtained the weight of the extruded contents (g). Then 5 ml of sterile saline was added and vortexed to mix well. Using saline, a series of 10-fold dilutions were made (1:10, 1:100, 1:1000, 1:10,000) and then 100 uL of the final dilution was plated on both types of media (MAC and MRS). Plates were then incubated at 37 °C for 24 hours and then evaluated. Pictures of each plate were taken, and the number of distinct colonies, streaks and hazy appearance were evaluated. Plates were stored at 5 °C. MAC medium: MacConkey Agar which is reported to be selective for Gram-negative bacteria (enterics); note: Klebsiella and Enterobacter produce mucoid colonies which appear moist, sticky, and slimy and these were found on many of the plates. MRS medium: De Man-Rogosa-Sharpe agar which is reported to be a selective medium for Lactobacilli.