The effect of dietary taurine supplementation on broiler performance, the cardiovascular system, and the incidence of ascites related mortalities

By

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Declaration

I, the undersigned, declare that this thesis, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me or another individual for a degree at this or any other tertiary institution.

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Abstract

The effect of dietary taurine supplementation on broiler performance, the cardiovascular system, and the incidence of ascites related mortalities.

The modern broiler has successfully been selected and bred year on year to grow faster, more efficient, and ultimately to be more profitable. Unfortunately, this rapid progress has over the years resulted in a whole range of physiological constraints such as Ascites or Pulmonary Hypertension Syndrome (PHS). These diseases or physiological constraints are directly related to the increased pressure placed on the cardiovascular and pulmonary system to keep up with the exceptionally high demand for rapid growth and development. Previous interventions implemented to control Ascites have not been entirely successful, placing profitability and sustainability of broiler farming operations under increased risk.

The objective of this study was to evaluate the effect taurine and different inclusion levels thereof on performance, the cardiovascular system and ascites related mortalities on broilers fed diets with or without poultry by-product meal. Six different feeds, that were scientifically formulated and produced with different taurine inclusion levels, half of them without PBY and the other half with PBY were fed to 2880 Ross 308 broilers, randomly allocated to 48 pens, with 8 replicated per treatment and 60 birds per pen. The six treatments were formulated to the same nutritional specifications, the only difference being the inclusion levels of taurine and poultry by-product meal, respectively. The six treatments ranged from a control without poultry by-product meal and no taurine (Treatment 1) to the highest taurine inclusion level with poultry by-product meal (Treatment 6). Over a 33-day growth trial, broiler performance and mortalities were measured, and physiological traits of the heart were measured once off at the end of the cycle.

Overall, the feed with the highest taurine inclusion level of 0.075%, and in conjunction with PBY, produced significantly greater bodyweights (BW) at weekly intervals from 14-33 days. All heart measurements and weekly mortality percentages, across all mortality categories, found no significant differences between treatments, although mortality categories showed some trends.

This study suggests that dietary taurine supplementation and the use of PBY can effectively be adopted as a means of improving broiler performance such as weekly bodyweights but may not avert the different physiological properties of the heart and the incidence of ascites-related mortalities.

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List of abbreviations

ADG	Average Daily Gain
AGP	Antibiotic Growth Promotant
BCS	Body Condition Score
BW	Body Weight
Ca	Calcium
Cl	Chlorine
CVS	Cardiovascular System
FCR	Feed Conversion Ratio
FI	Feed Intake
GIT	Gastro-Intestinal Tract
HW/BW%	Heart weight as a % of bodyweight
ICF	Intra-cellular Fluid
K	Potassium
Na	Sodium
P	Phosphorous
PBY	Poultry By-Product
PEF	Performance Efficiency Factor
PHS	Pulmonary Hypertension Syndrome
h^2	Heritability's
HW	Heart Weight
RBC	Red Blood Cells
RV	Right Ventricle
RV:HW	Right Ventricle to Heart weight ratio
RVF	Right Ventricular Failure
RVH	Right Ventricular Hypertrophy
SDS	Sudden Death Syndrome
TAU	Taurine

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Chapter 1

General Introduction

Ascites syndrome (AS), also known as pulmonary hypertension syndrome (PHS) remains one of the leading causes of high mortalities and lowered production efficiency in broiler farming, especially in Highveld regions of South Africa where many poultry farming operations are situated high above sea level (approx. 1500m and above). According to the South African Poultry Association (SAPA) in their surveillance period during July 2017 to December 2017, 34.1% of SA's broiler farming operations are situated in the highveld region (Gauteng & Mpumalanga regions). High elevation equates to lower oxygen availability in broiler houses, which in conjunction with poor house temperature and ventilation management mortality spikes and reduced flock performance are regularly seen. Ascites due to high elevation is caused by a variety of factors ranging from vasoconstriction meaning lower blood supply to the lungs, increased blood viscosity due to increased red blood cell formation and increased blood pressure in the lungs as the heart needs to pump more blood to supply the high oxygen requirement of the rapidly growing broiler (Julian et al., 2000, Reid et al., 1986). This ultimately results in right ventricular failure and subsequently right ventricle hypertrophy causing lymph fluid leakage into the peritoneal and eventually the abdominal space (Coello et al., 1985, Huchzermeyer et al., 1986).

The modern broiler (*Gallus gallus domesticus*) has also undergone rapid genetic progress for growth in the form of protein synthesis and improved feed efficiency and this all has allowed the broiler industry to reach market weights at earlier ages, increasing throughput (Dequypere et al., 2005, Hunton et al., 2006). A big negative impact of this rapid genetic progress is that the pulmonary and cardiovascular output capabilities of modern broilers have remained like that of broilers a few decades ago and this means modern broilers have reached or neared their maximum cardiac and pulmonary potential to sustain their rapid growth and development (Lorenzoni & Ruiz-Feria, 2006). Thus, the modern broilers "design" with its big breast muscles, relatively small abdominal space, place additional pressure on the air sacs and in conjunction with the limited lung and cardiac capacity all predispose broilers to potentially developing ascites once additional stressors are present (Julian et al., 1998, Balog et al., 2003). Approximately 2 decades ago a study by Balog, (2003) concluded that on average 5% of broilers that die are due to ascites, this percentages is most possibly even higher in the present due to higher stocking rates, higher energy dense feeds and genetically superior faster growing broilers than those of 20 years ago. This is a considerable amount of chicken meat that goes to waste and if rectified or lowered it could easily lessen the pressure already experienced by the demanding food supply chain.

Studies into many therapeutic strategies and altered management practices in the form of mineral compounds, water-based treatments, restrictive feeding, altered feed form, lower energy dense feeds, cold stress training of birds and altered light program have been thoroughly investigated with small positive advances but nothing considerably influential until now (Baghbanzadeh & Decuypere, 2008, Camacho-Fernandez et al., 2002, Kaoud et al., 2016, Ozkan et al., 2006).

In a future where, adequate global food production will be increasingly more problematic, physiological diseases such as ascites or the ascites related disorder will have to be better managed if not eradicated to meet demands. According to predictions food production will need to be doubled in output within the next 30 years with approximately 48% of this from animal source foods with poultry products contributing the largest fraction (Alexandratos & Bruinsma, 2012, FAO, 2017). Much of this increased food production needs to come from increased efficiency on farm due to limited space for expansion and "sustainable intensification" in order to not exploit and place additional pressure on natural resources (Foley et al., 2011).

Animal based foods contain much needed proteins and nutrients, which are needed to balance a proper diet (Murphy & Allan, 2003). Broiler meat is one of these critical foods needed due to its relative low cost, balanced nutritional content and is especially of importance in developing countries where a large proportion of the population fall below the middle class and need a more cost-effective source of protein-based foods to balance their diets and avoid malnutrition (Mottet & Tempio, 2017). Broiler meat production operations are also seen as more environmentally friendly than other livestock production systems as they place less strain on natural resources (Foley et al., 2011). The production of chicken meat can be considered one of the most environmentally friendly forms of meat production due to: most meat farmed per unit area (up to 60 kg/m^2), most efficient converters of feed to meat (FCR as low as 1.36), thus less nutrient wastage, efficient control and repurposing of excrement, less labour required (automated housing and feeding) and fast throughput.

Even though broiler mortalities related to ascites are not the leading cause to lower production efficiency they still have a considerable impact on the productivity of a broiler rearing operation. It is estimated that up to 5% of female broilers and 20% of male broilers die per cycle of ascites, predominantly due to male broilers inherent capacity for fast growth (Dewel et al., 1996, Balog et al., 2003). The most damaging effect of ascites on the broiler industry is the drop-in overall broiler performance due to sub-clinical ascites cases, where broilers have lowered average daily gain and feed conversion rates.

Taurine supplementation has gained interest over the last few decades due to its favourable effects on cardiovascular functions such as: anti-arrhythmic effects, hypotensive properties, contribution to calcium and sodium homeostasis, cardiac muscle control and the heart tissues ability to release cardiac taurine during ischemia and hypoxemia to minimize heart damage (Huxtable et al., 1992, Ruiz-Feria & Wideman, 2001). In previous studies taurine was found to improve growth performance, feed efficiency and the ability to reduce mortalities in a broiler cycle (Campbell & Classen, 1989). A study by Ruiz-Feria & Wideman, 2001 found that taurine supplementation in the water increases the free taurine concentrations in the blood plasma but not in the heart tissue but a study by Blair et al., 1991 found that a 0.1% taurine dietary inclusion level increases the amount of taurine in the cardiac tissue.

The aim of this project was to assess the effects that dietary taurine supplementation has on broiler performance, the cardiovascular system, and the incidence of ascites related mortalities under commercial conditions. The performance of broiler fed diets with or without poultry by-product meal and supplementation of taurine at different inclusions levels was assessed by comparing BW, FI, FCR, PEF and mortality after a 33-day rearing period. The effects of taurine on the broiler heart were also assessed at 33 days of age, on both males and females.

Hypothesis of the study

The first null hypothesis (H_0) of this study is that dietary taurine supplementation will have no effect on growth performance of Ross308 broilers.
The first alternative hypothesis (H_A) is that dietary taurine supplementation will improve growth performance of Ross308 broilers.

The second null hypothesis (H_0) of this study is that dietary taurine supplementation will have no effect on the incidence of ascites related mortalities of Ross308 broilers.
The second alternative hypothesis (H_A) is that dietary taurine supplementation will have an effect on the incidence of ascites related mortalities of Ross308 broilers.

The third null hypothesis (H_0) of this study is that dietary taurine supplementation will have no effect on the cardiovascular characteristics.

The third alternative hypothesis (H_A) is that dietary taurine supplementation will have an effect on cardiovascular characteristics.

The fourth null hypothesis (H_0) of this study is that dietary taurine supplementation from Poultry by-product meal will have no effect on the cardiovascular characteristics.

The fourth alternative hypothesis (H_A) is that dietary taurine supplementation from Poultry by-product meal will have an effect on cardiovascular characteristics.

Chapter 2

Literature Review

Taurine supplementation in broiler diets have proved in previous studies to improve broiler performance and lower ascites related mortalities when a flock is exposed to an environmental stressor such as high elevation, cold stress, or poor ventilation. Improved broiler performance is normally seen with improved feed conversion efficiencies and overall profitability of a flock. Taurine also exhibits some additional benefits such as the conjugation of bile acids and therefore aiding in lipid digestion and absorption. It also contributes towards important physiological processes such as neuromodulation, immunomodulation, and antioxidant effects. Taurine also serves as an osmolyte and plays a vital role in the development and functioning of the brain, central nervous system (CNS), muscle, heart, and retina (Huang *et al*., 2014). This review serves to provide a more detailed description and discussion around the effect of taurine on broiler performance, the cardiovascular system, and the incidence of ascites related mortalities in broiler flocks, under normal highveld conditions and if stressors are present.

2.1 Ascites Syndrome (AS)

Ascites or known as Pulmonary Hypertension Syndrome (PHS) is not a disease but rather a syndrome or injury that takes place due to changes in normal physiological processes that cause hypoxia. Hypoxia is a condition resulting from a lack of sufficient oxygen supply to the body, its organs and ultimately all tissues. It causes the cardiovascular and pulmonary system to work overtime in order to counter for the lack of sufficient oxygen supply, that could result in permanent organ damage, failure or death if the stressor is present past the point of recovery (Luger *et al*., 2003). Low oxygen supply is mainly caused by environmental factors, which in combination with broilers inherent capacity for fast growth that requires a lot of energy and therefore oxygen cause broilers to experience hypoxia more frequently than in the past with slower growing broilers (Baghbanzadeh & Decuypere, 2008).

The prevalence of ascites challenges in broiler flocks can be caused by a number of different factors and can be divided into four main groups of predisposing factors: Environmental influences, feed related factors, disease challenges and genetic factors. Ascites challenges are predominantly caused by environmental factors in broiler flocks, and it ranges from factors such as high-altitude farming (lower oxygen availability), poor house temperature and ventilation management, as well as inadequate lighting and on-farm management programs. Secondly, feed related factors that influence the growth rate of broilers and the responsible factor being rapid uncontrolled growth of broilers through important developmental phases in their growth cycle. Thirdly but highly important is the presence of certain disease challenges on a farm or more importantly in a broiler flock. Disease challenges include respiratory diseases and all other diseases that place the pulmonary and cardiovascular system under additional pressure and eventually increase the risk of ascites development

(Wideman, 1988). Lastly, genetic factors are not directly responsible for ascites but rather due to its indirect effects, such as the inherent genetic ability modern broilers possess for rapid growth and not so much fast development or increased growth of the very vital organs and structure that supports a broilers capability to grow and survive (Baghbanzadeh & Decuypere, 2008). The modern broiler has intensively been selected for faster rapid growth, larger meat portion sizes such as bigger breast and leg muscles. This intensive selection caused the rest of the broilers body to proportionally increase more in size than that of the organs (Vidyadaran *et al*., 1990).

Ascites is ultimately defined by the accumulation of clear fluid known as lymph fluid in the peritoneal spaces in irregular amounts that are either due to the over production or lowered rate of lymph fluid removal within the peritoneal spaces (Baghbanzadeh & Decuypere, 2008). It is mainly found around the heart and abdominal space with regular amounts ranging between 1-3 ml of lymph fluid within the pericardial sac, any amount above 4 ml is considered abnormal and could impose problematic effects on normal organ function (Kauod *et al*., 2016). Organ damage caused by ascites can either be diagnosed by right ventricular hypertrophy (RVH), heart ruptures, blood clot presence in the heart chambers, excessive plasma fluid accumulation in the pericardial sac or abdominal area and the presence of a flaccid heart (Ononiwu *et* al., 1979, Olkowski *et al*., 1999; Luger *et al*.,2003).

2.1.1 Aetiology of ascites

Ascites syndrome or known as pulmonary hypertension syndrome (PHS), is ultimately a heart defect or injury that is caused by altered physiological processes of the cardiovascular and pulmonary system that progresses in severity over time and if not treated or managed soon enough will result in permanent damage and death. Ascites is either initiated due to increased pulmonary vascular resistance or delayed pulmonary vascular vasodilation following stressors such as high elevation, cold or poor ventilation that cause broilers to experience hypoxia (Wideman & Bottje, 1993).

According to Balog *et al*., (2003) ascites occurs due to one or more physiological changes and as previously mentioned, results in the accumulation of lymph fluid either due to excess produced or insufficient fluid removal. The first change is the accumulation of fluid due to a drainage obstruction, secondly is due to a decrease in the plasma osmotic pressure, thirdly is the leakage of fluid due to increased vascular permeability and lastly a very influential change is where right ventricular failure (RVF) or liver damage causes increased portal pressure (Julian *et al*., 2005).

The onset of ascites or PHS can also be described by changes in the normal cardiopulmonary hemo-dynamics such as: increased cardiovascular activity and vasodilation to ensure sufficient oxygen transport to the tissues. Secondly pulmonary hypertension which is where the arterial pressure is higher than normal, and this is as a result of increased blood flow through a restricted pulmonary vascular system. Lastly hypotension follows as the right ventricle is incapable of increasing output to supply increased blood flow through the lungs (Wideman, 2000).

2.1.2 Effect of environmental factors on the occurrence of ascites

Environmental factors such as low environmental temperatures and high-altitude farming increase the incidence of ascites development in broiler operations (Baghbanzadeh & Decuypere, 2008). Low environmental temperatures cause vasoconstriction of the arteries and veins and could ultimately result in right ventricular failure (RVF) and eventually ascites when the stressor is present for a considerable amount of time. Rearing of broilers at high altitudes is often problematic and the incidence of ascites is higher due to atmospheric hypoxia that causes several physiological changes in broilers such as the effect on haemoglobin. Atmospheric hypoxia causes haemoglobin to have a reduced binding affinity with oxygen which means lower oxygen supply to tissues with tissue hypoxia as an end result (Julian, 2000). Thus, environmental factors are capable of placing additional pressure on broilers that are already near their minimum physiological oxygen needs even at their normal growth rates (Wideman *et al*., 2003). Lower oxygen supply to the body tissues cause increased cardiac output in order to increase blood flow to the lungs with increased blood pressure as a result. The increased cardiac output more specifically the right ventricular side of the heart eventually causes the heart and arteries to deteriorate over time with right ventricular failure (RVF), ascites and eventually death as a result (Julian, 2000).

Poor housing management as previously explained is predominantly the cause of ascites challenges on broiler farms. Poor ventilation is responsible for many of these ascites challenges as it contributes to ascites development either due to the lack of polluted air removal or due to the lack of clean fresh air replacement with sufficient oxygen concentrations. House temperatures if not properly monitored and recorded are also capable of causing ascites challenges and mostly due to broilers being exposed to extended periods of low environmental temperatures that as previously explained cause several alterations in physiological functions to compensate for the cold temperatures experienced. The incidence of ascites due to poor house temperature management is lower than in the past and will continue to decrease due to the modern house designs and automated environmental control systems implemented on broiler farms. These systems significantly reduce the amount of human error if setup well, but a degree of human intervention is still necessary as these systems have their failures.

Another environmental factor that could affect the occurrence of ascites is the type of lighting programs used on farm, as lighting programs indirectly affect broiler growth rates normally due to the “daylight” stimulation that stimulates broilers to spend more or less time feeding which is directly correlated to growth rate. Modern commercial broiler operations make use of lighting programs for the exact same reason, to effectively control growth rate through controlling time spent feeding. If poorly implemented the lighting program can cause permanent damage in a broiler flock, especially in the case of excessive daylight stimulation that will effectively cause over consumption and excessive growth rates that could alter important organ and structural developments in a broiler and lead to mortality spikes due to ascites.

Therefore, it is necessary to find the optimal balance between the many environmental factors that broilers live under to ensure a broiler flock can perform to their full potential and with as little stressors present as possible as these stressors are ultimately the causative factors.

2.1.3 Effect of dietary factors on the occurrence of ascites

A variety of dietary factors influence the incidence of ascites challenges in broilers flocks and nutritionists and producers have used this to their advantage. Manipulating the nutrient density and physical form of the feed has been a common method to control feed intake and subsequently growth which in turn reduces the incidence of ascites in broiler flocks (Baghbanzadeh & Decuypere, 2008).

Camacho *et al*., (2004) investigated the effects of managing growth rates and found that reducing the growth rates between days 3 - 14 of age, benefits a broilers health during those first two weeks when the growth rates are exponentially highest and the benefits of controlling growth rate early on is also seen until the later end of the production cycle when the growth rate is exponentially slower but birds are under the highest amount of pressure.

Feed restriction has been used successfully to reduce the occurrence of ascites in the broiler industry as it is able to reduces the strain placed on an already pressured pulmonary and cardiovascular system. Feed restriction is normally implemented during the peak growth rate phases in a broilers life cycle, but it is important to ensure that the feed restriction does not impair the growth rate to such an extent that the broilers don't reach the correct market weight at marketing (Ozkan *et al*., 2006). Another downside of using feed restriction is that the lower consumption of feed is directly coupled with lower intake of important medicinal products such as coccidiostats, antibiotic growth promotants (AGP), xanthophylls for proper pigmentation, minerals, vitamins and this in turn can negatively impact the health and profitability of a flock if the restriction is to excessive (Camacho-Fernandez *et* al., 2002, Baghbanzadeh & Decuypere, 2008).

A different approach than feed restrictions is the use of restrictive nutrient feeding methods as high nutrient dense diets are directly related to higher growth rates in broilers and place broilers under increased risk of developing ascites. Camacho-Fernandez *et al*., (2002) found that by reducing the nutrient density of diets moderately reduces the incidence of ascites related mortalities especially between the period of placement and week 3, but it is important as mentioned earlier to not impair growth to such an extent that it jeopardises future market weights and profitability of the flock.

Only in the last two decades did researchers notice the importance of poultry feeds physical form on feed intake and growth. Researchers and producers manipulated feeds by pelleting and crumbling them which in effect increases the size of ingestion from one bite/ peck compared to one bite/ peck of feed in mash form. Pelleting and crumbling of feeds in effect increases the density of the ingestion sizes by binding feed once in mash form in a pellet or crumble which is easily ingested by a broiler. Thus, researchers were able to further increase broiler growth rates after this discovery, but it indirectly contributed to higher incidences of ascites in the broiler industry. Thus, diets in mash form are able to reduce feed intake and thus growth rate but feeding mash diets are usually considered uneconomical, more feed wastage and capable of negatively affecting the overall profitability of a broiler operation (Baghbanzadeh & Decuypere, 2008). A study confirming these findings found that manipulation of feed form was able to reduce the occurrence of ascites related mortalities in flocks which received pelleted feeds opposed to the once normal mash feed (Bölükbasi *et al*., 2005).

A few other possible remedies to reduce the occurrence of ascites exist such as supplementing diets with L-carnithine, arginine, antioxidants & omega-3 fatty acids, which have shown to have positive effects on the cardiovascular and pulmonary system (Baghbanzadeh & Decuypere, 2008).

All these dietary factors have one goal in common and that is to reduce or control the rate of growth especially during the periods of a broilers life where important physiological and structural developments are critical.

2.1.4 Effects of genetic factors on the occurrence of ascites

The modern broiler has successfully been genetically selected for rapid growth and improved feed efficiencies over the last few decades, with much of this focusing on achieving increased rate of protein synthesis in order to reach market weights earlier and with less feed. The dilemma of this was that selection practices did not place the same amount of importance on increasing the rate of development of the cardiovascular and pulmonary system, resulting in the hearts and lungs to experience many metabolic diseases/ injuries in the modern broiler (Vidyadaran *et al*., 1990). When the broilers body grows disproportionately with that of the pulmonary and cardiovascular system, it usually causes broiler to develop hypoxia which could lead to ascites, as the lungs can't provide sufficient oxygen to the tissues for normal functions (Julian *et al*., 2000).

The modern broiler has a much smaller lung to body weight (BW) ratio than those broilers of a few decades ago and in conjunction with a broilers thick blood to gas barrier and large red blood cells (RBC) predispose the modern broiler to develop hypoxia and ascites much easier (Vidyadaran *et al*., 1990).

According to Baghbanzadeh & Decuypere, (2008) studies have found that fast-growing broilers experience a higher incidence of heart related issues such as ascites. Decuypere *et al*., (2005) suggested that data from studies indicated that ascites is not caused due to broilers increased requirement for oxygen because of higher growth rates but rather due to lower oxygen supply. This lower oxygen supply is either due to mismanagement of broiler houses or environmental factors as previously mentioned, but the true facts are that the modern broiler with its inherent capacity for rapid growth rates has higher oxygen requirements than those broilers of a few decades ago. Thus, currently all broiler rearing operations make use of broilers with rapid potential growth rates and thus high oxygen requirements to support this rapid growth rates are just met under normal management practices (Julian, 1993).

Furthermore, on a genetic level, modern male broilers have shown to be more susceptible to ascites and this is due to male's inherent genetic potential to grow more rapidly and be more efficient users of feed than females. As previously explained this place the pulmonary and cardiovascular system under increased risk to develop ascites due to increased oxygen demands that are not sufficiently met (Decuypere *et al*., 2000).

Over the past few decades researchers also investigated and found a number of genetic parameters that play major roles in ascites-related traits. Lubritz *et al*., (1995) & Moghadam *et al*., (2001) both found favourable heritability's (h^2) for the ratio of the right ventricular weight to total ventricular weight as well as favourable heritability's for fluid accumulation in the abdominal area. Moghadam also found that heart defects such as PHS and right ventricular failure as a trait are heritable, related to Ascites and positively correlated with broiler body weight. Maxwell *et* al., (1998) soon after concluded that Troponin T, a trait that indicates heart tissue damage is present in higher amounts with ascites related cases and also heritable. Thus, hopefully in the future broiler breeders will be able to implement the use of genetic markers and traits when selecting parent stock in order to possibly breed superior offspring with stronger cardiovascular and pulmonary characteristics and essentially broiler chicks less prone to ascites.

2.1.5 Effects of sex on the occurrence of ascites

As explained above, male broilers are more susceptible to developing ascites due to their inherent genetic capacity for high growth rates that require more energy and oxygen to be carried out, but due to the modern broilers design and the environment, they are reared under it all predisposes the male to higher chance of developing ascites. A study by Brigden & Riddell, (1975) found that approximately 75% of broiler mortalities are male broilers and the study of Bowes & Julian., (1988) a decade later also concluded that 70 – 80 % of SDS mortalities were males. With the rapid progress seen in the broiler breeding industry on in ovo sexing we soon might be farming mostly male broilers on farm, thus synonymously meaning higher chances of ascites challenges on farm due to placed flocks possibly consisting mostly of male broiler chicks. Thus, more emphases will soon need to be placed on effectively controlling ascites.

2.1.6 The different ascites related mortalities

Sudden death syndrome (SDS), also known as flip-overs are normally diagnosed based on a dead broiler with a good body condition score (BCS), a gastro-intestinal tract (GIT) full of feed, an empty gall bladder and the ability to not diagnose the reason for death to any other disease or injury (Bowes & Julian, 1988). SDS mortalities can mostly be identified prior to death by a state of uncontrolled wing flapping and SDS mortalities are mostly found horizontally dead on their backs (Bowes & Julian, 1988, Campbell & Classen, 1989). SDS can take place throughout a broiler cycle from placement until slaughter, with the amount of SDS mortalities usually increasing exponentially as the cycle continues, but according to a few studies completed, SDS mortalities peak during the period 2 - 4 weeks of age and are predominantly found to be males (Brigden & Riddell, 1975, Onuniwa *et al*., 1979, Riddell & Springer, 1985, Steele & Edgar, 1982). Most of the affected birds are usually males with 70 – 80 % of SDS mortalities found as male broilers (Bowes & Julian, 1988). It appears that SDS mortalities are caused firstly by heart damage which eventually leads to fluid build-up in the lungs (edema) and broilers succumb rapidly due to them being unable to breath effectively (Onuniwa *et al*., 1979).

2.1.7 Blood and organ characteristics of ascitic broilers

Ascitic broilers or broilers susceptible to ascites experience lower amounts of plasma protein - albumin which in effect can cause lower blood colloid oncotic pressures (Wise & Evans, 1975; Bowes *et al*., 1989). Lower plasma protein concentrations could be ascribed to lower feed intakes due to broilers experiencing stress or discomfort or due to lymph leakage in the liver (Balog, 2003). As previously explained, high elevations that cause atmospheric hypoxia in broilers reduce haemoglobins affinity to bind oxygen and as a result less oxygen deliverance to tissues that accelerates the rate of ascites development.

Ascites affects broilers primarily due to the large effect on the different vital organs. Some studies have found that broilers that succumbed to ascites had significantly heavier liver weights with this extra weight as a result of fat accumulation, similar to that of broilers experiencing fatty liver syndrome (Bowes & Julian, 1988, Whitehead & Randall, 1982). Some pathological studies have found alterations of the heart, lung, liver, and kidney tissue, but with many other finding none. The study by Onuniwa *et al*., (1979) revealed the following alterations to the different organ tissues: In both the heart and lung tissue degeneration of the tissue fibres were found which is caused primarily by edema and the infiltration of heterophils as a response to inflammation of the different tissues. In the liver and lungs, vascular congestion was found and additionally bleeding of different tissues within the kidney.

2.1.8 Ascites mitigating factors

Several mitigating factors have been found over the years to control the severity of ascites challenges in broiler operations, but normally once present it is very difficult to eradicate from a flock as the slight injuries already endured are normally irreversible. The different mitigating factors can mainly be categorized as non-feed related and feed related. Under non-feed related inputs, proper educated management practices on farm are the most important aspect as poor management practices will ultimately lead to ascites challenges on farm. Adequate on-farm management includes ensuring the following farm practices are carried out as close as possible to the standards, as received from the hatcheries/ breeding company: proper ventilation in the form of air removal and air replacement, optimum temperature profiles and correct lighting programs to ensure a healthy house environment for broilers that cause as little stress as possible. Many ascites challenges are caused by sub-optimal environmental management within broiler houses as broilers don’t handle any changes well.

Feed related inputs will include factors such as: the feeding of lower energy dense rations, altered feed form, fed either in the form of mash than the conventional pellet. Both these strategies aim towards controlling the rate of broiler growth through controlling the rate of feed intake. Broilers fed lower energy dense diets have lower growth rates and the same for broilers fed mash, they have significantly lower feed intakes than those reared with crumbled or pelleted feed due to the strong correlation between increased growth rates, higher feed intake and pelleted feeds.

All these above-mentioned management factors aim to lessen the load placed on an already pressured pulmonary and cardiac system by either avoiding an unhealthy house environment (providing a suitable house environment) or by reducing a broilers inherent capacity for rapid exponential growth, especially during growth phases where the pulmonary and cardiovascular system is under the most pressure. It is important to note that feed intakes should not be lowered to such an extent where it negatively influences the growth and thus profitability of a broiler cycle. A fine balance exists between adequate feed intake and growth rate; therefore, the weekly targets need to be studied before changes are made as each broiler rearing cycle has its own unique set of challenges.

2.2 Taurine

Taurine (2-aminoethanesulfonic-acid) is a sulphur containing free amino acid that is synthesized from methionine or cysteine and it forms part as one of the end products of sulphur metabolism. It has long been considered to only be the end product of sulphur metabolism (Sturman & Hayes, 1980, Huang *et al*., 2014). The name taurine was derived from the Latin name *Bos Taurus*, since it was first sampled and extracted from bovine bile of cattle (Huxtable, 1992). Two possible routes are involved in taurine synthesis in the liver: both pathways start with cysteine being oxidized to cysteine sulfinic acid, but in the one pathway cysteine sulfinic acid is oxidised further to cysteic acid which is eventually decarboxylated to form taurine (Jacobsen & Smith, 1968). In the other pathway cysteine sulfinic acid is converted to hypotaurine via decarboxylation and taurine is formed by further oxidation of hypotaurine (Jacobsen & Smith, 1968). Other tissues such as the brain, skeletal muscle, adipose tissue, and lungs are capable of synthesising Tau endogenously (Lambert *et al*., 2015).

Taurine is considered zwitterionic due its strong acid nature given by the sulfonate group and this causes taurine to have a very high-water solubility and low lipophilicity, in other words taurine dissolves easily in water but not in fats. Compared to most carboxylic amino acids taurine has a very slow rate of absorption through lipophilic membranes and its membrane regulatory actions is probably due to its ionic state (Huxtable, 1992). Taurine is mostly found in the cytoplasmic free amino acid pool of numerous body tissues, as well as in the muscle tissue (myocardium) of the heart. The myocardial tissue cells release taurine when the heart is in an ischemic state to slow tissue damage due to low oxygen supply (Allo *et al*., 1997). When compared to animal tissues, taurine is almost non-existent in plant sources (Martin *et al*.1966, Kataoka & Ohnishi, 1986; Ruiz-Feria *et al*., 1999).

Only during the last two decades has researchers found that taurine plays a more influential role in animal health and nutrition compared to the past where taurine was thought to be insignificant due to its presence in the excreta of broilers and to its lesser to no effect on energy contribution and protein synthesis in broilers (Lee *et al*., 2004, Redmond *et al*., 1998, Shim *et al*., 2009). Taurine either directly or indirectly influences the cardiovascular, nervous, and metabolic system (Dunnington *et al*., 1996). Broiler's taurine requirements are usually met as broilers are capable of producing sufficient levels from sulphur present in the body or feed, for normal maintenance and growth functions. Taurine requirements can differ depending on the amount of taurine required for bile acid conjugation or growth needs (Hayes & Sturman, 1983). Taurine contributes very little towards growth, and therefore taurine deficiencies are unlikely to cause growth related issues. In the past researchers have found that dietary taurine supplementation was advantageous in diets deficient of sulphur containing amino acids such as methionine and cysteine but evidence of increased performance due to taurine in maize and soybean type diets remain vague (Anderson *et al*., 1975).

In feed taurine are supplied in its highest amounts from animal by-products and its presence in grains is very little to completely negligible (Martin *et al*.1966, Kataoka & Ohnishi, 1986; Ruiz-Feria *et al*., 1999). Animal by-products contain variable amounts of taurine, with marine related animal products found to have the highest amounts, such as fishmeal (Spitze *et* al., 2003, Surai *et al*., 2019).

Closer to the 2000's numerous studies focused on the effect of taurine on broiler growth performance, but results were inconsistent and not conclusive. Thereafter, the role of taurine in poultry nutrition lost priority (Blair *et al*., 1991) and more recently, during the past decade, the interest about the effect of taurine in poultry and specifically broilers increased due to the alleviating effects seen on broilers found under stressful conditions, such as oxidative or heat stress (Surai *et al*., 2019). More recent research on the effects of taurine in broilers has been on its intestinal regulation, such as membrane stabilization, growth modulation factors, calcium homeostasis and conjugation of bile acids (Reshetnyak, 2013). The conjugation of bile acids is highly beneficial as it contributes towards improved digestion and absorption of fat (Redmond *et* al., 1998, Reshetnyak, 2013). Taurine has also been shown to exhibit numerous favourable immunological effects and therefore could be used with great benefits on newly placed young broiler chicks (Lee *et al*., 2004).

2.2.1 Effects of dietary taurine supplementation on broiler growth performance

Taurine as previously mentioned is not known for its effect on protein synthesis and contribution as an energy source but rather for its many therapeutic and immunological effects. With regards to growth performance parameters taurine has shown in past studies to have favourable effects on fat digestion and absorption in broilers which could improve feed intake and feed conversion efficiencies. Taurine improves fat digestion and absorption through aiding in the conjugation of bile acids, which in turn form bile salts in the liver which once excreted in the small intestine aid in the solubilization of fats for absorption (Tuft & Jensen, 1992). It has also been reported that the dietary taurine supplementation improves the growth performance of broilers who were fed diets deficient in sulphur (Anderson *et al*., 1975).

The effect of taurine supplementation on broiler growth performance specifically is sparse, inconsistent and many studies outdated. Furthermore, several studies have found that supplementing taurine in diets improves feed efficiency especially in the first week of age (Tufft & Jensen, 1992), increase ADG and decrease FCR (Zeng *et al*., 2009) and some have found that Tau can increase broiler growth performances, but it's important to note that some of these increased growth results are only seen during certain periods/ phases of broilers growth cycle (Dunnington *et* al., 1996, Monson, 1969, Lee *et al*., 2003). Other studies have found no effects on growth performance and feed efficiency where diets were supplemented with 0 - 0.6 % Taurine

(Miller *et al*., 1987) and this was in line with the study of Blair *et al*., (1991) that found no significant (P>0.05) effects on final body weights (BW) but rather significantly (P<0.05) improved feed efficiencies at six weeks of age in broilers fed taurine. A study by Campbell and Classen, (1989) also found no effects on broiler growth performances but improved feed efficiencies where diets were supplemented with 0 - 0.2 % taurine.

An interesting finding by Huang *et al*., (2014) displayed that despite the taurine unfavourable effects on intestinal development and structure in the broiler GIT, it still did not display decreased growth performance or increased mortalities. An earlier study had concluded that increased growth performance could possibly be explained by a complex relationship between a broiler's genetic potential and the level and source of taurine supplementation (Dunnington *et al*., 1996).

2.2.2 Effects of dietary taurine supplementation on the cardiovascular system

Taurine is found freely in the myocardial tissue of the heart with the concentrations peaking between the 7th day of incubation and hatch, thereafter the concentrations remain constant in the post hatch broiler (Dunnington *et al*., 1996). Taurine employs several effects on the cardiovascular system (CVS) as seen in Table 2.2 below. Researchers have found that Taurine is able to regulate the following processes in the heart: Balancing of the sodium (Na) and calcium (Ca) balance, regulation of the heart membrane structure and function as well intracellular fluid (ICF) osmolality (Allo *et al*., 1997). In mammals the taurine concentrations are the highest in the myocardial tissue and it is assumed this is the same for poultry, but it can't be claimed with accuracy (Ruiz-Feria *et al*., 1999). Studies on cats and turkeys have found that cats fed diets deficient of taurine develop cardiomyopathy, which is a condition where the heart struggles to pump adequate blood supply to the rest of the body. Cardiomyopathy usually deteriorates until blindness, heart failure and eventually death (Schaffer *et al*., 1982; Pion *et al*., 1987). In the past before the domestication of pets which we now know as dogs and cats, the importance of taurine in their diets were not known as pets were still evolutionary true carnivores and obtained sufficient amounts of taurine from their natural diets, being mostly muscle tissue (Spitze *et al*., 2003).

Table 2.1 The effect of Taurine supplementation on broiler growth performance parameters

Tau %	Age (d)	BW (g)	FCR	FI (g/d)	Location	Elevation (m)	Ref
Exp 1							
0.25	42	2442.9[a]	1.78[a]	100.9[a]	National Ilan	7	Lee *et al*., 2003.
0.5	42	2440.5[a]	1.75[a]	99.5[a]	university		
0.75	42	2360.2[a]	1.78[a]	98.0[a]			
Exp 2							
0.1	42	2532.0[a]	1.53[a]	90.3[ab]		7	Lee *et al*., 2003.
0.2	42	2463.7[a]	1.54[a]	88.3[b]			
0.3	42	2435.4[a]	1.51[a]	85.6[bc]			
0.4	42	2485.3[a]	1.41[a]	81.8[c]			
0	42	2.070	1.671[a]		Agric Canada	20	Blair *et al*., 1991.
0.025	42	2.043	1.627[b]		Res Station,		
0.05	42	2.071	1.643[ab]		Agassiz, British		
0.10	42	2.066	1.641[ab]		Columbia		
0	7	146	1.282		University of	200	Tufft & Jensen, 1992.
0.8	7	149	1.265		Georgie, Athens		
0	21	558	1.666				
0.8	21	549	1.639				
0	21	621	1.38		Unknown		Campbell & Classen,
0.05	21	615	1.37				1989.
0.10	21	603	1.38				
0.20	21	613	1.37				
			(3-6 wk)				
0	42	1956	2.08[a]				
0.05	42	1950	2.06[ab]				
0.10	42	1954	2.01[b]				
0.20	42	1961	1.97[b]				
		(g/b/d)					Alzawqari *et al*., 2016.
0	7-42	38.7	2.39	89.9			
0.25	7-42	39.5	2.38	91.5			
0.50	7-42	40.3	2.28	89.7			

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

An earlier study by Blair *et al*., (1991) aimed towards influencing the cardiac taurine concentrations by supplementing diets with 0.025 %, 0.05 % and 0.1 % dietary taurine and found that 0.05 % and 0.1 % dietary taurine positively increased the cardiac taurine concentrations opposed to the lower level of taurine and the control diet lacking taurine. A similar study found that dietary taurine supplementation (1mg/kg) in a diet lacking animal by-products minimally increased the cardiac taurine concentrations and a diet containing animal by-products with no taurine supplementation was able to significantly increase the plasma taurine concentrations but not that of the cardiac tissue. Furthermore, during the same study, they found that dietary B-alanine supplementation which is a Tau metabolism antagonist was capable of significantly ($P<0.05$) reducing cardiac taurine concentrations but not plasma taurine concentrations (Ruiz-Feria & Wideman, 2001). It is important to note that cardiac taurine concentrations seem to increase as broilers age (Blair *et al*., 1991). These studies are of importance as it shows that myocardial and plasma taurine content can effectively be manipulated and possibly to our favour, but more research is required.

Taurine supplementation has been reported by several studies to be directly linked with the calcium homeostasis of the cardiac tissue. Calcium concentrations are of high importance in the myocardial tissues as it is responsible for the regulation of normal heart contraction. Sawamura *et al*., (1983) found that oral taurine dosing of broiler chicks before slaughter extended the duration and strength of heart contractions of broilers fed lower calcium levels.

Table 2.2 The effects of taurine on the cardiovascular system (adapted from Huxtable, 1992)

Action	Function
Anti-arrhythmic	Suppress/reduce abnormal heart rhythms
Positively inotropic agents at low Ca	Increase muscular contraction strength
Negatively inotropic agents at high Ca	Weaken muscular contraction strength
Enhancement of digitalis inotropy	
Calcium paradox antagonism	
Hypotensive effect	Decrease blood pressure
Reduces lesion formation in Ca overload cardiomyopathy	
Reduces platelet aggregation	

2.2.3 Effects of dietary taurine supplementation on the incidence of ascites and sudden death related mortalities

In the past studies have investigated the relationship between taurine and sudden death syndrome (SDS) and concluded that taurine has little to no effect on the occurrence of SDS in broiler flocks. During these studies mortalities were not sorted between SDS and ascites, resulting in confusing conclusions about the effect of Tau on the incidence of ascites in broiler flocks (Blair *et al*., 1991, Campbell & Classen, 1989, Jacob *et al*., 1991).

The precise cause of SDS is not always clear but it is mostly caused by sudden death of healthy birds that displayed no other signs of sickness or disease. Heart damage is usually to blame, and this will include broilers with right ventricular failure (RVF) due to ascites, therefore it is of importance to perform detailed post-mortem investigations to determine the possible cause of death (Brigden & Riddell, 1975). An in-depth post-mortem investigation using microscopic evaluations on the myocardial tissue showed that SDS broilers had several alterations to the muscle fibres such as fibre degeneration and separation due to inflammation (Ononiwu *et al*., 1979). SDS broilers can also be identified prior to death by uncontrolled wing flapping and broilers succumbing to SDS are mostly found dead on their back, hence the name "flipovers" that can be used synonymously with the term SDS (Newberry *et al*., 1985). An unusual finding by a study found that broilers that succumbed to SDS displayed ruptured right atriums (Olkowski *et al*., 2007) but it is important to note that this is not a common finding and until now only a single event that we know of, if more mortalities were to be dissected it may increase in prevalence.

Broilers of all ages are susceptible to death due to SDS but peak mortalities due to SDS is after 4 weeks as the birds near marketing age and in a mixed sex flock it is not unusual that up to three quarters (75%) of broilers that succumbed are male broilers (Brigden & Riddell, 1975). According to Ross, if all management factors during the first week of broiler rearing such as adequate feed and water availability, quality and quantity and a favourable house environment (sufficient lighting, ventilation, temperature, stocking density) are provided the average broiler mortality percentage should be less than 0.7% within the first 7 days (Ross Broiler Management Guide, 2018). The rule of thumb for broiler mortalities as that the total average mortalities per week should not exceed the percentage as the week number, for example, the total average mortality percentage for a 5-week-old flock should be 5% or below.

An interesting study by Blair *et al*., (1990) found that the dietary protein source can effectively control the incidence of SDS. During the study they found that broilers receiving poultry by-product meal had lower amounts of SDS related mortalities and this can possibly be ascribed due to taurine's presence in the poultry by-product meal as it is from an animal source. The study by Spitze *et al*., (2003) found poultry by-product meal to have an average amount of 3270 mg taurine per kg dry weight, but amounts were highly variable between samples: 1894 – 5352 mg taurine/kg. The study by Campbell and Classen, (1989) saw lower mortalities and SDS in broilers who received dietary taurine, but there were no significant differences between the taurine supplemented mortality % and control thus more research needs to be done. A conflicting study by the same researcher Blair *et al*., (1991) found no significant ($P<0.05$) differences in total mortalities or those birds that succumbed due to SDS.

A few studies have investigated the relationship between cardiac taurine concentrations and the incidence of ascites related mortalities. The findings are not definitively clear, but some investigations have concluded that there is little to no relationship between cardiac taurine concentrations and the amount of SDS mortalities. A study by Jacob *et al*., (1991) proved the above conclusion by supplementing diets with Tau antagonists, which depletes or lower the Tau concentrations of the myocardium. The study found that effectively lowering the cardiac Tau concentrations does not increase the incidence of SDS mortalities, within a flock.

Rotter *et al*., (1985) analysed heart tissue calcium concentrations and found that broilers that succumbed to SDS had significantly lower heart calcium concentrations than a healthy culled broiler and this is in line with normal physiology, where a balanced calcium homeostasis is required for proper cardiovascular function.

In the current study we recorded and categorized mortalities either as SDS/ flip-over, ascites or other, to provide possible further detailed results.

Table 2.3 The effect of Tau supplementation on the incidence of ascites related mortalities in broilers

Tau %	Age (d)	Mortality (%)	SDS Incidence (%)	% SDS Total deaths	Location	Elevation (m)	Ref
0	21	3.5	1.7	47.6[a]	Agric Canada Res Station, Agassiz, British Columbia	20	Blair *et al.*, 1991.
0.025	21	5.3	2.3	43.8[a]			
0.05	21	4.8	2.0	41.1[b]			
0.1	21	4.8	2.2	44.8[a]			
0	42	9.3	5.5	58.9[a]			
0.025	42	12.5	5.5	44.0[a]			
0.05	42	10.5	4.2	39.7[b]			
0.1	42	9.8	4.2	42.4[a]			
0	42	8.97	6.09[a]		Unknown		Campbell & Classen, 1989.
0.05	42	6.40	3.69[b]				
0.10	42	6.56	4.65[b]				
0.20	42	6.89	4.00[b]				
0	21	2.5			University of Georgie, Athens	200	Tufft & Jensen, 1992.
0.8	21	2.5					

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

2.2.4 Effects of dietary taurine supplementation on broiler blood characteristics

A study by Porter *et al*., (1991) found that taurine concentrations in the blood plasma increases up to 3 times as much (40 – 117 µM) during the first few week's post hatch and remains relatively constant for the remainder of a broiler's life.

2.2.5 Taurine availability from animal sources

As previously mentioned, animal sources contain high amounts of taurine in the tissues compared to animal-based sources that contain very low to negligible amounts of taurine. Within animals the muscle, brain and internal organs contain the highest concentration of taurine (Van Gelder and Bèlanger, 1988).

The amount of taurine found in different animal tissues is variable and a study by Machlin & Pearson, (1957) measured taurine distribution and the amount of taurine taken up by different tissues after injecting chickens with Tau-S^{35}. During their studies they found that the chicken heart absorbs 34 times more and the leg muscle tissue 9 times more Tau-S^{35} compared to the breast muscle tissue. The same can be said for the gastrointestinal tract and spleen as they also contained high amounts of Tau-S^{35}. Other studies found that the mode of feed preparation especially heat treatment causes additional variability in the taurine content of feeds (Spitze *et al*., 2003)

2.2.6 Effects of dietary taurine supplementation on the gastro-intestinal tract and lipid digestion

The gastro-intestinal tract (GIT) determines the future potential and performance of a broiler as the GIT is responsible for the digestion and absorption of dietary constituents and converting them into body tissues and most importantly protein deposition in a broiler. Taurine is found in considerable amounts in the small intestine where it is known for its positive effects on several intestinal functions such as, bile acid conjugation in the liver (cholic & chenodeoxycholic acid), antioxidant effects, villi health and development as well as some contributions towards maintaining intestinal IGF-1 concentrations (Huxtable, 1992, Sturman, 1993, O'flaherty *et al*., 1997).

The absorption of dietary taurine sources in the gastro-intestinal tract is considered fast and efficient with a very high bioavailability (Sved *et al*., 2007) as Tau is transported by enterocytes via the brush border membrane system to the liver via the portal vein and in turn into the rest of the circulatory system (Schaffer & Kim, 2018). Tau absorption by tissues is facilitated by a Na^{+}/Cl^{-} dependant transporter known as Tau-T which is found in considerable amounts in the chicken embryo throughout embryogenesis and the tissues containing the highest Tau-T concentrations during the embryonic stage is the brain, heart and eye (Kim *et al*., 2006; Takahashi & Hatta, 2017).

Bile acid conjugation is seen as a favourable effect that taurine exerts within the GIT as this can promote the digestion and absorption of dietary fats especially saturated fats (Noy & Sklan, 2001). A study by Yuan & Wang, (2010) found that taurine negatively influenced intestinal development more specific duodenal and jejunal development in 6-week-old broilers as this dietary addition of taurine appeared to increase the serum bile acid concentrations to toxic levels, and also, found that it could induce mucosal damage (Yuan & Wang, 2010). The study by Yuan & Wang, (2010) furthermore concluded that dietary taurine supplementation increased the conjugated bile acid concentrations in the jejunum specifically at 7 days and as a result improved ($P<0.05$) the FCR’s during the first week of the trial. A later study by Huang *et al*., (2014) also concluded that taurine supplementation was able to negatively influence morphological developments such as mucosal functioning and development within the small intestine. Aspects that were unfavourably affected include decreased overall weight and length of the duodenum, jejunum, ileum, reduced villus depth and surface area as well as decreased crypt depths. Although these two studies results are inline, more research is needed specifically because the unfavourable effects on intestinal development did not appear to reduce broiler growth performance, feed efficiencies or mortality rate by any means which raises a few questions.

A study by Zeng *et al*., (2012) found that 0.15 % dietary taurine supplementation could increase ($P<0.05$) both lipase activity in the pancreas and small intestine which could possibly improve fat digestion. A more recent study investigated the effects that dietary Tau supplementation has on fat digestibility and found that 0.25 % and 0.55 % dietary Tau supplementation increases fat digestibility significantly (%) in broilers 21 days of age from 64.35 % in the control to 69.02 % ($P<0.05$) and 70.68 % ($P<0.05$) respectively. Increased digestibility percentages were also seen in broilers 42 days of age, 71.31 % in the control, 72.54 % and 74.42 % ($P<0.05$) for 0.25 % and 0.50 % Tau supplementation respectively (Alzawqari *et al*., 2016).

Therefore, increased growth performance and improved feed conversion rates are as a result of taurine that conjugates with bile acids such as cholic acid and chenodeoxycholic acid for example. This in turn stimulates increased fat emulsification which supports lipase activity and thus improved fat digestibility and absorption as a result (Haslewood, 1967). It is important to remember that pursuing improved fat digestibility is essential but not to such an extent that over production of bile acids causes toxicities that harm the GIT and future production of a flock.

2.2.7 Immunological effects of taurine

Taurine is known for many of its immunomodulatory effects in poultry and only during the last decade has interest towards taurine's positive therapeutic effects grown in the poultry research industry (Schuller-Levis & Park, 2004). During a study while analysing carcass and carcass component weights researchers found that dietary taurine supplementation resulted in significantly heavier ($P<0.05$) bursa weights. They found that 0.50 % dietary Tau supplementation caused approximately 58% heavier bursa weights than that of the control (Alzawqari *et al*., 2016). This is in line with previous findings by Schuller-Levis *et al*., (1990) and Wang *et al*., (2014) where Wang concluded that 0,05 % dietary taurine inclusion levels increased the bursa size significantly when compared to the standard control diets. If it is true that increased bursa weights cause increased B-cell production and as an affect improved immunity, it is something to pursue and initiate more investigations on. This would be of extreme importance especially in a constantly evolving world where dynamics have changed in the food and animal production industry, for example the increased interest towards implementing natural based treatments in broiler farming operations to gain improved immunity in our broiler flocks without the use of AGP's as a standard in broiler diets.

The Bursa of Fabricius is a lymph organ found above the tail of poultry and is responsible of very important immune system functions such as the development and maturing of B-lymphocytes needed for the development of innate immunity (Li *et al*., 2007, Wang *et al*., 2001). Thus, taurine appears to stimulate growth of the bursa and this is correlated positively with immunity. The B-cells found within the Bursa contains a highly complex taurine metabolism system that varies with the age of the bird and together with this taurine appears to control the Ca^{2+} uptake and ATPase activity within the maturing B-cells of the developing chick (Porter & Martin, 1992; Porter & Martin 1993).

The effect of taurine on broiler diseases has not been studied extensively in the past but an interesting finding by Lactera *et al*., (1992) found that 0.6 % dietary taurine supplementation increased the anti-Newcastle disease virus antibodies 14 days after vaccination.

2.3 Optimum Taurine inclusion level

The optimum taurine inclusion level is very difficult to summarise as many of these above-mentioned studies used very different inclusion levels. It is also difficult to quantify from which max inclusion level does dietary taurine supplementation cause toxic effects on GIT development and function. From an economic point of view supplementing diets with taurine firstly needs to be further examined and a summary on the return of investment needs to be made to see if the inclusion thereof justifies the added price of inclusion in the broiler diets.

Conclusion

The search for an effective method to reduce the incidence and severity of ascites challenges remains, and as explained in the literature study above, the effective way of doing so has still not been found. Ascites claims a large fraction of all on farm and off-farm mortalities in every cycle but the loss of production through sub-clinical cases also raises a concern. And due to the increasing demand for an affordable, nutritious protein product, current broiler farming operation will need to become more efficient and feed could be a fast-effective way of decreasing losses such as ascites challenges on farm.

Dietary taurine supplementation in broiler diets is not a new concept and has been experimented with high success in the last 50 years, with many positive contributions identified and found. Taurine supplementation shows potential through its many different routes but the precise accurate usage thereof in broiler diets lacks refinement. The use of protein derived by-products has been identified and is known for its inherent high taurine concentrations and past studies have found as explained above the many positive effects on improving broiler growth performance and reducing mortalities.

Therefore, the use of dietary taurine supplementation either from an animal by-product source such as PBY or a synthetic taurine source may effectively provide increased broiler performance through improving growth, feed efficiency or lessening losses due to mortalities, culls or condemnations.

Chapter 3

Materials and Methods

This project was approved by the Faculty of Natural and Agricultural Sciences Ethics Committee of the University of Pretoria (NAS295/2020).

3.1 Birds and housing

The trial was run at the poultry trial facilities at Daybreak Farms, Sundra, South Africa. Broilers were housed in a standard open-sided broiler house, fitted with tunnel ventilation. This house was divided into two separately controlled sides. Two thousand eight hundred and eighty (2880) vaccinated day-old Ross 308 chicks were purchased from the Daybreak Merinovlakte hatchery. On arrival at the trial house, a total of 60 chicks were randomly selected and allocated to one of 48 pens (24 pens per house). Each pen had an area of 3 m^2; however, the bell drinker used occupied an area of 0.407 m^2, resulting in a total usable area for the chicks of 2.593 m^2. Thus, the chicks were placed at a stocking density of 23 birds per m^2. The temperature profile that was followed from 2 days pre-placement to Day 33 is shown in appendix A; the lighting profile is shown in appendix B and the vaccination program is shown in appendix C. The bedding consisted of pine shavings, approximately 10 cm deep and extra pine shavings were added to the pen where needed throughout the trial due to spillages caused by the birds and water change. Each pen contained two tube feeders and a bell drinker of 12 L capacity.

The birds were monitored daily by the principal investigator and trial farm staff. There was farm personnel on the premises at all times throughout the trial to monitor the birds' comfort regarding heat, ventilation, feed, and water supply, as well as general health. Temperature and humidity loggers were installed in both sides at the beginning of the trial to ensure maximum comfort was maintained for the birds throughout the trial. The birds had *ad libitum* access to feed and water at all times.

3.2 Experimental design and treatments

Six different feeds formed part of the six different treatments, and each replicated eight times. Appendix D depicts they layout of the blocks and pens within the poultry house. Treatment 1 was considered as "standard" with treatment 2 and 3 branching out of treatment 1 but increasing respectively in taurine content. Treatment 4 was considered the "standard with PBY" and treatment 5 and 6 branching out of treatment 4 but increasing respectively in taurine content. All the treatments were applied continuously throughout the rearing period. The six treatments used were as follows: Treatment 1 (standard feed); Treatment 2 (standard feed plus 500 gram per ton Taurine); Treatment 3 (standard feed plus 750 gram per ton Taurine); Treatment 4 (standard feed plus PBY meal included at 3.5%); Treatment 5 (standard feed plus 3.5% PBY meal and 500 gram per ton Taurine) and Treatment 6 (standard feed plus 3.5% PBY meal and 750 gram per ton Taurine).

All feeds were formulated to the same specifications according to Ross 308 guidelines and were mixed for AFGRI Animal Feeds by one of their factories in AFGRI Animal Feeds Isando Mill (Kempton Park, 1600, South Africa) and were mainly maize-soybean meal diets. Feeds were then produced one at a time and all materials were hand weighed to ensure accuracy in measurement. Lines were flushed after the production of each feed to ensure no cross contamination of taurine and poultry-by-product meal over different treatments. A three-phase feeding program was used including a starter (S), grower (G) and finisher (F). The S was fed as an expanded crumble whereas the G and F were fed as pellets that were produced through a 4mm die. The S feed was fed from 0 to 17 days of age, G from 18 to 27 days of age and F from 28 to 33 days of age after which the birds were caught and taken to the abattoir. Appendix E depicts expected feed intake and feed allocation per pen per treatment, throughout the 33 days. Each treatment was formulated separately but to the same specifications to ensure consistency in the nutrient composition. The feeds were formulated on a least cost basis using Bestmix v3.32 201

Table 3.1 Treatments

Treatment	Label	Treatment	Tau Inlc %	Replicates
1	A	Control	0	8
2	B	500 g/ton Taurine	0.05	8
3	C	750 g/ton Taurine	0.075	8
4	D	Control with PBY	0	8
5	E	500 g/ton Taurine with PBY	0.05	8
6	F	750 g/ton Taurine with PBY	0.075	8

3.3 Measurements

3.3.1 Chemical analysis of feed samples

Analyses of feed samples for each phase was conducted using wet chemistry before the feed was delivered to the trial facility to ensure that the feeds had the same nutrient composition. Table 3.2 shows the wet-chemistry results that were obtained via analysis at the quality control laboratory on site for AFGRI Animal Feeds at the Isando feed mill in Isando (Isando, 1609, South Africa).

Table 3.2 Wet-chemistry results (% DM) obtained per phase analysed

Treatment	Phase	Protein	Moisture	Ca	Na	Cl	P	K
1	Starter	22.23	11.19	0.81	0.23	0.36	0.55	0.89
	Grower	21.52	11.08	0.69	0.20	0.34	0.49	0.81
	Finisher	22.16	10.68	0.83	0.20	0.35	0.47	0.99
2	Starter	20.59	12.44	0.72	0.21	0.37	0.51	0.79
	Grower	21.49	11.11	0.70	0.20	0.34	0.49	0.86
	Finisher	20.47	13.97	0.80	0.18	0.31	0.47	0.83
3	Starter	21.38	11.60	0.76	0.23	0.38	0.58	0.86
	Grower	21.54	9.07	0.64	0.21	0.35	0.50	0.87
	Finisher	22.51	9.98	0.91	0.20	0.34	0.49	0.95
4	Starter	21.39	11.24	0.80	0.28	0.40	0.52	0.86
	Grower	21.44	9.64	0.75	0.21	0.42	0.52	0.82
	Finisher	21.61	10.56	0.85	0.20	0.35	0.48	0.99
5	Starter	20.84	11.14	0.79	0.23	0.38	0.59	0.83
	Grower	20.92	10.94	0.70	0.19	0.36	0.52	0.76
	Finisher	22.60	10.41	0.80	0.21	0.38	0.52	0.86
6	Starter	21.13	11.46	0.81	0.22	0.38	0.53	0.86
	Grower	19.02	11.24	0.77	0.20	0.36	0.51	0.79
	Finisher	20.65	13.44	0.70	0.17	0.30	0.44	0.78

3.3.2 Performance measurements

3.3.2.1 Bodyweight (BW)

Broilers were weighed weekly to obtain average BW for each individual pen of 60 birds. All the birds in a pen were weighed collectively in a crate, which was tared before every weighing, and the average bodyweight was then calculated by dividing the recorded value by the number of birds in the pen. The day-old chicks were weighed at placement and then repeated weighings at 7, 14, 21, 28 and 33 days of age.

3.4.2.2 Feed intake (FI)

Weekly feed intake was measured by weighing out a specific amount of feed at the beginning of the phase into bins in front of each pen. Two tube-feeders in each pen were kept full by adding feed from the bins and at the end of each week the feeders and bins were weighed to determine the amount of remaining feed that was left over at the end of the week. The weekly weighing of feed intake occurred at the same time as the weighing of the birds. Cumulative feed intake was calculated by the summation of the weekly feed intakes.

3.4.2.3 Feed conversion ratio (FCR)

The cumulative FCR was calculated by dividing the cumulative FI of the pen by the total BW gained per pen, over the experimental period and was corrected for mortality by adding the bodyweight gain of the mortalities during the week to the bodyweight gain of the pen during the week.

3.4.2.4 European performance efficiency factor (PEF)

The PEF value is a calculated value incorporating all of the performance factors and is regarded as a good measure of overall performance, for commercial purposes.

PEF = (Liveability % x Mass (kg)/ Age in Days x FCR) x100

3.4.2.5 Mortalities

The trial house was inspected twice daily; any mortalities were removed, weighed, and recorded as either death due to ascites, sudden death syndrome (SDS) or other/unknown.

3.5 Physiological measurements

At the end of the trial (33 days) one male and one female broiler per treatment replicate was randomly selected which resulted in 16 birds (8 male & 8 females per treatment) being selected for physiological measurements per treatment. These birds were then culled ethically by cervical dislocation and weighed, dissected and further physiological measurements were taken at the Daybreak trial houses by trained personal.

3.5.1 Total heart weight

During dissection the heart of each bird was removed, cleaned of most visceral fat and non-heart tissue. Thereafter the hearts were stored in buffered formalin (10%) for later weighing. The hearts were later weighed with a small digital scale accurate to 0.01 grams. The weights were measured in grams and recorded.

3.5.2 Heart weight as a percentage of bodyweight (HW/BW%)

The weight of the heart (in grams) was divided by the bodyweight of the bird (in grams) and multiplied by one hundred to equal the HW/BW%.

3.5.3 Right ventricle weight

After weighing the heart, the heart was cut laterally one third up from the apex of the heart to expose the right ventricular cavity. After the right ventricular cavity diameter and right ventricle wall thickness was recorded the right ventricle was carefully removed with a sharp surgical scissor and thereafter weighed and recorded. Please refer to appendix F for visual representations.

3.5.4 Right ventricle to heart weight ratio (RV:HW)

After weighing the right ventricle weight (in grams) was compared with the total heart weight (in grams) to determine the right ventricle to heart weight ratio.

3.5.5 Right ventricle cavity diameter

As mentioned previously the heart was cut laterally exactly one third up from the apex to expose the right ventricle cavity where the diameter was measured in millimetres (mm) with an electronic calliper/ vernier.

3.5.6 Right ventricle wall thickness

After exposing the right ventricle cavity, the right ventricle wall thickness was measured and recorder in millimetres (mm) with an electronic calliper/ vernier.

3.6 Mortality measurements

The trial houses were inspected twice daily for mortalities. Mortalities were collected throughout the trial and weighed and categorized as either death due to ascites, sudden death syndrome (SDS) or other/unknown causes.

3.6.1 Ascites mortalities

Mortalities were categorized as death due to ascites when a mortality displayed one of the following signs: Water belly, which is double checked by dissection to confirm that either the pericardial sac or abdominal area contains a water like yellow fluid.

3.6.2 Sudden death syndrome mortalities

Mortalities were categorized as death due to SDS when a mortality displayed one of the following signs: Uncontrolled wing flapping prior to death or when a mortality was found lying flat on its back with no other visual signs or reasons for death.

3.6.3 Other/ unknown mortalities

Mortalities that did not fit the description for either ascites or SDS was recorded in this category.

3.7 Statistical analysis

Data was analysed using randomized block design (RBD) which was used for the application of the various replications within treatments (the different treatments are nested in blocks and blocks in houses). Data were analysed using the statistical program GenStat® (VSN International, 2017). Tukey's 95% confidence interval was applied to measure and determine significance between the different treatments as well as treatment means and standard error. Repeated Measures such as the weekly measurement Analysis of Variance with the generalized linear model was used.

The generalized linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + L_j + TL_{ij} + e_{ij}$$

Where Y = variable studied during the period

μ = overall mean of the population

T = effect of the ith treatment

L = effect of the jth level

TL = effect of the ijth interaction between treatment and level

e = error associated with each Y

The Generalized linear models (GLM) used extend the usual regression analysis to cater for non-normal distributions. They also incorporate a link function that defines the transformation required to make the model linear.

Chapter 4

Results

4.1 Performance data

4.1.1 Bodyweight (BW)

Table 4.1 illustrates the weekly BW (g) of the broilers of the six different dietary treatment diets and table 4.2 shows the ADG (g) of the broilers of the six different dietary treatment diets.

Day old

There were no significant differences (P>0.05) in BW between treatments on day 0.

Day 7

There were no significant differences (P>0.05) in 7-day BW's between treatments with different taurine dietary inclusion levels.

Day 14

Varying levels of dietary taurine caused significant differences (P<0.05) in 14-day BW's of Ross 308 broilers. Broilers that received Treatment 6 were significantly (P<0.05) heavier on 14 days of age than broilers that received Treatment 1, 2, 3 and 4. Broilers that received Treatment 5 were significantly (P<0.05) heavier on 14 days of age than broilers that received Treatment 1, 3 and 4. Broilers that received Treatment 2 were significantly (P<0.05) heavier on 14 days of age than broilers that received Treatment 4. There was no significance (P>0.05) in 14-day BW's between the rest of the treatments.

Day 21

Varying levels of dietary taurine caused significant differences (P<0.05) in 21-day BW's of Ross 308 broilers. Broilers that received Treatment 5 and 6 were significantly (P<0.05) heavier on 21 days of age than broilers that received Treatment 1, 3 and 4. Broilers that received Treatment 2 were significantly (P<0.05) heavier on 21 days of age than broilers that received treatment 4. There was no significance (P>0.05) in 21-day BW's between the rest of the treatments.

Day 28

Varying levels of dietary taurine caused significant differences (P<0.05) in 28-day BW's of Ross 308 broilers. Broilers that received Treatment 2, 5 and 6 were significantly (P<0.05) heavier on 28 days of age than broilers that received Treatment 4. There was no significance (P>0.05) in 28-day BW's between the rest of the treatments.

Day 33

Varying levels of dietary taurine caused significant differences (P<0.05) in 33-day BW's of Ross 308 broilers. Broilers that received Treatment 6 were significantly (P<0.05) heavier on 33 days of age than broilers that received the control without taurine supplementation Treatment 1 and 4. There was no significance (P>0.05) in 33-day BW's between the rest of the treatments, which all contained some level of taurine.

In summary, broilers that received different levels of dietary taurine showed significant (P<0.05) differences in BW every week after week 2 throughout the trial.

Table 4.1 Weekly BW (g) (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)					
		0	7	14	21	28	33
Control	0	44.69	183.2	427.8	827.4	1450	1973
		(±1.0585)[a]	(±6.170)[a]	(±10.67)[cd]	(±30.71)[bc]	(±46.46)[ab]	(±48.98)[b]
500 g/ton	0.05	44.44	178.5	446.8	867.6	1508	2036
		(±0.9713)[a]	(±5.426)[a]	(±12.35)[bc]	(±44.71)[ab]	(±28.33)[a]	(±27.09)[ab]
750 g/ton	0.075	44.62	180.1	428.0	816.2	1448	1996
		(±1.0388)[a]	(±7.704)[a]	(±12.04)[cd]	(±32.65)[bc]	(±45.76)[ab]	(±66.93)[ab]
Control with PBY	0	44.50	175.9	420.2	806.1	1415	1964
		(±0.7440)[a]	(12.024)[a]	(±20.68)[d]	(±65.47)[c]	(±54.39)[b]	(±60.84)[b]
500 g/ton with PBY	0.05	44.67	175.8	465.7	899.8	1493	2016
		(±1.1443)[a]	(±10.658)[a]	(±22.30)[ab]	(±53.02)[a]	(±69.15)[a]	(±88.07)[ab]
750 g/ton with PBY	0.075	44.85	176.1	473.0	911.4	1517	2070
		(±0.9267)[a]	(±9.086)[a]	(±11.41)[a]	(±32.89)[a]	(±35.43)[a]	(±57.12)[a]
$\overline{x}$		44.6	178.3	443.6	854.6	1471.8	2009.2

[1]Column means with the same superscript are not significantly (P>0.05) different from each other.

Day 0-7

No significant (P>0.05) differences at 7 days of age were found in ADG of Ross 308 broilers between the different dietary treatments.

Day 7-14

Significant differences (P<0.05) were found in ADG of Ross 308 broilers at 14 days of age that were fed treatments with varying levels of taurine. Broilers that received Treatment 5 and 6 had significantly (P<0.05) higher ADG than Treatment 1, 2, 3 and 4 up to 14 days of age. Broilers that received Treatment 2 had significantly (P<0.05) higher ADG that Treatment 1, 3 and 4 up to 14 days of age. The rest of the treatments showed no significant differences (P>0.05) in ADG from one another up to 14 days of age.

Day 14-21

Significant differences (P<0.05) were found in ADG of Ross 308 broilers at 21 days of age that were fed treatments with varying levels of taurine. Broilers that received Treatment 5 and 6 had significantly (P<0.05) higher ADG that Treatment 3 and 4 up to 21 days of age. The rest of the treatments showed no significant differences (P>0.05) in ADG from one another up to 21 days of age.

Day 21-28

No significant (P>0.05) differences at 28 days of age were found in ADG of Ross 308 broilers between the different dietary treatments.

Day 28-33

No significant (P>0.05) differences at 33 days of age were found in ADG of Ross 308 broilers between the different dietary treatments.

Table 4.2 Average Daily Gain (ADG) (g) (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	**Taurine %**	**Age (Days)**				
		0-7	7-14	14-21	21-28	28-33
Control	0	19.79	34.93	57.0	88.91	104.7
		(±0.987)[a]	(±1.070)[a]	(±3.530)[ab]	(±6.075)[a]	(±7.190)[a]
500 g/ton	0.05	19.16	38.32	60.11	91.43	105.7
		(±0.716)[a]	(±1.114)[b]	(±6.017)[ab]	(±4.352)[a]	(±7.111)[a]
750 g/ton	0.075	19.36	35.41	55.46	90.21	109.6
		(±1.105)[a]	(±1.517)[c]	(±3.663)[b]	(±3.325)[a]	(±7.156)[a]
Control with PBY	0	18.77	34.90	55.12	87.0	109.6
		(±1.652)[a]	(1.497)[c]	(±8.260)[b]	(±8.472)[a]	(±5.311)[a]
500 g/ton with PBY	0.05	18.73	41.41	62.02	84.74	104.7
		(±1.416)[a]	(±1.741)[a]	(±7.190)[a]	(±8.076)[a]	(±11.611)[a]
750 g/ton with PBY	0.075	18.75	42.41	62.63	86.52	110.6
		(±1.267)[a]	(±1.159)[a]	(±4.017)[a]	(±7.224)[a]	(±6.531)[a]
$\bar{x}$		19.09	37.90	48.33	69.17	86.07

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.1.2 Cumulative feed intake (FI)

Table 4.3 illustrates the cumulative feed intake of Ross 308 broilers that received different treatments with varying levels of taurine.

Day 0-7

Significant differences (P<0.05) were found in FI of Ross 308 broilers up until 7 days of age, that were fed treatments with varying levels of dietary taurine. Broilers that received Treatment 5 and 6 had significantly (P<0.05) lower FI that Treatment 2 and 3 up until 7 days of age. The rest of the treatments showed no significant differences (P>0.05) in FI from one another.

Day 0-14

Significant differences (P<0.05) were found in FI of Ross 308 broilers up until 14 days of age, that were fed treatments with varying levels of dietary taurine. Broilers that received Treatment 1 had significantly (P<0.05) lower FI that Treatment 2 and 6 up until 14 days of age. The rest of the treatments showed no significant differences (P>0.05) in FI from one another.

Day 0-21

No significant (P<0.05) differences up until 21 days of age were found between FI between the different dietary taurine treatments.

Day 0-28

No significant (P<0.05) differences up until 28 days of age were found between FI between the different dietary taurine treatments.

Day 0-33

No significant (P<0.05) differences up until 33 days of age were found between FI between the different dietary taurine treatments.

Table 4.3 Cumulative feed intakes (g/bird) (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		0-7	0-14	0-21	0-28	0-33
Control	0	145.9	521.1	1060	2037	2798
		(±5.499)[ab]	(±25.94)[b]	(±37.31)[a]	(±73.48)[a]	(±113.5)[a]
500 g/ton	0.05	156.0	554.5	1073	2049	2829
		(±6.122)[a]	(±10.02)[a]	(±30.16)[a]	(±77.81)[a]	(±121.2)[a]
750 g/ton	0.075	154.7	530.0	1083	2019	2774
		(±12.938)[a]	(±20.69)[ab]	(±67.75)[a]	(±79.59)[a]	(±122.4)[a]
Control with PBY	0	146.8	532	1106	2039	2794
		(±8.401)[ab]	(±22.64)[ab]	(±73.28)[a]	(±663.21)[a]	(±108.4)[a]
500 g/ton with PBY	0.05	139.8	551.2	1089	2015	2751
		(±7.842)[b]	(±19.42)[ab]	(±71.31)[a]	(±67.07)[a]	(±96.3)[a]
750 g/ton with PBY	0.075	136.4	557.9	1130	2078	2841
		(±10.310)[b]	(±17.81)[a]	(±91.84)[a]	(±48.32)[a]	(±62.8)[a]
$\overline{x}$		125.7	463.8	934.4	2039.5	2398.1

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.1.3 Cumulative feed conversion ratio (FCR)

The FCR (g feed/ g BW) was comparable with normal commercial FCR and showed significant differences (P<0.05) between treatments when varying levels of dietary taurine were fed to Ross 308 broilers. Significant differences (P<0.05) in FCR were found throughout the duration of the trial with every week showing significant differences on cumulative FCR. Table 4.4 illustrates the differences in FCR, calculated weekly throughout the trial between Ross 308 broilers that were fed treatments feeds with varying levels of dietary taurine.

Day 0-7

The 7-day cumulative FCR of Ross 308 broilers fed different treatments with varying dietary taurine levels, differs significantly (P<0.05) from one another. Treatment 6 had significantly (P<0.05) lower 7-day FCR's that Treatment 2. No significant (P>0.05) differences in 7-day FCR's were found between the rest of the treatments.

Day 7-14

The 14-day cumulative FCR of Ross 308 broilers fed different treatments with varying dietary taurine levels, differs significantly (P<0.05) from one another. Treatment 5 and 6 had significantly (P<0.05) lower 14-

day FCR's that Treatment 4. No significant (P>0.05) differences in 14-day FCR's were found between the rest of the treatments.

Day 14-21

The 21-day cumulative FCR of Ross 308 broilers fed different treatments with varying dietary taurine levels, differs significantly (P<0.05) from one another. Treatment 2, 5 and 6 had significantly (P<0.05) lower 21-day FCR's that Treatment 4. No significant (P>0.05) differences in 21-day FCR's were found between the rest of the treatments.

Day 21-28

The 28-day cumulative FCR of Ross 308 broilers fed different treatments with varying dietary taurine levels, differs significantly (P<0.05) from one another. Treatment 2 and 5 had significantly (P<0.05) lower 28-day FCR's that Treatment 4. No significant (P>0.05) differences in 28-day FCR's were found between the rest of the treatments.

Day 28-33

There were no significant (P>0.05) differences in cumulative FCR on 33 days of age between any of the different dietary treatments.

In summary, significant (P<0.05) differences in cumulative FCR were found on every week throughout the trial except the last week. All cumulative FCR's were commercially acceptable and thus no values were regarded as outliers.

Table 4.4 Cumulative feed conversion rates (g feed/g BW) (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		0-7	0-14	0-21	0-28	0-33
Control	0	1.054	1.362	1.356	1.452	1.452
		(±0.03950)[ab]	(±0.08960)[ab]	(±0.06066)[ab]	(±0.07874)[ab]	(±0.07522)[a]
500 g/ton	0.05	1.16	1.379	1.307	1.400	1.420
		(±0.02033)[a]	(±0.02584)[ab]	(±0.06786)[b]	(±0.04705)[b]	(±0.05728)[a]
750 g/ton	0.075	1.148	1.383	1.407	1.441	1.425
		(±0.15335)[ab]	(±0.05838)[ab]	(±0.11112)[ab]	(±0.09138)[ab]	(±0.10244)[a]
Control with PBY	0	1.121	1.418	1.463	1.488	1.457
		(±0.05797)[ab]	(±0.05429)[a]	(±)[a]	(±0.05972)[a]	(±0.05854)[a]
500 g/ton with PBY	0.05	1.070	1.310	1.275	1.392	1.397
		(±0.07472)[ab]	(±0.03170)[b]	(±0.07942)[b]	(±0.04249)[b]	(±0.05484)[a]
750 g/ton with PBY	0.075	1.040	1.303	1.304	1.412	1.403
		(±0.06261)[b]	(±0.03941)[b]	(±0.10192)[b]	(±0.03653)[ab]	(±0.04107)[a]
$\bar{x}$		1.09	1.36	1.352	1.43	1.43

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

Table 4.5 Weekly FCR (g feed/g BW) (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		0-7	0-14	0-21	0-28	0-33
Control	0	1.054	1.537	1.353	1.575	1.644
		(±0.03950)[ab]	(±0.13070)[ab]	(±0.1375)[ab]	(±0.1779)[a]	(±0.1558)[a]
500 g/ton	0.05	1.163	1.486	1.242	1.524	1.713
		(±0.02033)[a]	(±0.03356)[ab]	(±0.1310)[b]	(±0.0763)[a]	(±0.0826)[a]
750 g/ton	0.075	1.148	1.516	1.430	1.484	1.626
		(±0.15335)[ab]	(±0.09105)[ab]	(±0.1903)[ab]	(±0.1380)[a]	(±0.1978)[a]
Control with PBY	0	1.121	1.578	1.518	1.543	1.627
		(±0.05797)[ab]	(±0.07017)[a]	(±0.2946)[a]	(±0.2216)[a]	(±0.1474)[a]
500 g/ton with PBY	0.05	1.070	1.420	1.244	1.567	1.639
		(±0.07472)[ab]	(±0.01915)[b]	(±0.1541)[b]	(±0.1234)[a]	(±0.1672)[a]
750 g/ton with PBY	0.075	1.040	1.420	1.306	1.572	1.613
		(±0.06261)[b]	(±0.04455)[b]	(±0.2032)[ab]	(±0.1267)[a]	(±0.0782)[a]
$\bar{x}$		1.09	1.49	1.35	1.54	1.64

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.1.4 European performance efficiency factor (PEF)

Significant (P<0.05) differences were found in PEF right throughout the 33-day trial period between Ross 308 broilers that were fed diets with varying taurine inclusion levels. Table 4.6 illustrates the weekly PEF of Ross 308 broilers fed different levels of dietary taurine levels.

Day 7

No significance (P>0.05) was found between Ross 308 broilers at 7 days of age between any of the different treatments.

Day 14

Ross 308 broilers that received Treatment 5 and 6 had significantly (P<0.05) higher 14-day PEF values than broilers that received Treatment 1, 2, 3 and 4. No significant (P>0.05) differences were found between the rest of the treatments at 14 days of age.

Day 21

Ross 308 broilers that received Treatment 5 had significantly (P<0.05) higher 21-day PEF values than broilers that received Treatment 1, 3 and 4. Broilers that received Treatment 6 had significantly (P<0.05) higher 21-day PEF values than broilers that received Treatment 3 and 4. Broilers that received Treatment 2 had significantly (P<0.05) higher 21 day PEF values than broilers that received Treatment 4. No significant (P>0.05) differences were found between the rest of the treatments at 14 days of age.

Day 28

Ross 308 broilers that received Treatment 2,5 and 6 had significantly (P<0.05) higher 28-day PEF values than broilers that received Treatment 4. No significant (P>0.05) differences were found between the rest of the treatments at 28 days of age.

Day 33

No significant (P>0.05) differences were found between the rest of the treatments at 33 days of age.

In summary, significant (P<0.05) differences were found in weekly PEF between Ross 308 broilers fed diets with varying levels of dietary taurine. The weekly PEF values favoured treatments with higher dietary taurine inclusion levels.

Table 4.6 PEF (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		0-7	0-14	0-21	0-28	0-33
Control	0	246.8	222.2	284.2	345.6	370.0
		(±16.49)[a]	(±19.43)[b]	(±23.98)[bcd]	(±31.86)[ab]	(±29.61)[a]
500 g/ton	0.05	218.4	229.7	312.**4**	374.6	395.0
		(±8.08)[a]	(±11.17)[b]	(±32.72)[abc]	(±18.86)[a]	(±22.34)[a]
750 g/ton	0.075	228.1	220.5	276.1	354.1	390.9
		(±33.81)[a]	(±16.60)[b]	(±32.05)[cd]	(±36.82)[ab]	(±46.83)[a]
Control with PBY	0	223.8	209.5	262.3	331.3	371.7
		(±25.98)[a]	(±16.60)[b]	(±55.17)[d]	(±26.09)[b]	(±28.94)[a]
500 g/ton with PBY	0.05	235.9	253.1	336.3	378.1	398.6
		(±28.31)[a]	(±16.73)[a]	(±35.04)[a]	(±27.85)[a]	(±29.59)[a]
750 g/ton with PBY	0.075	240.7	256.8	328.3	372.9	401.7
		(±19.96)[a]	(±11.12)[a]	(±27.27)[ab]	(±18.27)[a]	(±21.49)[a]
$\bar{x}$		232.28	231.96	299.93	359.43	387.98

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.2 Physiological data

4.2.1 Heart analysis

Table 4.7 illustrates all the different heart measurements recorded for male Ross 308 broilers at 33 days of age that were fed treatments with different dietary taurine inclusion levels. All birds culled were selected based on body weight as close as possible to mean BW between all six treatments.

Table 4.7 Male broiler heart characteristics 33 days (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	BW (g)	HW (g)	HW/BW %	RV (g)	RV: HW	RV Cavity dia (mm)	RV Wall Thick (mm)
Control	0	2170	9.402	0.4332	1.884	0.1990	1.636	2.469
		(±138.4)	(±1.0126)	(±0.03907)	(±0.4180)	(±0.02765)	(±1.478)	(±1.0639)
500 g/ton	0.05	2162	9.212	0.4259	1.704	0.1852	0.644	1.951
		(±144.0)	(±0.8601)	(±0.02419)	(±0.2256)	(±0.02049)	(±0.946)	(±0.6584)
750 g/ton	0.075	2196	9.195	0.4185	1.691	0.1833	1.688	2.186
		(±109.1)	(±0.9068)	(±0.02942)	(±0.2965)	(±0.02193)	(±1.117)	(±0.6462)
Control with PBY	0	2180	9.612	0.4410	1.768	0.1835	1.201	1.822
		(±135.3)	(±1.1583)	(±0.04629)	(±0.3122)	(±0.01956)	(±1.502)	(±0.6422)
500 g/ton with PBY	0.05	2290	9.596	0.4181	1.781	0.1844	0.703	1.802
		(±102.0)	(±1.1730)	(±0.03800)	(±0.3561)	(±0.01827)	(±0.908)	(±0.8937)
750 g/ton with PBY	0.075	2180	9.091	0.4176	1.746	0.1927	1.549	1.931
		(±123.1)	(±0.6346)	(±0.03057)	(±0.1206)	(0.01922)	(±1.859)	(±0.6998)

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.2.1.1 Male heart weights

No significant (P>0.05) differences were found in male Ross-308 broiler heart weights between any of the different treatments with different dietary taurine inclusion levels at 33 days of age.

4.2.1.2 Male heart weight to Body weight percentage

No significant (P>0.05) differences were found in male Ross-308 broiler heart weight to body weight percentages between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.3 Male right ventricle weight

No significant (P>0.05) differences were found in male Ross-308 broiler heart right ventricle weights between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.4 Male right ventricle to heart weight ratio

No significant (P>0.05) differences were found in male Ross-308 broiler right ventricle to heart weight ratios between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.5 Male right ventricle cavity diameter

No significant (P>0.05) differences were found in male Ross-308 broiler heart right ventricle cavity diameters between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.6 Male right ventricle wall thickness

No significant (P>0.05) differences were found in male Ross-308 broiler heart right ventricle wall thickness between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

Table 4.8 illustrates all the different heart measurements recorded for female Ross 308 broilers at 33 days of age that were fed treatments with different dietary taurine inclusion levels. All birds culled were selected based on body weight as close as possible to mean BW between all six treatments.

Table 4.8 Female broiler heart characteristics 33 days (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	BW (g)	HW (g)	HW/BW %	RV (g)	RV: HW	RV Cavity dia (mm)	RV Wall Thick (mm)
Control	0	1924	7.435	0.3872	1.500	0.2006	1.377	1.73
		(±87.5)	(±0.6956)	(±0.04044)	(±0.2889)	(±0.02404)	(±1.308)	(±0.5562)
500 g/ton	0.05	1920	7.421	0.3851	1.467	0.1932	1.265	1.953
		(±133.4)	(±1.4302)	(±0.05792)	(±0.5556)	(±0.03329)	(±1.382)	(±0.5562)
750 g/ton	0.075	1970	7.848	0.3984	1.459	0.1867	1.316	1.555
		(±127.8)	(±0.8923)	(±0.03323)	(±0.1341)	(±0.01543)	(±1.021)	(±0.7052)
Control with PBY	0	1986	7.894	0.3984	1.481	0.1855	0.990	1.549
		(±99.4)	(±0.8841)	(±0.05018)	(±0.4733)	(±0.04241)	(±1.185)	(±1.0498)
500 g/ton with PBY	0.05	2062	7.718	0.3734	1.580	0.2012	1.226	1.623
		(±111.5)	(±0.8155)	(±0.02466)	(±0.4982)	(±0.04330)	(±1.125)	(±0.7264)
750 g/ton with PBY	0.075	1910	7.175	0.3761	1.361	0.1904	1.299	1.758
		(±120.9)	(±0.3935)	(±0.01591)	(±0.0990)	(0.01805)	(±1.101)	(±0.4713)

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.2.1.7 Female heart weights

No significant (P>0.05) differences were found in female Ross-308 broiler heart weights between any of the different treatments with different dietary taurine inclusion levels at 33 days of age.

4.2.1.8 Female heart weight to Body weight percentage

No significant (P>0.05) differences were found in female Ross-308 broiler heart weight to body weight percentages between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.9 Female right ventricle weight

No significant (P>0.05) differences were found in female Ross-308 broiler heart right ventricle weights between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.10 Female right ventricle to heart weight ratio

No significant (P>0.05) differences were found in female Ross-308 broiler right ventricle to heart weight ratios between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.11 Female right ventricle cavity diameter

No significant (P>0.05) differences were found in female Ross-308 broiler heart right ventricle cavity diameters between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.12 Female right ventricle wall thickness

No significant (P>0.05) differences were found in female Ross-308 broiler heart right ventricle wall thickness between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

Table 4.9 illustrates all the different heart measurements recorded for male and female Ross 308 broilers combined at 33 days of age that were fed treatments with different dietary taurine inclusion levels. All birds culled were selected based on body weight as close as possible to mean BW between all six treatments.

Table 4.9 Male and female broiler hearts combined characteristics 33 days (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	BW (g)	HW (g)	HW/BW %	RV (g)	RV: HW	RV Cavity dia (mm)	RV Wall Thick (mm)
Control	0	2047	8.418	0.4103	1.692	0.1998	1.507	2.101
		(±)	(±)	(±)	(±)	(±)	(±)	(±)
500 g/ton	0.05	2041	8.317	0.4055	1.586	0.1892	0.954	1.952
		(±)	(±)	(±)	(±)	(±)	(±)	(±)
750 g/ton	0.075	2083	8.522	0.4084	1.575	0.1850	1.502	1.871
		(±)	(±)	(±)	(±)	(±)	(±)	(±)
Control with PBY	0	2083	8.753	0.4197	1.624	0.1845	1.096	1.686
		(±)	(±)	(±)	(±)	(±)	(±)	(±)
500 g/ton with PBY	0.05	2176	8.657	0.3958	1.681	0.1928	0.964	1.713
		(±)	(±)	(±)	(±)	(±)	(±)	(±)
750 g/ton with PBY	0.075	2045	8.133	0.3969	1.554	0.1916	1.424	1.844
		(±)	(±)	(±)	(±)	(±)	(±)	(±)

[12]Column means with the same superscript are not significantly (P>0.05) different from each other.

4.2.1.13 Heart weights

No significant (P>0.05) differences were found in Ross-308 broiler heart weights between any of the different treatments with different dietary taurine inclusion levels at 33 days of age.

4.2.1.14 Heart weight to Body weight percentage

No significant (P>0.05) differences were found in Ross-308 broiler heart weight to body weight percentages between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.15 Right ventricle weight

No significant (P>0.05) differences were found in Ross-308 broiler heart right ventricle weights between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.16 Right ventricle to heart weight ratio

No significant (P>0.05) differences were found in Ross-308 broiler right ventricle to heart weight ratios between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.17 Right ventricle cavity diameter

No significant (P>0.05) differences were found in Ross-308 broiler heart right ventricle cavity diameters between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.2.1.18 Right ventricle wall thickness

No significant (P>0.05) differences were found in Ross-308 broiler heart right ventricle wall thickness between any of the different treatments fed with different dietary taurine inclusion levels at 33 days of age.

4.3 Postmortem data

No significant differences (P>0.05) were observed in mortality at any stage through the rearing period. This can be seen in tables 4.10, 4.11, 4.12 & 4.13 that illustrate the effect that taurine supplementation has on the incidence of ascites, flip-over and other related mortalities respectively.

Table 4.10 illustrates the cumulative ascites mortality rates (%) of Ross 308 broilers fed diets with different taurine inclusion levels.

Table 4.10 Cumulative ascites mortality percentages per week (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	**Taurine %**	**Age (Days)**				
		7	14	21	28	33
Control	0					
		0.41667	0.41667	0.8333	0.8333	2.083
500 g/ton	0.05	(±0.7715)	(±0.7715)	(±1.2599)	(±1.2599)	(±2.136)
		0	0	0	0	0.625
750 g/ton	0.075	(±0)	(±0)	(±0)	(±0)	(±0.863)
		0	0	0	0	0.833
Control with PBY	0	(±0)	(±0)	(±0)	(±0)	(±1.260)
		0	0	0.4167	0.4167	1.875
500 g/ton with	0.05	(±0)	(±0)	(±0.7715)	(±0.7715)	(±2.588)
PBY		0	0	0	0.2083	1.458
	0.075	(±0)	(±0)	(±0)	(±0.5893)	(±1.391)
750 g/ton with		0	0	0.2083	0.6250	0.625
PBY		(±0)	(±0)	(±0.5893)	(±0.8626)	(±0.863)

Table 4.11 illustrates the cumulative flip-over mortality rates (%) of Ross 308 broilers fed diets with different taurine inclusion levels.

Table 4.11 Cumulative flip-over mortality percentages per week (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		7	14	21	28	33
Control	0	0	0	0.2083	0.2083	0.208
		(±0)	(±0)	(±0.5893)	(±0.5893)	(±0.589)
500 g/ton	0.05	0	0	0.6250	0.6250	1.042
		(±0)	(±0)	(±0.8626)	(±0.8626)	(±1.240)
750 g/ton	0.075	0	0	0.6250	0.8333	1.042
		(±0)	(±0)	(±0.8626)	(±1.2599)	(±1.240)
Control with PBY	0	0	0	0.4167	0.4167	0.417
		(±0)	(±0)	(±0.7715)	(±0.7715)	(±0.772)
500 g/ton with PBY	0.05	0.20833	0.2083	0.2083	0.2083	1.042
		(±0.5893)	(±0.5893)	(±0.5893)	(±0.5893)	(±1.240)
750 g/ton with PBY	0.075	0.20833	0.4167	1.0417	1.2500	2.292
		(±0.5893)	(±0.7715)	(±0.8626)	(±1.1785)	(±2.171)

Table 4.12 illustrates the cumulative “other/ unknown” mortality rates (%) of Ross 308 broilers fed diets with different taurine inclusion levels.

Table 4.12 Cumulative other mortality percentages per week (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		7	14	21	28	33
Control	0	0.41667	0.4167	1.0417	1.0417	1.2500
		(±0.7715)	(±0.7715)	(±1.2400)	(±1.240)	(±1.179)
500 g/ton	0.05	0	0.2083	0.8333	1.0417	1.6667
		(±0)	(±0.5893)	(±1.7817)	(±1.768)	(±2.520)
750 g/ton	0.075	0	0	0.2083	0.6250	0.6250
		(±0)	(±0)	(±0.5893)	(±1.240)	(±1.240)
Control with PBY	0	0	0.2083	0.4167	0.6250	0.6250
		(±0)	(±0.5893)	(±0.7715)	(±0.863)	(±0.863)
500 g/ton with PBY	0.05	0	0	0.2083	0.4167	0.4167
		(±0)	(±0)	(±0.5893)	(±0.772)	(±0.772)
750 g/ton with PBY	0.075	0	0	0.2083	0.4167	0.8333
		(±0)	(±0)	(±0.5893)	(±1.179)	(±1.260)

Table 4.13 illustrates the cumulative total mortality rates (%) of Ross 308 broilers fed diets with different taurine inclusion levels.

Table 4.13 Cumulative total mortality percentages per week (±standard error of the mean) of Ross 308 Broilers that received different dietary taurine inclusion levels

Taurine level	Taurine %	Age (Days)				
		7	14	21	28	33
Control	0	0.8333	0.8333	2.083	2.083	3.542
		(±1.2599)	(±1.2599)	(±2.136)	(±2.136)	(±2.430)
500 g/ton	0.05	0	0.2083	1.458	1.667	3.333
		(±0)	(±0.5893)	(±1.652)	(±1.543)	(±2.520)
750 g/ton	0.075	0	0	0..833	1.458	2.500
		(±0)	(±0)	(±1.260)	(±1.877)	(±2.357)
Control with PBY	0	0	0.2083	1.250	1.458	2.917
		(±0)	(±0.5893)	(±1.179)	(±1.391)	(±2.782)
500 g/ton with PBY	0.05	0.2083	0.2083	0.417	0.833	2.917
		(±0.5893)	(±0.5893)	(±0.772)	(±1.260)	(±1.477)
750 g/ton with PBY	0.075	0.2083	0.4167	1.458	2.292	3.750
		(±0.5893)	(±0.7715)	(±0.589)	(±1.768)	(±2.136)

4.4 Pearsons' linear correlation coefficients (-1<r<1) of heart characteristics, cause of mortality and 33d performance parameters of Ross-308 broilers

Note: these correlation coefficients are an indication of the strength of the linear relationships between pairs of measurements. Table 4.14 illustrates the correlation coefficients between performance parameters of Ross-308 broilers used in this trial and quality parameters used to evaluate heart characteristics, cause of mortality and as well the different performance parameters.

Table 4.14 The 33d Correlation coefficients between and within performance traits, heart traits and mortalities used to evaluate the effect of dietary taurine supplementation on Ross-308 broilers respectively

Parameters	BW33	GAIN 33	CFI33	FI33	CFCR 33	FCR3 3	PEF3 3	HW	HW_ BW %	RV	RV: HW	RVCa vitdia	RVWallt hick	Ascites %	Flip%	Other %	TMort %
BW33	-																
GAIN33	0.5515	-															
CFI33	0.7406	0.3756	-														
FI33	0.7273	0.9660	0.0012	-													
CFCR33	0.1157	0.2682	0.0010	0.0201	-												
FCR33	0.9274	0.0515	0.0010	<0.001	0.0061	-											
PEF33	0.0265	0.2267	0.0133	0.1065	<0.001	0.0323	-										
HW	0.4538	0.3955	0.2475	0.5075	0.1378	0.2751	0.1270	-									
HWBW%	0.3202	0.5635	0.0533	0.0392	0.0148	0.0416	0.0200	0.0088	-								
RV	0.8710	0.0951	0.0263	0.2853	0.0449	0.0537	0.0525	<0.001	0.0139	-							
RV: HW	0.7209	0.0630	0.0064	0.2130	0.0490	0.0276	0.0701	0.0292	0.0540	<0.001	-						
RVCavitydia	0.5943	0.2497	0.2418	0.1236	0.4859	0.0480	0.6896	0.2252	0.0494	0.1978	0.2574	-					
RVWallthick	0.9535	0.1056	<0.001	0.0129	0.0018	<0.001	0.0113	0.1162	0.0114	0.0044	<0.001	0.0888	-				
Ascites%	0.0537	0.1825	0.2830	0.8462	0.0303	0.5689	0.0083	0.0371	0.1190	0.0387	0.1154	0.9618	0.1710	-			
Flip%	0.0035	0.5338	0.1925	0.2065	0.8102	0.5299	0.4155	0.6426	0.7686	0.8919	0.5086	0.2800	0.3779	0.1230	-		
Other%	0.7607	0.3064	0.1605	0.5144	0.3213	0.2980	0.3639	0.5421	0.5836	0.2044	0.1373	0.4878	0.2686	0.3338	0.6165	-	
TotalMort%	0.6197	0.2708	0.0027	0.2753	0.0411	0.1335	0.0785	0.1248	0.2036	0.0100	0.0038	0.6552	0.0091	0.0378	0.3928	0.0255	-

Table 4.14 Discussion:

As expected, many strong positive correlations exist between 33-day bodyweight and other performance parameters such as 33-day feed intake, as higher feed intakes usually relate to higher 33-day bodyweights. Meaningful strong positive correlations between 33-day bodyweight and heart characteristics exist such as the strong positive correlations with right ventricle weight and right ventricle to heart weight ratio. This is in line with our expectations as we expected heavier birds at 33-days of age to have heavier right ventricle weights and subsequently larger right ventricle to heart weight ratios. Another strong positive correlation with 33-day bodyweight is the correlation with the total number of mortalities after 33 days and other mortalities at 33 days. This also draws the conclusion that heavier birds are more likely to die, and when comparing the 33-day bodyweight with the flip over and ascites mortality percentages the correlations are positively weak. My conclusion is that a larger percentage of the total and other mortality percentages must fall under ascites and flip over as not all mortalities are easy to categorize under cause of death.

When looking at the heart characteristics a few meaningful correlations can also be seen. A strong positive correlation exists between the following parameters: heart weight and total flip overs, right ventricle cavity diameter and total ascites related mortalities. This is also as expected as birds under pressure are likely to develop enlarged hearts that will result in flip overs, another correlation we expected is the strong positive correlation between right ventricle cavity diameter and ascites related mortalities, as birds in an ascitic state develops right ventricular hypertrophy and therefore heavier right ventricle weights.

Chapter 5

Discussion

The use of taurine in poultry nutrition has been studied extensively, with a wide scope, over the last few decades. This resulted in many inconclusive answers and remaining questions. The effect of dietary taurine supplementation on ascites, in broilers specifically, has not been extensively in the past and as with previous studies many conflicting results exist. A few studies found no influence and other positive effects of dietary taurine supplementation on broiler performance, cardiovascular characteristics, and ascites related mortalities. This specific study agrees to some previous findings but also disagrees with many of them. This study examined three aspects of dietary taurine supplementation in broiler nutrition: the first aspect being taurine's effect on broiler performance, more specifically growth-related parameters. The second aspect being taurine's effect on cardiovascular characteristics, more specifically the heart and its measurements. And lastly the effect of Tau supplementation on mortalities within a broiler cycle.

5.1 Performance of broilers

5.1.1 Bodyweight and Average Daily Gain

In this study, the broilers that received dietary taurine supplementation showed the greatest growth performance results and more specifically those subjected to the higher inclusion levels of Tau in the diet. This study also found that Tau supplementation only increases broiler BW significantly after two weeks of age. This is contradicting of earlier studies that found that broilers are influenced by Tau supplementation to a greater extent earlier in their life (study). Although it appears that dietary taurine supplementation increases broiler BW, it is not obviously clear from which point on does dietary taurine supplementation prove worthwhile, or what level of dietary taurine supplementation with or without poultry by-product meal could serve as the golden bullet researchers are after. The results of this study still contradict many others that found no effect of dietary taurine supplementation on broiler BW (Campbell & Classen, 1989; Blair *et al*., 1991; Tufft & Jensen, 1992; Lee *et al*., 2003). During the study 0.075% dietary Tau in the diet containing PBY showed significantly (P<0.05) higher BW than both control treatments containing no Tau at 14, 21 and 33 days of age, irrespective of the presence of PBY or not. This disagrees with earlier studies which found no significant increases in broiler BW for all inclusion levels of Tau, ranging from 0.025% to 0.8% Tau in the diet (Campbell & Classen, 1989; Blair *et al*., 1991; Tufft & Jensen, 1992; Lee *et al*., 2003). The effect of dietary taurine source remains unclear, with more in-depth research needed to form an accurate conclusion.

As for average daily gains (ADG) significant differences were seen during week 2 where 0.05 % Tau with PBY and 0.075% Tau with PBY had significantly (P<0.05) higher ADG than all other treatments.

5.1.2 Feed Intake and Feed Conversion Ratio's

The effect of dietary Tau supplementation on feed intake (FI) has not yet been well investigated, but according to an earlier study increasing dietary Tau levels in the diet significantly ($P<0.05$) decreases feed intake (Lee *et al*., 2003). It is important to note that the study by Lee *et al*., (2003) experimented with high Tau inclusion levels: 0.1 – 0.4 %, which could possibly explain lower intakes due to detrimental effects on feed palatability. In this current study we found that increasing levels of Tau, 0.05 % without PBY and 0.075% with PBY significantly ($P<0.05$) increased cumulative feed intakes during 0 – 14 days when compared to the control without PBY. For the rest of the trial, feed intakes were constant with no significant differences which is in line with two earlier studies completed by Alzawqari *et al*., (2016) and another study by Lee *et al*., (2003), completed at lower Tau inclusion levels, 0.025 – 0.075 %.

In the past a few studies evaluated the effect of dietary Tau supplementation on lipid digestion and absorption and found that supplementation thereof was able to significantly increase bile acid conjugation (Noy & Sklan, 2001). As a result, bile acid conjugation causes better emulsification of lipids and as an end result improved feed conversion ratio's, theoretically. In this current study we found that 0.075% dietary Tau with PBY was able to significantly ($P<0.05$) lower FCR from week 1 - 3 when compared to the control diet with PBY and no Tau. A lower Tau inclusion level of 0.05% with PBY was also able to significantly ($P<0.05$) decrease FCR but from week 2 – 4, when compared to the control diet with PBY and no Tau. The diet with 0.05% Tau without PBY was also able to significantly ($P<0.05$) decrease FCR from week 3 – 4, when compared to the control diet with PBY and no Tau. These findings agree with previous findings especially those that found significantly improved feed conversion efficiencies within the young broilers first few weeks of life (Tufft & Jensen, 1992, Zeng *et al*., 2009). Although the current study and a few previous studies only found improved FCR in the first few weeks, other studies found significantly improved feed efficiencies in older broilers aged 6 weeks of age (Miller *et al*., 1987, Campbell & Classen, 1989, Blair *et al*., 1991). This shows clearly that dietary taurine supplementation is able to significantly improve feed efficiencies ($P<0.05$) and can possibly be explained by improved lipid digestion and absorption when reviewing past literature. Some more recent investigation into taurines effect on broiler performance, found that high levels of supplementation can lead to toxic effects if the serum bile acid concentrations are dangerously high. Both Yuan & Wang, (2010) and Huang *et al*., (2014) found that these toxic levels could induce mucosal damage and cause delayed GIT development or permanent development thereof, but a very interesting finding is that although all these negative effects are caused, decreased performance and increased mortality rates are not observed. It could be that the short lifespan of broilers doesn't allow the toxic levels to have any sudden or prolonged effects on performance or cause mortality before slaughter.

5.1.3 Performance Efficiency Factor (PEF)

The performance efficiency factor (PEF) serves as a means of summarising broiler performance within a cycle by considering the BW, FCR and mortalities of broilers. We used the PEF values to compare the results of the treatments to the commercial PEF values found in the South African broiler industry and unfortunately none of the previous studies reviewed in this study incorporated PEF values. In this study we found the PEF to be comparable to many commercial values, validating that the treatments used in this study are able to produce competitive results in the broiler production industry.

In general, there were significant (P<0.05) differences between treatments that received taurine and those treatments free of taurine (control). Broilers that received 0.05 % and 0.075% Tau with PBY had significant (P<0.05) higher PEF than the control with PBY and no Tau, for week 2 to 4. More significant differences were seen between treatments but none with a clear trend as that seen within the treatments containing poultry by-product meal.

5.2 Cardiovascular characteristics

Extensive amounts of research have been done on the effect of ascites in broilers on the heart as a whole and its different muscles or valves, but no research that we know off has been completed on the effect of varying levels of taurine in the diet on heart characteristics. It is widely known that ascitic broilers all suffer from varying degrees of right ventricular failure and that such broilers usually have enlarged right ventricles. None of the studies reviewed in this study analysed heart characteristics, similar measurements of the heart exist in studies that researched the physiological changes of the heart in ascitic broilers,

During this study we found no significant (P<0.05) effects of taurine on any of the heart characteristics, but a number of strong positive correlations were found between several parameters measured in this study (as seen in table 4.14) and the right ventricle of the heart. 33-day BW were correlated to a few right ventricle characteristics, firstly, a strong positive correlation (0.8710) was found with right ventricle weights (RV) and a strong positive correlation (0.9535) with RV wall thickness indicating that heavier bodyweights will produce heavier and thicker right ventricles. Furthermore, RV weights were found to be strongly correlated (0.8919) to sudden death syndrome (SDS) mortalities, indicating that heavier right ventricular weights increase the incidence of SDS mortalities in broilers. Lastly, the RV cavity diameters were found to have strong positive correlation (0.9618) with Ascites mortalities and the heart eight (HW) to BW ratio had a strong positive correlation (0.7686) with SDS mortalities. These findings were expected when reviewing previous studies that found that heavier birds more frequently experience cases of ascites and possibly as a result of right ventricular hypertrophy (RVH) that causes the heavier and thicker RV weights, a higher incidence of SDS and ascites related mortalities.

5.3 Mortalities

No significant (P>0.05) differences were seen in the mortality rates of Ross 308 broilers fed varying levels of taurine in the diet throughout the 33-day trial. Only 3 studies reviewed in this paper investigated the effect of taurine on mortality rate as well as the effect of dietary Tau supplementation on the incidence of sudden death syndrome mortalities (Campbell & Classen, 1989, Blair *et al*., 1991, Tufft & Jensen, 1992).

Several strong positive correlations were found between the different mortality categories and measured parameters in this study that agrees to many results found in previous studies and some of them are reviewed in this paper. As previously discussed, strong genetic correlations exist between a few heart characteristics and the different mortality categories. A strong positive correlation (0.8462) exists between feed intakes during the last week of the cycle (day 28 – 33) and Ascites as well as a strong positive correlation (0.8102) between cumulative FCR and SDS mortalities. These correlations confirm what has already been documented by previous studies that very high feed intakes and very efficient feed conversions in broilers contribute to higher amounts of ascites and SDS mortalities in broiler flocks. This can possibly be explained by correlation between high feed intakes and rapid growing broilers whose cardiovascular and pulmonary system is under higher pressure to sustain growth and maintenance.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

The effects of dietary taurine supplementation on broiler performance has been explored moderately in the past, with many of those studies at least 5 decades old. This study aimed to create more insight on the effect that taurine supplementation has in broiler performance and the effect on ascites related mortalities. Dietary taurine supplementation in this study did however show that the highest inclusion level of taurine 0.075% in conjunction with poultry by-product meal will produce significantly heavier broilers from week 2 until slaughter age of 33 days when compared to the 2 control treatments with no taurine supplementation irrespective of PBY being present or not. The study did not find dietary taurine supplementation to have significant effects on broiler cardiovascular, pulmonary properties and ascites-related mortalities. However, a few very meaningful correlations were found during this study with many heart characteristics that are similar to ascitic broiler showing strong positive correlations with ascites and SDS related mortalities. Positive correlations were also found between low FCR and high feed intakes and SDS and Ascites related mortalities. This study indicated that taurine supplementation does show potential in broiler diets to improve performance and overall profitability but the effect on ascites warrants more in-depth investigation.

Due to the ever-present pressure placed on broiler production systems to become more efficient in order to prove more productive the use of dietary additions such as taurine need to be found. This is of importance to ensure broiler production systems are able to supply the rapid growing demand for affordable, healthy, and nutritious protein sources. The results of this study have shown that dietary taurine supplementation may have positive effects on broiler production and the overall profitability of such system. The end user will benefit by the use of taurine as it ensures the product they buy remains profitable and cost effective.

6.2 Critical Review and Recommendations

1. Extended research is needed to accurately quantify the effects that dietary taurine supplementation has on the cardiovascular and pulmonary system of broilers. The results of this study show that large variance is found specifically within the physiological measurements of the heart. Further research is needed to accurately assess the effects.

2. Based on the results of this trial the optimum dietary inclusion level of taurine is 750 gram per ton or 0.075% in conjunction with poultry by-product meal in the ration.

3. The trial was conducted in Sundra, Mpumalanga within the highveld region of South Africa, in the middle of winter, during the time of year when most ascites challenges are seen on broiler farms. In this study very low mortality rates were seen and taurine’s possible positive contributions towards controlling ascites related mortalities might only come forward in more ascites challenged broiler flocks/ cycles.

4. However, no significant (P>0.05) differences were found between the level of dietary taurine supplementation, heart characteristics and ascites related mortalities, meaningful and valuable strong positive correlations were found between heart characteristics, broiler performance parameters and ascites related mortalities. Indication of true trends were therefore found.

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Appendix

Appendix A Temperature profile of the trial house from 2 days before placement to slaughtering at 33 days

Day	Target floor temperature (°C, 50 % rH[1])
1 day before placement to 2	35.5
3 to 5	34.5
6 to 8	33.5
9 to 11	29.7
12 to 14	27.2
15 to 17	26.2
18 to 20	25.0
21 to 23	24.0
24 to 33	23.0

[1]rH=Relative Humidity

Appendix B Lighting program of the trial house from placement of the Ross broiler chicks to slaughter at 33 days-of-age

Day	Controller's set point			
	Lights on	Lights off	Hours of Daylight	Hours of Darkness
1 to 3	00:00	23:00	23	1
4 to 8	00:00	21:00	21	3
9 to 11	05:00	22:00	17	7
12 to 15	05:00	20:00	15	9
16 to 33	05:00	19:00	14	10

Appendix C Vaccination program (New Castle Disease and Infectious Bronchitis) of the Ross 308 broilers during the trial

Age (days)	Vaccination	Method	Trade name	Supplier
Hatchery	NCB[1]	Spray	Avinew	Merial South Africa (Pty) Ltd
Hatchery	IB[2]	Spray	Bioral H120	Merial South Africa (Pty) Ltd
10-12 days	NCB	Water	TAbic VH	Phibro Animal Health
10-12 days	IB	Water	TAbic MB	Phibro Animal Health
16-18 days	NCB	Water	Avinew	Merial South Africa (Pty) Ltd

[1]NCB = New Castle Disease
[2]IB = Infectious Bronchitis

Appendix D Layout of the pens and blocks in the trial house with the random treatment allocations to each pen

Block Design

	HOUSE A						HOUSE B				
	PEN	TREAT	TREAT	PEN			PEN	TREAT	TREAT	PEN	
Block 1	1	C	E	24	Block 4	Block 1	1	F	D	24	Block 4
	2	F	B	23			2	B	F	23	
	3	D	C	22			3	C	B	22	
	4	B	F	21			4	A	A	21	
	5	A	D	20			5	D	C	20	
	6	E	A	19			6	E	E	19	
Block 2	7	E	F	18	Block 3	Block 2	7	C	B	18	Block 3
	8	C	E	17			8	B	A	17	
	9	D	D	16			9	A	F	16	
	10	F	C	15			10	F	E	15	
	11	A	A	14			11	E	C	14	
	12	B	B	13			12	D	D	13	

[1]TREAT=Treatment
[2]Number 1-10 = treatment number

Appendix E The feeding phases with feeding periods and expected intakes per bird.

Feed (3 phases)	Feeding period (days)	Feed intake (g/bird)	Feed allocation/pen (kg)
Starter	17	750	45
Grower	10	1200	72
Finisher	6	950	57

Appendix F Heart Dissection Visual representations