The effect of zilpaterol hydrochloride as feed additive on the growth performance and carcass characteristics of broilers

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Declaration

I, **Unity Joubert** declare that the dissertation, which I hereby submit for the degree M.Sc. Agric at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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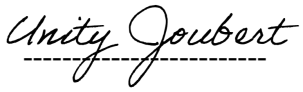

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List of Abbreviations

ADG	Average daily gain
ATP	Adenosine triphosphate
BW	Body weight
c-AMP	Cyclic AMP
CNS	Central nervous system
CY	Carcass yield
D	Dopamine
E	Epinephrine
FA	Fatty acids
FCR	Feed conversion ratio
FI	Feed intake
GH	Growth hormone
ICV	Intra-cerebroventricular
IGF-I	Insulin growth factor-I
ISOP	Isoproterenol
NE	Norepinephrine
NEFA	Non esterified fatty acids
NPY	Neuropeptide Y
NST	Non-shivering thermogenesis
PKA	Protein kinase A
POMC	Pro-opiomelanocortin
SE	Standard error
T_3	Thyroid hormone
TAG	Triacylglycerodes
TG	Triglyceroles
UCP	Uncoupling proteins
VFA	Volatile fatty acids
ZH	Zilapterol hydrochloride
β - AR	β-adrenergic receptors
βARK	β-adrenergic receptor kinase
α - MSH	α-Melanocortin stimulating hormone

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Abstract

The effect of zilpaterol hydrochloride (ZH), a β_2-adrenergic agonist, on growth, feed intake and carcass characteristics of broilers were examined. Broilers were raised in an environmentally controlled broiler house for 35 days. A standard commercial starter and grower diet was fed until day 28 when the experimental period started. The starter diet was fed for the first 14 days, followed by the grower diet from day 15 to 28 days (14 days). The experimental diet was administered for 7 days from day 28 to day 35. A completely randomised design with a 4 x 2 factorial arrangement was used for the four experimental diets containing different levels of ZH (0 mg/kg, 5 mg/kg, 7 mg/kg and 9 mg/kg) and fed to male and female broilers reared separately. Weekly body weight (BW) and feed intake (FI) measurements were taken and data was used to calculate feed conversion ratio (FCR). Two birds per pen were selected for sampling on day 36. Carcass weights, as well as individual portion measurements were taken for breast, thighs, drumsticks, wings and abdominal fat pad. The control group had the best performance values for BW, FI and FCR (P <0.05). The highest inclusion level of 9 mg/kg ZH resulted in significantly lower performance values. By contrast, ZH had a positive effect on carcass characteristics. Supplementation of ZH at a concentration of 5 mg/kg ZH improved carcass yield in both male and female birds, whereas 7 mg/kg ZH treatment resulted in the highest thigh and breast yields (P <0.05). Zilpaterol hydrochloride reduced abdominal fat deposition in females supplemented with 5 mg/kg ZH (P <0.05), however, higher inclusion levels of ZH resulted in increased fat deposition in both males and females. A sexual dimorphic effect was observed in the study. Female birds seemed to respond better to ZH supplementation compared to males, evidenced by their higher carcass yield and lower fat deposition. Based on the results, the selected ZH inclusion rates were too high, which caused a negative response on growth performance and fat deposition. However, supplementation of ZH at a concentration of 5 mg/kg ZH improved carcass yield.

Chapter 1

Introduction

Throughout history, humanity has constantly been challenged to increase food production to sustain the endless demand of a continuously increasing world population. The demand for poultry, specifically chicken meat, has increased dramatically when compared to other sources of animal meat (Moran, 2004). Genetic modifications, improved management and nutritional applications have led to the expansion of the commercial poultry industry (Havenstein *et al*., 2003). Selecting birds with higher growth rates have been the prime selection trait since the 1950s, but this selection strategy is unfortunately accompanied by higher feed intakes, fat deposition and greater environmental pollution (Tallentire *et al*., 2016). Birds with a faster growth rate have an increased appetite and therefore, a higher feed intake per day, which result in chickens containing a higher amount of carcass fat. Excess fat is perceived as an undesirable constituent of meat, which decreases carcass yield and consumer preference (Webb & O'Neill, 2008). Production efficiency depends on the efficiency of feed utilisation, which ultimately determines profit. Consequently, selection for improved feed efficiency became the principal selection trait instead of focusing on body weight alone. As a result, the number of days and the amount of feed required to raise a broiler to slaughter weight have reduced, which has a significant impact on broiler production and the environment (Havenstein *et al*., 2003). Benefits of improved feed conversion ratio (FCR) include lower production costs, improved carcass quality, less waste excretion into the environment, as well as reduced pressure on grazing lands and world feed supplies such as grain (Sillence, 2004).

Emerging and promising scientific developments today not only allow us to increase food production, but the efficiency of animal and plant production as well. Major challenges such as consumer acceptance and the European ban on hormonal substances in ruminant species as well as the use of antibiotics as growth promoters in monogastric species have forced researchers to develop new technologies to improve the efficiency of animal production (Sillence, 2004). Metabolic modifiers such as β-adrenergic agonists have been studied for their role as lean-enhancing agents in animals (Anderson *et al*., 2004). The main responses to β-adrenergic agonist treatment are enhanced skeletal growth and reduced fat content in animals. In addition, supplementation of these substances are normally accompanied by an increased growth rate and feed efficiency (Mersmann, 1998). β-agonists stimulate muscle growth through increased muscle protein synthesis, reduced muscle degradation or a combination of both (Yang & McElligott, 1989). According to Morgan *et al.* (1989), β-agonists enhance muscle hypertrophy by altering the proteolytic activity in muscle, which results in reduced protein degradation. Observed body fat reduction in broilers from β-agonist supplementation is relatively small and mostly inconsistent (Fiems, 1987). According to Yang & McElligott (1989), chicken adipocytes do not have a large binding affinity to β-agonist and therefore, do not induce

lipolysis of fat tissue, unlike mammals. In poultry, the liver is the main site for *de novo* synthesis of fatty acids (FA), where adipose tissue merely serves as a storage site for triacylglycerodes (TAG). Therefore, β-agonists have an indirect response on adipose tissue deposition in broilers by increasing the sensitivity of the liver to β-agonist stimulation. Work done by Harris *et al.* (1988), suggested that mixed α- and β-adrenergic agonists are more effective repartitioning agents to use in chickens.

Only two β-agonists, namely zilpaterol hydrochloride (a β_2-agonist) and ractopamine (a β_1-agonist) have been approved for their use as feed additives to enhance growth performance, feed efficiency and carcass leanness in sheep, cattle and pigs. Recently, zilpaterol hydrochloride (ZH) received considerable attention due to reports indicating that the supplementation of ZH elicits a greater response in growth and carcass characteristics when compared to other β-agonists (López-Carlos *et al*., 2010). This is due to the fact that skeletal muscle tissue consist predominantly of β_2-receptors and therefore, has a greater affinity for β_2-agonists such as ZH (Yang & McElligott, 1989; Young *et al*., 2000b; Lynch & Ryall, 2008). In addition, ZH is rapidly eliminated from the system and therefore does not pose any risk of toxic residues which could be harmful to the consumer (Dunshea *et al*., 2005; Dikeman, 2007).

The general goal for any commercial broiler production system is to reduce the time for broilers to reach market weight in order to reduce feed and management costs (Rogers, 2013). The supplementation of β-agonists improve growth rate and feed efficiency of animals. As a result, broiler producers are able to produce birds that reach target weight in a shorter period. In addition, broilers will require less feed, which allows producers to decrease production costs. However, the general response to β-adrenergic agonists treatment in broilers are generally low and inconsistent, which can be attributed to the fact that broilers have reached their genetic maximum and therefore, biologically unable to improve (Reeds & Mersmann, 1991).

The aim of the present study was to determine the efficacy of ZH supplementation on broiler growth and carcass weight. Different concentrations of ZH were supplemented to broiler diets with the objective to determine which concentration level will result in the greatest response. H_0: The supplementation of ZH would have no effect on broiler performance and carcass characteristics. H_1: Supplementing ZH to broilers would result in higher body weights, feed intake and feed efficiency, as well as improved carcass yield.

Chapter 2

Literature review

2.1 Introduction

Research regarding the control of fat and lean deposition in livestock has escalated because it is linked to product quality and the efficiency of feed utilisation (Webb & O'Neill, 2008). Measures to improve the efficiency of feed utilisation is imperative to animal producers because feed cost affects profit to a large extent (Sillence, 2004). Feed additives are used worldwide to improve the nutrient status of animals and to increase growth performance, feed intake (FI) and the efficiency of feed utilisation. Improved feed efficiency can be accomplished by administering metabolic modifiers that changes the ratio of protein to fat deposition (Yen, 1995; Wenk, 2000). Fat deposition is energetically more efficient than muscle deposition, however, the fattening animal requires more energy per unit of gain due to the high caloric density of fat (Rattray & Joyce, 1976; Eggert & Nielsen, 2006). Increased fat gain results in lower feed efficiency and consequently, decreased farm profit. Therefore, increasing the ratio of protein gain in animals will improve the efficiency of feed utilisation, which results in less feed required to produce one kilogram of meat.

In recent decades, the application of genetic selection in broiler lines for commercial use has dramatically increased (Richards & Proszkowiec-Weglarz, 2007; Tallentire *et al*., 2016). Selecting birds with a higher growth rate has been the leading selection trait since the 1950s, however, this selection strategy is accompanied by a higher FI, fat deposition and greater environmental pollution (Tallentire *et al*., 2016). Birds with a faster growth rate have an increased appetite and therefore a higher FI per day, which significantly affects production costs (Richards, 2003). In addition, consumers have recently become more aware of their health and the quality of food they consume (Schönfeldt & Gibson, 2008). Excess fat is perceived as an undesirable constituent of meat, which decreases carcass yield and consumer preference (Webb & O'Neill, 2008). Research indicates that consuming high amounts of fat increases the risk of cardiovascular diseases, colon cancer and obesity (Kheiri *et al*., 2011a). Therefore, the emphasis has shifted away from fat quantity to fat quality (MacRae & Lobley, 1991). On the other hand, excessive feed intakes result in excess nutrients not utilised by the body, which are excreted in poultry manure and responsible for the harmful acidifying emissions (Sillence, 2004).

One specific metabolic modifier that has gained significant interest is the use of β-adrenergic agonists. β-agonists have been studied for more than three decades for their effects as lean-enhancing agents (Anderson *et al*., 2004). Originally, β-agonists were used for the treatment of bronchospasms in human patients and horses (Mersmann, 1998). However, increasing research studies showed that the administration of β-agonists also enhanced skeletal muscle mass and decreased body fat in humans and animals (Mersmann, 1998). The supplementation of β-agonists have proved to be effective in enhancing feed efficiency and meat quality in cattle, sheep, pigs, chickens and turkeys

(Anderson *et al*., 2004). β-agonists stimulate a biological response by binding to a specific β-adrenergic receptor (β-AR) found on the external surfaces of cells (Strydom *et al*., 2009). β-agonists are also referred to as repartitioning agents due to their ability to alter specific metabolic signals in muscle and fat cells (Anderson *et al*., 2004). This mechanism of action causes the nutrients to be redirected towards muscle instead of fat, which improves the efficiency of animal production (Anderson *et al*., 2004). Therefore, β-agonists influence protein and lipid metabolism resulting in pronounced increases in muscle protein synthesis and a reduction in fat deposition (Badino *et al*., 2008).

The degree of response to β-agonists varies significantly between animal studies (Fiems, 1987). Variations in response are related to species differences, disparities in tissue distribution, structure of receptor subtypes and the mode of action of applying different β-agonists (Mersmann, 1995). This review will highlight the effects of β-agonist treatment on animal growth and feed intake. The mechanism of action related to β-ARs, their function and the factors that influence animal response, will be discussed.

2.2 Modern poultry industry

The commercial poultry industry as it is known today, has started more than 50 years ago and since then the production of poultry meat increased significantly (Hocking, 2014). Genetic modifications, improved management and nutritional applications have led to the expansion of the modern poultry industry (Havenstein *et al*., 2003). Over the last 50 years, commercial genetic selection pressure has produced chickens with exceptional growth rates that are highly feed efficient and are able to yield high carcass weights (Tallentire *et al*., 2016). As a result, the number of days and the amount of feed required to raise a broiler to slaughter weight has reduced, which have a significant impact on broiler production and the environment (Havenstein *et al*., 2003). Zuidhof *et al.* (2014) studied the effect of genetic selection on the growth and efficiency in two unselected strains and one selected Ross 308 strain. The study revealed that from 1957 to 2005, broiler growth had increased by 400% and feed efficiency reduced by 50%. Producers are now able to produce poultry meat at relatively the same price as in the 1950s (Havenstein *et al*., 2003).

Recently, the demand for poultry, specifically chicken meat, has increased dramatically when compared to other sources of meat (Moran, 2004). It is predicted that global poultry consumption will increase by 27% in 2023 (Zuidhof *et al*., 2014). Chicken meat is currently one of the best sources of protein, especially in underdeveloped countries due to its affordability and accessibility. In developing countries, the rapid population growth, the change in lifestyle factors and consumer perception has mainly contributed to the higher demand for poultry meat (Moran, 2004). In sub-Saharan Africa in 2017, South Africa was the largest poultry producer with a consumption of 38.37 kg per capita per annum (Department of Agriculture, Fisheries and Forestry, 2017). In the developed world, between 1960 and 2004, the US consumer index for poultry products increased at half the rate when compared

to other meat products (Zuidhof *et al*., 2014). The increased demand for poultry meat may be attributed to consumer perception as poultry meat is perceived as healthy, nutritious, low in fat and contains more desirable unsaturated fatty acids (UFAs) when compared to other sources of animal meats (Buyse *et al*., 1991).

2.3 **Nutritional and hormonal regulation of muscle growth and feed intake of animals**

In order to fully understand the effect of the β-adrenergic agonists on growth and feed intake in animals, the following section will briefly discuss the different types of muscle and the energy and hormonal requirements that stimulate growth in animals.

2.3.1 Energy metabolism in skeletal muscle

Muscle synthesis is the primary determinant of body weight (BW). Animal BW, nutrition, hormonal status and muscle fibre type alters the energy expenditure of muscle, which is a major contributor to the maintenance energy requirements of animals (Hocquette *et al*., 1998). Nutrient sources for muscle energy originate from arterial flow, *de novo* synthesis in the liver and energy stores in the form of muscle glycogen and adipose tissue (Hocquette *et al*., 1998). Muscle growth depends on intra-muscular fuels, namely glycogen and triglycerides (TGs) as well as extra-muscular energy sources, such as glucose, lactate, non-esterified FAs (NEFAs), volatile FAs (VFAs), TGs and ketones. However, the potential contribution of each nutrient to muscle oxidation varies between ruminants and monogastric animals (Hocquette *et al*., 1998).

Muscle fibres are classified according to their contractile properties, metabolic characteristics, oxidative capacity and enzyme activity, which have an effect on muscle energy expenditure (Hocquette *et al*., 1998). Three main fibre types exist in animals, which are distinguished based on their metabolic properties known as types I (slow-oxidative), IIA (fast-twitch oxido-glycolytic) and IIB (fast-twitch glycolytic) (Litt Miller, 2007). Muscle fibre number is fixed at birth and any further increase in muscle growth (hypertrophy) is attributed to an elevated muscle size or a shift in the fibre type (Knobel, 2014). In addition to nutrients, muscle requires oxygen for growth and therefore, relies greatly on the oxidative capacity of muscle (Khajali & Wideman, 2016). The oxidative capacity of muscle has a major influence on the muscle's metabolic rate, which is greater in red skeletal muscle (Allouh, 2007). Muscle oxygen supply depends on blood flow, arterial oxygen concentration, the tissue's absorption rate, the level of myoglobin and mitochondrial capacity (Hocquette *et al*., 1998). Oxygen is transported via the blood in haemoglobin and stored within myoglobin in muscle cells. Myoglobin is a protein molecule that stores oxygen to be used as an energy source for extended muscle activity (Knobel, 2014). Therefore, the myoglobin content is higher in oxidative muscle (red muscle) as compared to glycolytic muscle (white muscle) due to a higher concentration of mitochondria (Knobel, 2014).

Species have different muscle oxidative capacities because they contain different ratios of a specific muscle fibre type at varying structural locations. The oxidative capacity of species, in decreasing order, is horses = sheep > cattle > rabbits > pigs > chickens (Hocquette *et al*., 1998). Muscles used for extended periods of activity, such as standing and walking, are composed of fibres known as slow-twitch type I (Decuypere *et al*., 2000). These fibres are red in colour due to their high myoglobin content (Decuypere *et al*., 2000). Red fibres have a narrow diameter which is adapted to aerobic (oxidative) metabolism (Dransfield & Sosnicki, 1999). White muscle fibres are known as fast-twitch which, is referred to as types IIA, IIB and IIX isoforms which stores glycogen as an energy source (Decuypere *et al*., 2000). White fibres have larger cross-sectional diameters and are adapted to anaerobic (glycolytic) metabolism used for brief bursts of activity (Dransfield & Sosnicki, 1999). Cattle meat is dark red in colour because their muscles are constantly being used for walking and standing, and therefore, contains more type I muscle fibres (Knobel, 2014). In the case of chickens, their thigh and leg muscles are extensively used and thus consist mostly of red fibres (type I). However, the meat from their breast and wings are white and contains fast glycolytic type IIB fibres (Dransfield & Sosnicki, 1999). Therefore, the oxidative capacity is larger in red muscle, higher in smaller animals and greater in active animals.

2.3.2 Endocrine mechanisms involved in the regulation of growth in animals

Several hormones are involved in the regulation of metabolic processes within animals, including growth hormone (GH), thyroid hormone (T_3), insulin, glucocorticoids and sex steroids (Sejresen & Vestergaard Jensen, 1987). These hormones function to maintain biological homeostasis and facilitate growth in animals and are activated in response to a specific biological need (Timmerman, 1987). In addition, the metabolic response may involve more than one hormone at a time and is coordinated via complex feedback loops (Richards, 2003).

Growth hormone is the major hormone involved in regulating growth in animals (Sejresen & Vestergaard Jensen, 1987). Growth hormones are mainly involved in muscle protein synthesis and degradation to stimulate muscle growth in animals. Generally, GHs are an antagonist to insulin activity, as it inhibits lipogenesis and stimulates lipolysis in adipose tissue (Hocquette *et al*., 1998). Furthermore, GH concentrations are higher in fast-growing breeds of cattle, sheep and pigs, whereas the concentration of GH is inversely related to growth in chickens (Sejresen & Vestergaard Jensen, 1987). According to Hocquette *et al.* (1998), red muscles are more sensitive to GH stimulation compared to white muscles. This is in agreement with Burke *et al*. (1987), who administered GH to chickens and found no significant effect on growth, because chickens predominantly have white glycolytic muscle type (Burke *et al.,* 1987). In addition, GH levels are higher in males in comparison to females, which explain the large differences observed in BW between sexes.

Growth hormone receptors are found in adipose tissue, liver and bone, but not in muscle (Sejresen & Vestergaard Jensen, 1987). Growth hormones have a direct and indirect effect on growth

in animals. Growth hormones act directly on adipose tissue and the liver by stimulating lipolysis and the hepatic secretion of somatomedin (also known as IGF-I) (Sejresen & Vestergaard Jensen, 1987). However, blood insulin concentrations and the nutritional status of animals influence somatomedin release. For example, when circulating insulin levels are low, GH binding to the liver is inhibited, which in turn blocks somatomedin release and consequently alters growth in animals (Sejresen & Vestergaard Jensen, 1987). Alternatively, GHs have an indirect effect on the peripheral tissues by increasing tissue resistance to insulin and thereby inhibiting glucose uptake by adipose tissues (Hocquette *et al.*, 1998). Therefore, the main effects of GH release are reduced fat deposition and increased muscle growth.

Lipogenesis is controlled and regulated by insulin, glucagon and T_3, where insulin and T_3 stimulate lipogenesis and glucagon inhibits lipogenesis (Richards & Proszkowiec-Weglarz, 2007). Insulin is secreted in response to a high blood glucose concentration, which stimulates energy storage in muscle and adipose tissue by activating lipogenesis. Furthermore, insulin improves the supply of nutrients to muscle by increasing arterial blood flow and decreasing blood glucose through the inhibition of hepatic gluconeogenesis and lipolysis (Hocquette *et al*., 1998). High growth rates and carcass leanness are associated with increased muscle insulin sensitivity and glycolytic muscle energy metabolism (Hocquette *et al*., 1998). Some animals, like chickens, are considered to be more resistant to insulin, however, the cause of their resistance is unclear (Ashwell & McMurtry, 2003). Insulin resistance results in reduced glucose uptake for muscle energy production. Glucose is then converted into FAs and stored in adipose tissue as TGs. Consequently, carcass growth is inhibited and fat deposition is increased (Hocquette *et al*., 1998).

2.3.3 Neuro-hormonal mechanisms involved in regulating feed intake of animals

Animals have an inherent ability to sustain a constant BW by regulating their FI and energy expenditure (Tachibana & Tsutsui, 2016). Intensive selection of animals for growth and meat production have been accompanied by significant changes in their FI (Moran, 2004). According to Richards (2003), modern broilers are unable to regulate FI to maintain homeostasis and are thus susceptible to overconsumption. High feed intakes lead to excessive fat deposition and other health related conditions, such as leg problems and reproductive inefficiency (Richards, 2003). As a result, poultry species do not utilise their feed efficiently which increases cost and reduces farm profit (Richards, 2003). It is therefore essential to understand the mechanisms regulating FI in order to produce animals more efficiently. Richards & Proszkowiec-Weglarz (2007) published an extensive review on specific genes regulating FI and how gene expression is controlled by nutritional and hormonal secretions. A variety of signalling molecules including neuropeptides, hormones, nutrients and metabolites are produced in response to changes in nutrition (Richards & Proszkowiec-Weglarz, 2007). Interestingly, the authors also identified a relationship between the appetite and energy balance

of animals through a hormone called leptin, which serves as a link between peripheral energy stores and the central nervous system (CNS) to regulate feeding behaviour.

The CNS is the key regulator of FI that can be divided into two pathways known as the short-term and long-term regulators of FI (Richards, 2003). These pathways involve neural sites in the brainstem and hypothalamus that receive information from peripheral tissues (Richards, 2003). The short-term regulator of FI, referred to as the peripheral satiety system, transmits meal-related signals from the gastrointestinal tract (GIT), pancreas and liver. These signals are generally short-lived and unable to alter long-term changes in energy maintenance and BW (Richards & Proszkowiec-Weglarz, 2007). The long-term system transmits information to the hypothalamus on the available energy stores, which involves both neural and endocrine pathways. The hypothalamus contains numerous peptidergic molecules that serve as the signalling components of the energy status (Tachibana & Tsutsui, 2016). These signalling molecules can be divided into orexigenic (stimulates energy intake) and anorexigenic (inhibits energy intake) (Tachibana & Tsutsui, 2016).

Metabolic pathways are integrated with neuroendocrine pathways via the CNS to regulate FI in animals (Richards, 2003). Metabolites, such as glucose, FAs and amino acids produced from specific metabolic pathways serve as signalling molecules for the energy status of animals (Denbow, 1999). Changes in blood glucose levels stimulate insulin release, which in turn signals the brain to increase or decrease FI. Similarly, FA synthesis is regulated by insulin and serves as a signalling molecule of energy balance. When animals are in a positive energy balance, their insulin and T_3 levels are high, which inhibits feed intake. Moreover, changes in energy mass from enhanced TG production stimulates leptin secretion, which in turn affects FI and alters energy homeostasis in animals (Richards, 2003). In mammals, circulating levels of leptin and insulin are the major signalling molecules for energy status (Richards & Proszkowiec-Weglarz, 2007). Similar effects have been demonstrated in goldfish, Asian blue quails and sparrows, however, less is known about poultry species (Tachibana & Tsutsui, 2016). Leptin is a hormone that is produced from adipose tissue and plasma concentrations of leptin remain relatively constant, unlike insulin. Tachibana & Tsutsui (2016) reported that central and peripheral injections of leptin caused an increase in energy expenditure of animals and reduced their feed intake, body weight and adipose tissue. The function of leptin requires binding to specific receptors, which are expressed in both central and peripheral tissues (Denbow, 1999). As plasma leptin concentration increases in response to high energy stores, catabolic pathways are activated and anabolic pathways are inhibited (Richards & Proszkowiec-Weglarz, 2007). The opposite occurs in situations where adipose tissue stores are low. Low plasma leptin concentration stimulates anabolic pathways, which in turn increases feeding behaviour. Therefore, there is a clear link between the metabolic and endocrine pathways involved in the feeding behaviour of animals. The role of insulin and leptin in the long-term regulation of feed intake in animals are shown in Figure 2.1.

In addition, other signalling molecules, referred to as neuropeptide genes, have been shown to regulate feeding behaviour in animals, namely neuropeptide Y (NPY) and the proopiomelanocortin

(POMC) genes (Shiraishi *et al*., 2008). These genes respond to changes in the energy status of animals and are expressed in the brain of both mammals and avian species (Richards, 2003). More specifically, NPY has an orexigenic effect in animals, thus stimulating FI, whereas POMC inhibits FI (Shiraishi *et al*., 2008).

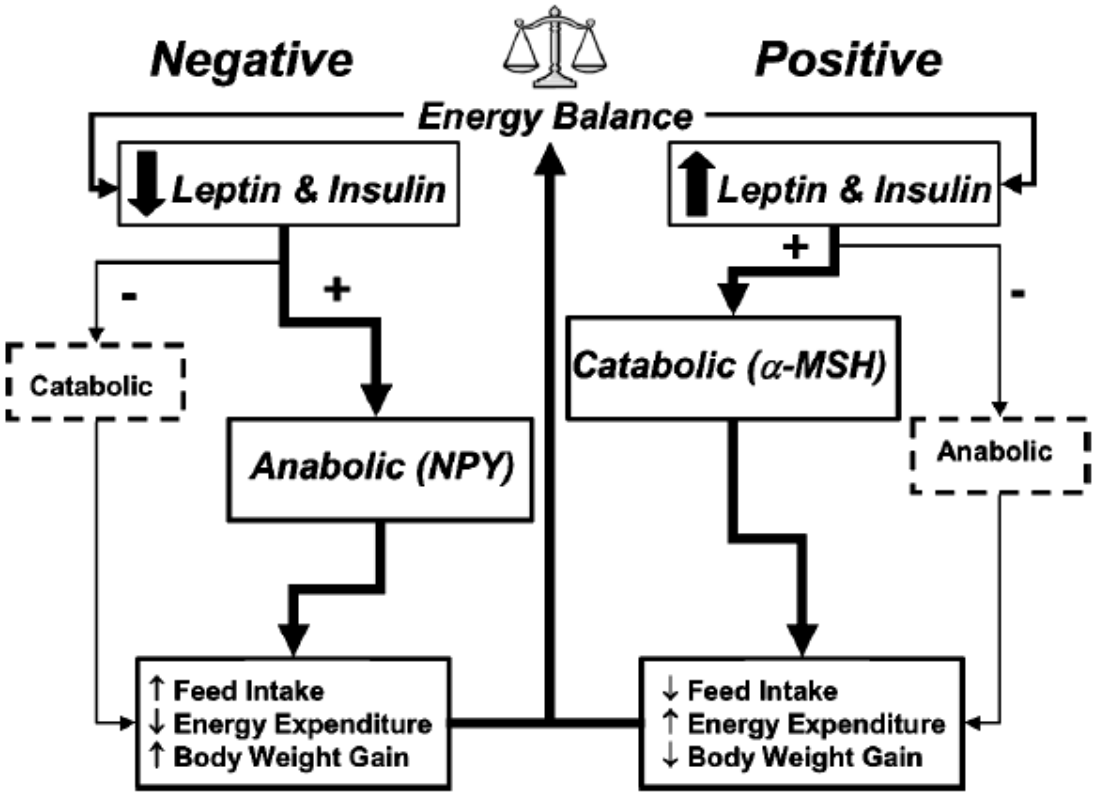

Figure 2.1: Long-term regulation of FI and energy balance in poultry. Leptin is released in response to the size of the energy (fat) stores (NPY: Neuropeptide Y, α-MSH: Melanocyte Stimulating Hormone (Richards, 2003)

2.4 **Endogenous catecholamines**

Catecholamines, namely epinephrine (E), norepinephrine (NE) and dopamine, are known to function as short-term emergency hormones in response to stress (Timmerman, 1987). However, increasing evidence suggests that catecholamines also play a crucial role in the long-term metabolic processes (Sejresen & Vestergaard Jensen, 1987). These hormones have both paracrine (neurotransmitter) and sympathetic (hormonal) functions, which influence metabolism and growth in animals (Timmerman, 1987). Catecholamines (Figure 2.2) are chemical mediators with an aromatic ring and an amine group in their structure (Smith, 1998). During stress, catecholamines are released and transmitted throughout the body followed by the binding and stimulation of adrenergic receptors. Catecholamine's actions are activated when it binds to specific α- and β-AR found on target tissues, however, α- and β-receptor stimulation often antagonises each other (Berdeaux & Stewart, 2012).

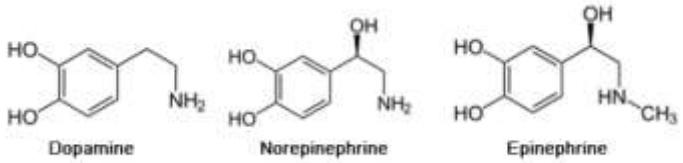

Figure 2.2: Chemical structure of naturally occurring catecholamines; epinephrine, norepinephrine and dopamine (Smith, 1998)

Catecholamines act directly on specific target tissues, such as adipose and muscle cells, and indirectly through endocrine hormone secretion. Neurotransmitters and hormones are the physiological components found in humans and animals that transmit information from one organ or target tissue to another (Buttery & Dawson, 1987). Neurotransmitters, namely NE, form part of the sympathetic nervous system, which are found in the nerve endings of cells and are released upon stimulation of specific nerves. On the other hand, hormones are synthesised in the adrenal glands and transported in blood, followed by binding to specific receptors on target organs. Catecholamines induce catabolic effects in metabolism by stimulating lipolysis in adipose tissue, glycogenolysis in muscle and liver, inhibiting glucose uptake from peripheral tissues and reducing insulin secretion (Virden & Kidd, 2009). However, increasing evidence has demonstrated that catecholamines also have an anabolic action on muscle, which formed the rationale behind the invention of β-adrenergic agonists (Beerman, 1987).

2.5 **β-adrenergic agonists**

β-adrenergic agonists, are synthetic compounds that share common structural qualities to naturally occurring catecholamines, however, they differ in their chemical and pharmacokinetic characteristics (Smith, 1998). The chemical structures of β-adrenergic agonists are shown in Figure 2.3. These synthetic compounds are modified by substituting an alkyl on the amine of the nitrogen molecule (Smith, 1998). The aromatic rings are essential for biological activity and are substituted with halogens, amines, hydroxyl, hydroxyl-methyl and cyano groups (Smith, 1998). The chemical substitutions influence the longevity and efficacy of β-agonists to their receptors. In addition, the pattern of substitution plays a major role in determining the route of metabolism, rate of absorption and elimination in the tissues (Smith, 1998).

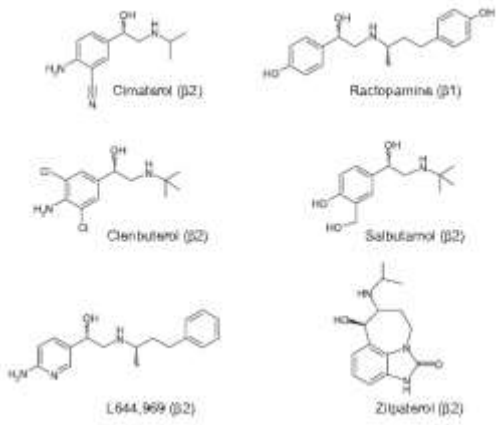

Figure 2.3: Chemical structures of synthetic β-adrenergic agonists Receptor specificity is indicated in brackets (Anderson *et al*., 2004)

The general response to β-agonist activation includes an increase in the heart rate, blood pressure and renal blood flow (Timmerman, 1987; Mersmann, 1998). As a result, blood flow to the digestive organs is reduced, and circulation to skeletal muscle and the heart is increased (Katica *et al*., 2016). In addition, bronchi in the lungs expand, which leads to an increased respiration rate and elevated blood oxygen concentration (Anderson *et al*., 2004). From a metabolic point of view, β-agonists stimulate an increase in glucose release from the liver, which is converted into energy in muscle. Furthermore, β-agonists increase the release of FAs by stimulating lipolysis (Katica *et al*., 2016). β-agonists produce a greater response than naturally occurring catecholamines as they are supplemented at higher effective doses and are more specific to β-ARs. Due to their modified chemical structure, β-agonists are able to cross the blood-brain barrier and therefore not subjected to the rapid deactivation by liver transferases after oral administration. As a result, β-agonists have a greater response for a longer period of time (Mersmann, 1998).

2.5.1 The mechanism of action of β-adrenergic agonists

A complex is formed when a synthetic β-agonist binds to a β-AR and initiates the activation of G-proteins (Fiems, 1987). The most well documented β adrenoceptor signalling pathway involves the cAMP-protein kinase A (PKA) signalling pathway, which is responsible for the anabolic response of skeletal muscle to β-AR stimulation (Mersmann, 1998). The Gs protein complex activates adenyl cyclase to produce cyclic-AMP (cAMP) from ATP. According to Young *et al.* (1999), cAMP is linked to the regulation of gene expression in muscle and adipocyte tissues. cAMP then binds and activates PKA, which in turn phosphorylates the receptor to produce intracellular protein enzymes (Page, 2004). These protein enzymes are responsible for the activation of lipase, which is the primary component involved in lipolysis (Young *et al*., 1999). The mechanism of action of β-agonist is illustrated in Figure 2.4.

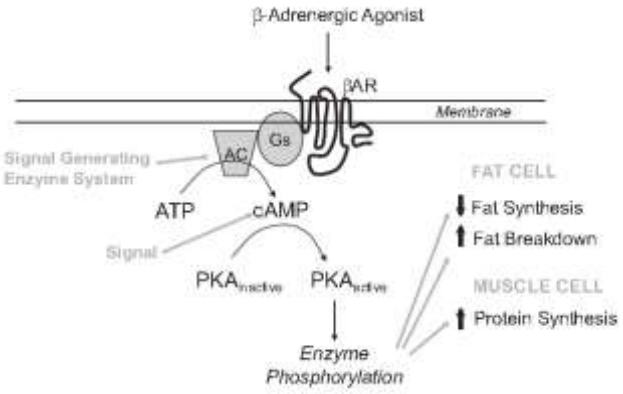

Figure 2.4: Mechanism of action of β-adrenergic agonists β-adrenergic receptors are activated after binding of β-agonists to the cell receptors followed by coupling to G-proteins G-proteins in turn stimulate adenylyl cyclase to convert ATP to cAMP (Anderson *et al*., 2004)

2.5.2 β-adrenergic receptors

β-agonists cannot pass through cell membranes, unlike anabolic steroids, and ligand binding occurs via extracellular binding of the cell (Mersmann, 1995). β-adrenergic receptors are found in all mammalian tissues and organs, however, the density and affinity of receptors depends on the physiological state of animals and differs between species (Minneman *et al*., 1980).

Two types of adrenergic receptors exist, namely β (beta) and α (alpha) receptors, which are further divided into β_1, β_2 and β_3, and α_1 and α_2 (Johnson, 2006). β-adrenergic receptors are classified according to different subtypes in order to understand their physiological function (Frielle *et al*., 1988). Stimulation of these receptors results in a contraction or relaxation of tissue and subsequent release of metabolic substances. β-receptors belong to both the hormonal and the sympathetic nervous system and play a regulatory role in cardiovascular, respiratory, metabolic and reproductive function (Johnson, 2006). According to Timmerman (1987), the β_1-receptors belong to the sympathetic nervous system, whereas the β_2 subtype is a hormone receptor. β-receptors are found in the heart, blood vessels, uterus, fat cells and skeletal muscle (Table 2.1)

Table 2.1 The density of β_1- and β_2-receptors in different tissues and organs, and their response to β-adrenergic agonist stimulation (Timmerman, 1987)

Organ	Receptor type	Response to stimulation
Heart	β1 > β2	Positive inotropic
Blood vessels	β2 > β1	Relaxation
GI tract	β2 > β1	Relaxation
Bronchial muscle	β2 > β1	Relaxation
Uterus	β2 > β1	Relaxation
Kidneys	β1 > β2	Renin release
Fat cells	β2 > β1	Lipolysis
Skeletal muscle	β2 > β1	Glycogenolysis
Pancreas	β2 > β1	Insulin release
Liver	β2 > β1	Glycogenolysis

The response to β-adrenergic agonists is elicited based on the involvement of adrenergic receptors found in the CNS and most peripheral tissues (Katica *et al.*, 2016). Almost every mammalian cell type has β-AR embedded in its plasma membrane and contains more than 400 amino acids in a continuous chain with 65-70% homology between the three β-AR subtypes (Mersmann, 1998; Pearen *et al.*, 2009). All adrenoceptors belong to the guanine nucleotide G-protein-coupled receptor (GPCR) family, which is the largest group of cell surface receptors found in mammals (Brown *et al.*, 1976). All GPCRs and adrenergic receptors are similar in structure, with seven transmembrane α-helices (Figure 2.5). These helices form three extracellular loops, namely the NH_2 terminus and three intracellular loops including the COOH terminus (Johnson, 2006). The third intracellular loop is important for the activation of receptors.

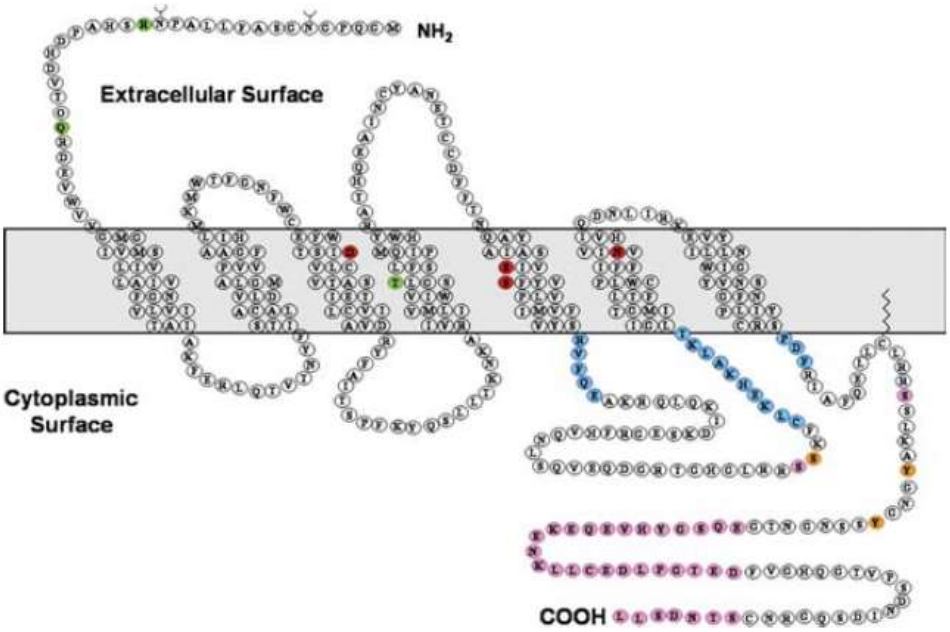

Figure 2.5: The structure of a β_2-adrenergic receptor (Johnson, 2006)

2.5.3 The regulation of β-adrenergic receptors through receptor desensitisation

Due to variations found in receptor structure and distribution between tissues, the resulting physiological responses are complex and difficult to determine (Mersmann, 1998). β-adrenergic receptor function is maintained through processes that support receptor density, which includes synthesis and downregulation of receptors (Young *et al*., 2000a). Mechanisms that alter receptor activity include receptor desensitisation, phosphorylation and internalisation (Lynch & Ryall, 2008). The phosphorylation of β-adrenergic receptors is illustrated in Figure 2.6. It has been demonstrated that continuous stimulation of β-agonists causes desensitisation or inactivation of receptors (Lohse *et al*., 1990). This is accomplished through the phosphorylation by specific kinases, namely β-adrenergic receptor kinase (βARK) and PKA (Lynch & Ryall, 2008). These kinases separate the receptor from G proteins leading to downregulation of adenylyl cyclase, which prevents the receptor from sending signals via the cAMP cyclic pathway (Harris, 2013). Furthermore, receptor activity is altered through the internalisation of a receptor which can no longer bind to agonists, nor are they coupled to the G protein complex to stimulate adenylyl activity (Lohse *et al*., 1990; Harris, 2013).

The degree of desensitisation differs between receptor subtypes ($\beta_3 > \beta_1 > \beta_2$) where β_3-receptors are less subjected to inactivation and β_2 are more readily inactivated (Badino *et al*., 2008). The density of β-receptors changes with the stage of cell differentiation and with the hormonal status of animals (Fève *et al.,* 1990; Lohse *et al.*, 1990). In addition, extended or continuous administration of β-agonists increases the risk of receptor inactivation and the degree of desensitisation differs between tissues and organs (Lohse *et al*., 1990). For example, the amount of β-ARs present on porcine adipose tissue decreased by 50% after 24 days of ractopamine feeding, whereas skeletal muscle receptor density decreased by only 20% (Spurlock *et al*., 1994). Spurlock *et al.* (1994), suggested that the higher body weights obtained in the ractopamine supplemented group are due to the lower desensitisation of muscle receptors, which resulted in an increase in the muscle mass of pigs. Similarly, Lynch & Ryall (2008) have indicated that the downregulation of muscle receptors are influenced by the age of an animal and it is specific tissue. Lynch & Ryall (2008) supplemented a β-agonist to both young and old rats to compare the degree of receptor inactivation between heart and skeletal muscle. The results from the study demonstrated a lower degree of receptor desensitisation in fast-twitching muscles in older rats as compared to younger rats. However, receptor desensitisation in the heart muscle was greater in older rats. The authors concluded that the β-adrenergic signalling pathway is preserved in fast-twitch muscle and the degree of inactivation depends on age.

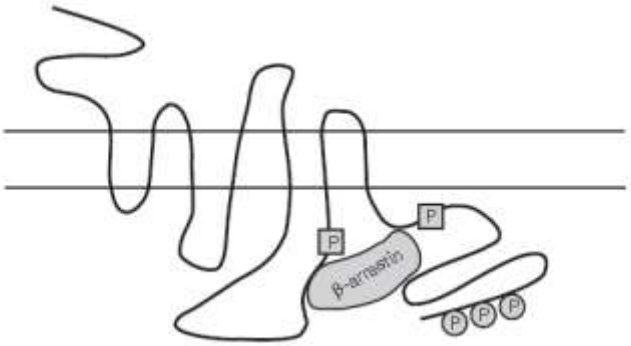

Figure 2.6: Phosphorylation of β-adrenergic receptors Receptors are phosphorylated by specific kinases, which result in an increase in the binding of β-arrestin This, leads to the uncoupling and internalisation of the receptor (Moody *et al.,* 2000)

2.5.4 The response to β-adrenergic agonist supplementation on muscle and adipose tissue

When β-agonists occupy specific β-ARs on adipocyte cells, biochemical signals are produced within the cells that inhibit fat synthesis and increases fat degradation (Figure 2.7) (Page, 2004). However, when β-agonists bind to receptors found on muscle cells, protein synthesis is increased and protein degradation is inhibited (Sillence, 2004). As a result, the rate of muscle deposition is greater than fat deposition, which leads to leaner animals (Anderson *et al*., 2004). In young growing animals, nutrient (energy) intake is directed towards muscle gain and as the animal reaches maturity, the ingested nutrients are directed towards fat deposition instead of muscle. For this reason, β-agonists are administered during the final stages of the feeding period to prevent increased fat deposition in mature animals (Anderson *et al*., 2004; Badino *et al*., 2008). Observed improvements in performance are evident during the initial period of supplementation. Thereafter, the response to β-agonist treatment diminishes due to receptor downregulation caused by extended receptor stimulation (Lohse *et al*., 1990).

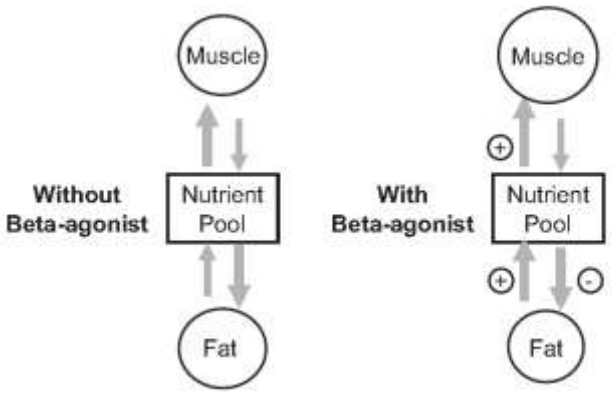

Figure 2.7: β-agonists are used as repartitioning agents in animals due to the ability of β-agonists to alter specific metabolic signals, which results in the repartitioning of nutrients towards muscle, instead of fat (Anderson *et al.,* 2004)

2.5.4.1 Direct effect of β-adrenergic agonist on muscle tissue

The most evident response to β-agonist stimulation is improved muscle mass through hypertrophy, fibre changes and an increase in lean deposition (Byrem *et al*., 1998; Moody *et al*., 2000). Muscle induced hypertrophy is mediated through β-ARs found on the surface of muscle cells. The hypertrophic response was demonstrated by MacLennan and Edwards (1989) who injected clenbuterol (β_2-agonist) and propranolol (antagonist) into rat peripheral tissue (MacLennan & Edwards, 1989; Sillence, 2004). The ability of clenbuterol to increase muscle hypertrophy was blocked by the antagonist, thereby proving the involvement of β-ARs in stimulating muscle growth.

β-agonists promote muscle protein accretion, which depends on the rate of protein synthesis *versus* the rate of protein degradation. It is unclear whether β-agonists increase protein synthesis, decreases protein degradation or a combination of both, which results in increased protein deposition (Buhr, 1992; Sillence, 2004; Dunshea *et al*., 2005; Dikeman, 2007). Reduced urinary nitrogen (N) excretion in animals was observed when animals was supplemented with β-agonists, which supports the idea that agonists enhance protein accretion through reduced protein degradation (National Research Council, 1994). Furthermore, β-agonists do not depend on dietary protein and energy intake, which suggest that their primary mechanism of action is through reduced protein degradation because protein synthesis requires a balanced diet (MacRae & Lobley, 1991). In addition, β-agonists may induce muscle hypertrophy by altering the proteolytic activity within muscle (Mersmann, 1998). Muscle proteolytic enzyme concentrations are used as indicators of muscle activity to determine protein degradation rates. Results from various animal studies indicate that it is either the reduction of protease enzymes (calpains) or an increase of protease inhibitors (calpastatin) that cause an increase in protein deposition (Bardsley *et al*., 1992). Alternatively, β-agonists stimulate muscle protein synthesis by improving the protein to DNA ratio, which is an indication of the physiological size of muscle (Grant et al., 1990). However, Morgan *et al*. (1989) failed to demonstrate any significant differences in the DNA concentration of muscle in chickens treated with cimaterol. This was supported by Kim & Sainz (1992), who found a reduced DNA concentration in lambs treated with β-agonists suggesting that an increase in DNA is not a prerequisite for muscle hypertrophy. The variability in response between animal studies may be due to the type of β-agonist used, the receptor specificity for β_1 - or β_2 -agonists in different species or the dose and duration of supplementation (Dixon *et al.*, 1987; Sillence, 2004). According to Moody *et al.* (2000), β_2-receptors stimulate protein synthesis and degradation, while β_1-receptors only stimulate protein synthesis.

Muscle growth depends on the free energy available for the synthesis and degradation of protein, where protein synthesis requires more energy than protein degradation (Gebre *et al*., 2012). Therefore, any changes in energy supply will alter the rate and amount of muscle protein deposition. One of the major factors influencing the muscle energy expenditure is the muscle's metabolic type, namely oxidative versus glycolytic muscle (National Research Council, 1994). Protein synthesis and degradation are higher in red oxidative muscle types compared to white glycolytic muscle types,

which increases muscle expenditure of animals and therefore growth (Hocquette *et al*., 1998). As a result, an increase in muscle growth is associated with an increase in glycolytic metabolism. The administration of β-agonists has been found to modulate oxidative metabolism, glucose transport and mitochondrial function (Hocquette *et al*., 1998).

2.5.4.2 Indirect effect of β-adrenergic agonists on muscle

β-agonists have an anabolic response related to increased weight and protein deposition (MacRae & Lobley, 1991). β-agonists increase the metabolic rate of animals by increasing their heart rate, arterial blood flow and nutrient flow to muscle for protein synthesis (Mersmann, 1998). This is evidenced by increased blood flow to the hind limbs of cattle, and the skeletal muscle and adipose tissue in pigs (Mersmann, 1995). Partitioning nutrients towards muscle improves the balance between supply and demand, and thus less amino acids need to be catabolised (MacRae & Lobley, 1991). As a result, β-agonists are less sensitive to amino acid availability as their requirements are already closely met by absorption. In addition, β-agonists have a protein sparing effect on energy metabolism by enhancing N retention, which results in increased muscle protein synthesis and reduced amino acid oxidation (National Research Council, 1994). The administration of β-agonists improved N retention in the hindquarters of steers due to an increase in blood flow (MacRae & Lobley, 1991). Interestingly, although oxygen utilisation by hindquarters increased, glucose uptake did not, which proved that the energy supplied for protein synthesis came from lipid oxidation and not protein (MacRae & Lobley, 1991).

In addition, β-agonists improve muscle protein at the expense of protein deposition in other organs (Figure 2.8). β-agonist supplementation is therefore efficient in meat producing animals, but not in animals selected for other production traits such as milk and wool. The response to β-agonist treatment in species will thus differ as the requirement for amino acids depends on the type of animal production and their physiological state (MacRae & Lobley, 1991).

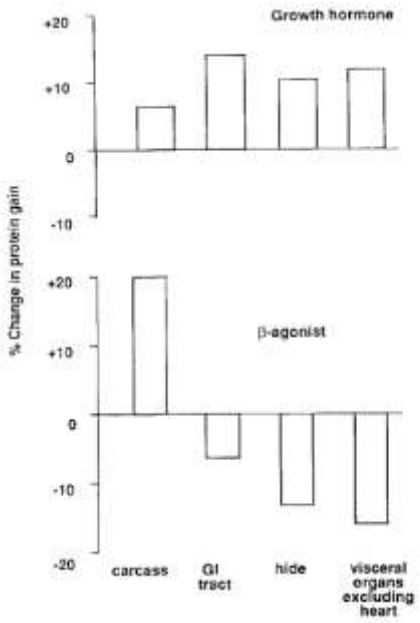

Figure 2.8: The difference between growth hormone and β-agonist treatment in altering protein deposition in different tissues (MacRae & Lobley, 1991)

2.5.4.3 Effect of β-adrenergic agonists on adipose tissue

One of the responses to β-agonist supplementation is a reduction in body fat. However, the outcomes are less potent when compared to the muscular effects due to the lower density of the β-receptors found on adipose cells (Badino *et al*., 2008). Anderson *et al.* (2004) reported that the mechanisms used to facilitate muscle growth from β-agonist treatment are not the same as those used in adipose tissue. The anabolic effects of clenbuterol on muscle were blocked by an adrenergic antagonist, however, the lipolytic effect of clenbuterol was not affected (Anderson *et al*., 2004). The study showed that the clenbuterol treated group had reduced body fat deposition compared to the control, which clearly indicated the different mechanisms of action in adipose tissue. Furthermore, results from *in vitro* studies in several species have demonstrated elevated concentrations of plasma NEFA which indicated that β-agonists have the ability to stimulate the adipocyte lipolytic system (Mersmann, 1998). The stimulation of β-adrenergic receptors and cAMP activates rate-limiting enzymes involved in lipolysis, which in turn inactivates specific lipogenic enzymes involved in the *de novo* synthesis of FA and TGs (Anderson *et al*., 2004). In addition, β-agonists decrease the responsiveness of adipose receptors to insulin stimulation, which further decreases the uptake and storage of TGs (Moody *et al*., 2000).

The mechanisms of action of β-agonists and their response may vary across species due to different lipid regulatory mechanisms that exist, specifically between ruminants and monogastric animals (Yang & McElligott, 1989). The response to β-agonists in avian species has been variable and mostly inconsistent when related to body fat reduction (Gwartney *et al*., 1991). In contrast to

mammals, chicken adipocytes are generally insensitive to β-agonist stimulation and therefore indirectly reduce adipose tissue deposition. These differences observed in adipose tissue deposition are related to energy metabolism and storage between species (Buyse *et al*., 1991). Lipids present in chicken blood differ from those of mammals because it is derived from the intestinal absorption from ingested lipids, hepatic synthesis and mobilised fat (Ansari-Pirsaraei *et al*., 2007). In avian species, the liver is the main site for *de novo* FA synthesis, which is then transported to adipose tissue for storage as TGs. Generally, abdominal fat deposition can be reduced by either inhibiting the absorption of dietary fat and FA synthesis in the liver or by increasing β-oxidation of FAs, which decreases the size and number of adipose cells (Fouad & El-Senousey, 2014). Studies have shown that, unlike mammals, β-agonists decrease adipose tissue mass in poultry by reducing cell size rather than cell number (Buyse *et al*., 1991). Furthermore, an increased heart rate due to β-agonist stimulation, followed by an elevation in the blood flow to organs and fat tissues, resulted in an enhanced NEFA being carried away from the adipocytes (Mersmann, 1995). Therefore, β-agonist indirectly enhances the lipid degradation process.

2.5.5 Hormonal response to β-adrenergic agonist treatment

β-adrenergic agonists influence hormonal secretion in a positive or negative way depending on the dose, species and type of agonist used (Beerman, 1987). The response in hormonal secretion to β-agonist treatment is variable across experimental studies and therefore, depends on the physiological state of the animal. Literature suggests that catecholaminergic systems have a profound effect on the secretion of hormones and the resulting response differs between species (Sejresen & Vestergaard Jensen, 1987). Exogenous administration of GH produced similar effects in growth and carcass composition as β-agonists, however, the resulting responses are independent of each other (Johnson *et al.*, 2014). Growth hormone supplementation induces muscle hypertrophy through satellite cell proliferation and DNA synthesis (Beerman, 1987). In contrast, β-agonist induces muscle hypertrophy through increasing muscle size (Baxa, 2008). According to Buyse *et al.* (1991), there is a β-adrenergic inhibition of GH release in the domestic fowl but no consistent effects were observed on GH, T_3, IGF-I or corticosterone levels in broilers (Buyse *et al*., 1991). In sheep and cattle, cimaterol treatment increased both GH and T_4 levels, whereas IGF-I concentrations were increased (Beerman, 1987). In contrast, Young *et al*. (1995) observed no significant effect on GH and IGF-I secretions after β-agonists supplementation. However, Moody *et al.* (2000) reported irregular GH secretion cycles, and concluded that β-agonist treatment modifies growth patterns by altering normal GH secretions.

β-agonists and insulin have opposite effects on metabolic pathways in the liver, skeletal and adipose tissues. It has been suggested that the repartitioning effects are due, in part, to the opposing effects of β-agonists on insulin responsiveness in fat *versus* muscle tissue (Moody *et al*., 2000). Chronic supplementation of a β-agonist differs from acute administration, which demonstrates their

specific mechanisms of action (Beerman, 1987). Chronic administration of cimaterol in steers significantly reduced plasma insulin concentration, however glucose uptake in muscle was not altered (Beerman, 1987). According to Page (2004), β-agonists improved the sensitivity of muscle receptors to insulin's ability to increase glucose uptake. On the other hand, acute cimaterol supplementation resulted in increased insulin levels in steers (Beerman, 1987). The elevated insulin concentration was accompanied by high plasma glucose concentrations due to an increase in hepatic and skeletal glycogenolysis stimulated by cimaterol. According to Beerman (1987), the level of insulin increased either due to an elevation in glucose levels, or due to the negative feedback mechanisms regulating insulin concentration.

Reports have indicated that the glucocorticoid and T_3 hormones regulate the number and function of β-ARs (Johnson, 2006). However, other studies involving the administration of β-agonist in combination with T_3 and glucocorticoid hormones failed to prove their direct involvement in muscle hypertrophy (Beerman, 1987; Hamano *et al.,* 1999b). Beerman (1987) reported that T_3 concentration was slightly increased in lambs fed cimaterol and theorised that T_3 has an indirect effect on β-agonist's response by enhancing the efficiency of lipolytic hormones. However, according to MacRae & Lobley (1991), the elevated T_3 levels observed in a similar study may be due to an increased metabolic rate and heat production in response to treatment. Furthermore, a study by Hamano *et al.* (1999b), demonstrated that the response to clenbuterol treatment was improved when T_3 was administered in broilers. This was evidenced by the greater response in growth and carcass composition in T_3 treated birds as compared to the control. Based on the results, it was found that thyroxine stimulated the effect of clenbuterol through an indirect interaction by increasing protein turnover rates (Hamano *et al.,* 1999b). These findings are in agreement with Baxa (2008) which stated that the resulting benefits of combining hormones and β-agonist treatments could be due to the additive or complementary effects on different tissues, instead of intracellular interactions.

2.5.6 The effect of β-adrenergic agonist supplementation on feed intake

Response in FI to β-agonist treatment involves direct (tissue specific) and indirect (endocrine) changes related to fat and muscle metabolism (Mersmann, 1998). Alterations in muscle and fat metabolism depends on the physiological state of the animal and differs between mammals and avian species (Hamano *et al.*, 1999a). The effect of β-agonists on FI has been variable and inconsistent between animal studies (Baghbanzadeh & Decuypere, 2008; Kheiri *et al.*, 2011b; Baghbanzadeh *et al.*, 2015). When β-agonists are used to alter growth rates in animals, it is natural to assume that a higher FI is required to stimulate the increased growth rates. However, numerous studies have demonstrated a reduced FI (Buyse *et al.*, 1991; Strydom *et al*., 2009), while others have reported an increase (Buyse *et al*., 2010; Kheiri *et al*., 2011a; b) or no effect (Boostan *et al*., 2015). β-adrenergic agonists are similar in structure and function to endogenous catecholamines. Hence, β-agonist are able to produce a similar response in feeding behaviour via a direct interaction with receptors in the brain

and possibly the liver (Howes & Forbes, 1987). Furthermore, based on the numerous controversies observed in FI between animal studies, the resulting response might be associated with an indirect effect of β-agonist treatment related to endocrine function. In addition, the type of β-agonist used and the dose and duration of treatment will ultimately determine the response in growth and FI between and within species (Reeds & Mersmann, 1991).

2.5.6.1 Direct effect of β-adrenergic agonists on feed intake

β-adrenergic agonists are non-catecholamines and therefore differs from natural catecholamines based on their absorption and elimination rates (Smith, 1998). β-adrenergic agonists can have a central effect on FI as it is supplemented at higher effective doses and ability to cross the blood brain barrier more easily as compared to E or NE (Howes & Forbes, 1987). Research has indicated that β_1-agonists stimulated FI in poultry species (Denbow *et al*., 1986), whereas β_2-agonists stimulated FI in broilers (Bungo *et al*., 1999) and layers (Tachibana *et al*., 2009). Interestingly, β_3-agonists reduced feeding in chickens fed *ad libitum*, but not in restricted feeding conditions (Tachibana *et al*., 2003).

Research in feed and water intake in animals mostly involved α-adrenergic receptors, however, increasing evidence also indicated the presence of β-adrenergic satiety centres in the hypothalamus of animals (Denbow *et al*., 1986). According to Baghbanzadeh *et al.* (2015), brain adrenergic synaptic transmission plays an important role in FI regulation. Like mammals, catecholamines are found in high concentrations in the CNS of birds and have been shown to be involved in the feeding behaviour of animals (Denbow, 1999). Studies have shown that changes in brain NE levels can either increase or decrease feeding depending on the site of application (Hagemann *et al*., 1998). Hypothalamic microinjections of NE increased FI in both fed and restricted rats (Grossman, 1960), whereas NE injections resulted in reduced feeding behaviour in chickens (Baghbanzadeh *et al.*, 2015). Grossman (1960) concluded that the NE induced FI in rats are due to the stimulation of the β_2-adrenergic receptors located in the hypothalamic regions of the brain. Similarly, intracerebroventricular (ICV) catecholamine injections were shown to increase FI in pigeons (Ravazio *et al*., 1990) and fast-growing broilers (Denbow *et al*., 1986). On the other hand, ICV catecholamine injections had no effect in slow-growing chickens and decreased feeding behaviour in turkeys (Denbow, 1983). According to Hagemann *et al.* (1998), the variations in response observed between avian species are thought to be the result of increased selection for fast-growing birds, which caused differences in catecholamine circuitry in the hypothalamus.

2.5.6.2 Indirect effect of β-adrenergic agonists on feed intake

The effects of hormonal manipulations on energy metabolism and FI have been widely studied across all species (Richards, 2003). Increasing evidence supports the idea that naturally occurring catecholamines play a major role in metabolic processes, not only after sudden stress

stimuli, but also on basal plasma hormone levels (Timmerman, 1987). Therefore, in the long-term, β-adrenergic agonists may have a profound effect on the feeding behaviour of animals.

The liver has been shown to be a major regulator of FI by either stimulating or inhibiting the release of metabolites from metabolic pathways. This may be in the form of glucose, FAs or amino acids, which serve as signalling molecules of the energy status in animals (Richards, 2003). For example, Denbow (1999) reported that hepatic injection of glucose suppressed FI in birds. Furthermore, the liver contains both α- and β-receptors, and is therefore subject to β-agonist stimulation (Howes & Forbes, 1987). Howes & Forbes (1987) performed a series of studies to determine the physiological action of E on FI in birds. Salbutamol (β_2-agonist) and E injections at various sites resulted in a reduced FI in birds. The authors concluded that E has a hepatic action on FI in birds, however, the study failed to prove the exact mechanism of action. Therefore, the binding of β-adrenergic agonists to receptors found in the liver may have an indirect effect on feeding behaviour by stimulating specific metabolic pathways (Howes & Forbes, 1987).

Alternatively, it has been proposed that the change in chicken feeding behaviour from β-agonist treatment is related to an indirect response to circulating hormone levels, such as insulin, leptin, glucagon and thyroid hormones (Reeds *et al*., 1988; Mersmann, 1998). Insulin is the major hormone regulating blood glucose concentration in all animals (Reeds & Mersmann, 1991). Therefore, specific metabolic pathways are either stimulated or inhibited depending on the concentration of insulin in the blood. When animals consume feed their blood glucose levels increase which stimulate insulin secretion which in turn signals specific satiety centres within the brain to reduce FI (Ashwell & McMurtry, 2003). This holds true when blood insulin levels are low. The satiety receptors in the brain are therefore inactivated, causing animals to increase their FI. The administration of β-agonists has been reported to decrease (Beerman, 1987), increase (Yang & McElligott, 1989) or have no effect (Fiems, 1987) on insulin concentrations in animals. In situations where β-agonist causes a reduction in blood insulin levels, it is possible that animals will increase their FI to sustain their metabolic needs. Research studies has demonstrated that insulin interacts with orexigenic and anorexigenic peptides within the brain of mammals, which controls their feeding behaviour (Shiraishi *et al*., 2008). Shiraishi *et al.* (2008) performed a study on insulin control in chicks and concluded that central insulin injections suppressed FI via melanocortins. Insulin increased the expression of the POMC genes and decreased the expression of the NPY genes, which inhibits and stimulates FI, respectively (Shiraishi *et al*., 2008). Therefore, β-agonist treatment may indirectly alter feeding behaviour in animals through changes in insulin release.

Alternatively, leptin is a signalling hormone that is able to sense the energy status of animals and is therefore able to alter their feeding behaviour (Kearns & McKeever, 2009; Tachibana & Tsutsui, 2016). Studies have shown that leptin has an anorexigenic effect in animals, where high circulating levels of leptin cause a reduction in FI. For instance, adipose-specific cytokines leptin and adiponectin have shown to influences appetite and energy balance in horses (Kearns & McKeever,

2009). Leptin is secreted from adipose tissue and the concentration of leptin depends on the size of energy stores and remains relatively constant. Animals that have an inherently lower adipose tissue mass will have a lower concentration of circulating leptin as compared to animals that are obese. One of the main effects of β-agonist supplementation is reduced adipose deposition by inhibiting lipogenesis and stimulating lipolysis (Schiavone *et al*., 2004). As a result, animals will have lower fat energy stores available and consequently, lower circulating levels of leptin, which signals the brain to increase FI (Tachibana & Tsutsui, 2016). A study by Kearns & McKeever (2009) showed that the aadministration of clenbuterol increased adiponectin and decreased leptin concentrations in horses. Therefore, β-agonist treatment alters feeding behaviour of animals by indirectly inhibiting leptin secretion through reduced adipose tissue deposition.

2.6 Benefits of using β-adrenergic agonists in the commercial industry

The beneficial effect of β-agonist supplementation concerns many aspects of the animal production industry, as well as the consumers and the environment (Figure 2.9). The use of a β-adrenergic agonists allows for higher slaughter weights while maintaining the same level of lean mass (Dikeman, 2007). β-agonists can be used as an effective management tool to improve leanness and decrease fat in animals selected for traits other than meat production, such as reproduction efficiency and increased longevity (Anderson *et al*., 2004). Furthermore, producers are able to manufacture meat containing a lower amount of fat, which is more acceptable to consumers (Anderson *et al*., 2004). Additionally, other aspects of the meat production industry also benefit from the use of β-agonists, such as meat packers and processors that receive higher yielding carcasses with low-fat content (Anderson *et al*., 2004). Supplementation of β-agonists improve the efficiency of feed utilisation which ultimately increases production profit by decreasing feed cost (Anderson *et al*., 2004). Improved FCR benefits the environment due to less land required to produce the feed needed by meat producing animals. In addition, β-agonists improves N retention and therefore, reduces the amount of gas excreted into the environment (Reeds *et al*., 1988; Moran, 2004).

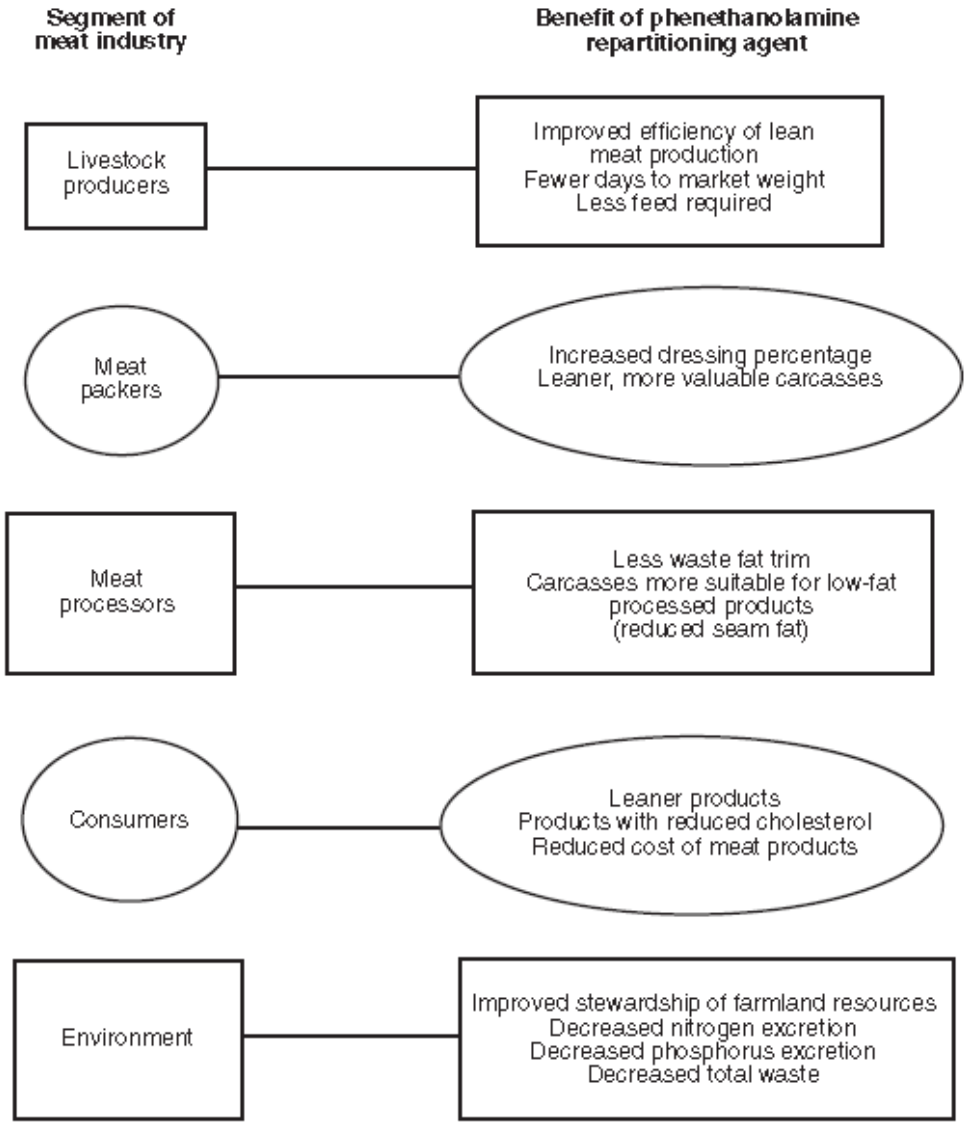

Figure 2.9: Schematic representation of the benefits for each sector from feeding β-agonists to animals (Anderson *et al*., 2004)

2.7 Factors contributing to the variation in response to β-adrenergic agonist supplementation between animals

Extrapolating results from *in vitro* studies to *in vivo* could be misleading and thus, increase the risk of under-or overestimating the response to treatment. Even though *in vitro* studies accurately describe the mechanism of action of β-agonists, the quantitative use of results is incorrect (Mersmann, 1998). This is due to animal and environmental factors that could influence the response *in vivo*. Therefore, to obtain quantitative data, *in vivo* studies have to be performed in a given species following a specific experimental design and then validated *in vitro* (Mersmann, 1998). β-agonist supplementation has shown to improve performance in cattle, sheep, pigs, chickens and turkeys (Fiems, 1987). However, the degree of response varies significantly between animal studies.

Variations in response are related to species differences and distinctions in tissue distribution, structure of receptor subtypes and the mode of action when different β-agonists are applied (Mersmann, 1995).

2.7.1 Variation in receptor structure

The primary amino acid sequences of a protein differ across species as a result of deletions, insertions or substitutions occurring over the years (Dixon *et al*., 1987). These alterations cause modifications of the primary amino acid structure and leads to a change in protein function. Consequently, the primary amino acid structure of different receptor subtypes will also differ across and within the same species (Young *et al*., 2000b). Research indicates that β-receptors shares 70-75% homology across species. However, β_1, β_2 and β_3-receptors share only 40-50% homology with each other within a given species (Mersmann, 1995). Additionally, within a single animal, the primary amino acid structure of a specific receptor subtype could differ between tissues, which further explain the various pharmacological responses observed in scientific studies (Devic *et al*., 2001). Moreover, some β-agonists can bind to both β_1- and β_2-adreneric receptors (Frielle *et al*., 1988).

2.7.2 Variation in density and affinity to target tissues

The amount and distribution of receptors vary across species (Young *et al*., 1999). This could alter the response to β-agonist treatment even if the receptor subtypes were identical in structure (Brown *et al*., 1976). Skeletal muscle is predominantly composed of β_2-receptors and heart muscle contains mainly β_1-receptors (Mersmann, 1998). Therefore, supplementing a β_2-agonist will not stimulate an increase in heart muscle to the same extent as skeletal muscle. Similarly, rat adipocytes contain β_3-receptors, whereas humans do not, and will therefore show variations in response if a β_3-agonist is administered (Tachibana *et al*., 2003). To add to the complexity of determining an exact response, individual cells within the same species could have different distributions of receptor subtypes (Brown *et al*., 1976).

Moreover, some β-agonists can bind to both β_1- and β_2-adreneric receptors (Frielle *et al*., 1988). Red muscles are mainly present in the thighs and wings of chickens and contains a higher density of β-receptors as compared to white muscle found in the breast tissue (Young *et al*., 2000b). This was also proved in a similar study by Hamono *et al.* (2004) who supplemented clenbuterol to broilers and observed a greater response in thigh muscle weight compared to breast muscle weight.

2.7.3 Dose and duration of treatment

The affinity to β-agonists is determined by the dose and efficacy of the type administered (Frielle *et al*., 1988). Metabolic modifiers are only effective for a limited period of time after which the response will reach a plateau (Lees, 1981). This is due to receptor downregulation following prolonged exposure, which decreases their sensitivity to β-agonist activation (Lohse *et al*., 1990). The

degree of receptor downregulation determines the duration of treatment, which varies between species and the type of agonist used. As a result, the use of metabolic modifiers is limited to the finishing phase and supplemented for two to three weeks depending on the species. Studies have indicated that there is no economic benefit of feeding β-agonists for longer than the specified administration period because the response starts to diminish (Dalrymple *et al*., 1984). In swine, the increase in average daily gain (ADG) began to plateau after three weeks (Sainz *et al*., 1993), whereas in poultry, the growth response started to diminish after two weeks (Dalrymple *et al.,* 1984; Sainz *et al.*, 1993).

Studies have shown that a desired response can be achieved at lower dosage levels (Dalrymple *et al.*, 1984). For example, weight gain was improved in broilers at treatment inclusion levels below 1 mg cimaterol/kg compared to 2 mg cimaterol/kg inclusion level (Dalrymple *et al*., 1984). The higher concentrations (2 mg/kg) resulted in a lower growth rate due to a reduction in FI. However, carcass leanness and dressing percentage continued to improve at higher concentrations (Dalrymple *et al*., 1984). Furthermore, differences in β-agonist specificity and potency add to the variation in response between species (Frielle *et al*., 1988). According to Asato *et al*. (1984), cimaterol is a more potent repartitioning agent in poultry when compared to clenbuterol. This is evidenced by the greater magnitude of response observed in the growth and feed efficiency when cimaterol was supplemented.

2.7.4 Sex, age and the genotype of animals

In broilers, females tend to have a greater response to β-agonist treatment compared to males as females have a higher tendency to deposit fat (Rehfeldt *et al*., 1997). Besides the effects seen in poultry (Dalrymple *et al*., 1984) and swine (Dunshea *et al.*, 1993), little data has indicated a sexually dimorphic response in ruminants. Rehfeldt *et al*. (1997) fed 1 mg clenbuterol/kg and found improved ADG and final BW, and a reduced FCR in males. However, in females, clenbuterol only reduced the abdominal fat pad. Skeletal muscle weights were also enhanced in both sexes with greater increases in fibre diameter in females when compared to males. According to Rehfeldt *et al.* (1997), sex-related fibre size response to β-agonists are subject to the initial fibre number. Increases in diameter are smaller at greater fibre numbers and *vice versa*. Therefore, males contain a greater number of muscle fibres and have a lower increase in fibre diameter in response to clenbuterol supplementation when compared to females.

Furthermore, the repartitioning effects of β-agonists allow more energy to become available for protein accretion resulting in less abdominal fat deposition (Rehfeldt *et al*., 1997). Therefore, the greater final carcass protein yields obtained in both sexes were essentially due to the increase in BW in males and changes in body composition in females (Rehfeldt *et al*., 1997). Similarly, in Japanese quails, gender x β-agonist interaction was observed at 7 mg salbutamol/kg supplementation (Zare Shahneh *et al*., 2012). Salbutamol improved FI and FCR to a greater extent in females as compared to males. Breast, thigh and liver weight were also significantly higher in females. However, the chemical

composition of the breast muscle did not differ between males and females (Zare Shahneh *et al*., 2012).

Reports have suggested a genotype x β-agonist interaction, however, results are contradictory (Mitchell *et al.*, 1990; Warriss *et al.*, 1990; Dunshea *et al.*, 1993). According to Schinckel *et al.* (2002), the magnitude of response to ractopamine supplementation was greater in pigs selected for increased lean growth. In contrast, inferior genotypes responded to a larger extent than superior genotypes to ractopamine administration (Mitchell *et al*., 1990; Warriss *et al.*, 1990). Similarly, Dunshea *et al.* (1993) observed greater protein deposition in gilts and barrows as compared to boars. The age of animals, also have an influence on the magnitude of response to supplementation. β-agonists are generally administered during the finishing phase of the feeding period when animals reach maturity. Therefore, a greater response will be observed in more mature animals as they tend to deposit more fat (Dunshea *et al.*, 1993). In addition, younger animals have a lower receptor density at the target tissue when compared to older animals, and will therefore show a low or no response to treatment (Fiems, 1987).

2.7.5 Species variation

Chickens are generally insensitive to β-agonist stimulation and increasing research has been undertaken to determine the cause and solution for their unresponsiveness (Hamano *et al*., 1999b; Schiavone *et al*., 2004; Badino *et al*., 2008). β-ARs are found in all mammalian cells, however, less is known about the distribution in avian species (Yarden *et al*., 1986). Similarities observed in growth response, increased blood flow and hormone release between avian and mammalian species indicates some similarity in β-AR distribution between these species. Based on the research of β-ARs in turkeys, the pharmacological properties of avian β-ARs are not similar to those of mammalians, which makes comparisons across species more difficult (Young *et al*., 2000b). Shappell *et al.* (2000) used turkey satellite cells (*in vitro*) to determine their response to ractopamine at the cellular level. The authors concluded that ractopamine does not act directly via the β-ARs on muscle cells, which means β-agonists stimulate changes in muscle characteristics through extra-muscular effects by increasing blood and amino acid uptake in muscles (Shappell *et al*., 2000). As a result, the response in growth and carcass characteristics will differ between species due to the variations in the mechanisms of action of β-agonists in different target tissues.

2.7.6 Dietary nutrient requirements

β-adrenergic agonist supplementation alters the normal protein and fat deposition of animals, thereby changing their daily nutrient requirements (Yen *et al*., 1990). An enhanced growth response will not occur if nutrient intake is limited. Animals that are fed β-agonists require an increase in dietary protein to accommodate the elevated rates of muscle deposition (Reeds & Mersmann, 1991). Studies indicate a linear response in growth and carcass composition with increasing levels of dietary

protein when β-agonists are administered (Hamano *et al*., 1998a). Hamano *et al.* (1998a) demonstrated that an increase in BW from clenbuterol treatment was dependent on protein intake. The effect of clenbuterol and various concentrations of protein (220, 240 or 260 g protein/kg feed) were examined. The study revealed an increase in the performance and carcass characteristics when 240 and 260 g protein/kg was fed (Hamano *et al.*, 1998a). These findings agree with a previous study that showed that the combination of a low protein diet (110 g CP/kg) and clenbuterol markedly decreased the BW and FI of broilers (Hamano *et al.*, 1998b). Similarly, Dunshea *et al.* (1993) demonstrated a higher protein requirement in swine treated with ractopamine in order to stimulate the growth-promoting effect of β-agonists. Therefore, absolute protein intake is the limiting factor on growth rate and β-agonist response (Mersmann & Reeds, 1991).

Another limiting factor affecting β-agonist response is the energy concentration of the diet (Hamano *et al*., 1998c). Kim *et al.* (1991) proved that a diet containing both high protein (190 g CP/kg) and high energy (13.4 MJ/kg) resulted in the highest BW and feed efficiency in broilers. The authors concluded that the most efficient protein concentration to improve performance in β-agonist-treated animals is the one that increases consumption. Similarly, Mitchell *et al.* (1990) showed that the response to ractopamine treatment in pigs was influenced by protein level and limited by dietary energy intake. The results indicated that ractopamine is unable to depress lipid deposition and increase protein deposition if energy is limited (Mitchell *et al*., 1990). To achieve maximum benefit from β-agonist supplementation, it is essential to provide a balanced protein and energy diet (Reeds *et al*., 1981). Animals will either increase or decrease FI in order to sustain their metabolic requirements. Therefore, diets deficient in either protein or energy will alter the response to treatment of β-agonists.

2.8 Response of poultry to different types of β-adrenergic agonists

2.8.1 Ractopamine

Ractopamine is a β_1-agonist and is approved for the use as a feed additive in swine and cattle diets (Radunz, 2011). Only a few studies have been conducted to determine the effect of ractopamine in modern broiler strains (Kheiri *et al.*, 2011b). A study by Kheiri *et al*. (2011a) on ractopamine supplementation in female broilers resulted in no significant improvements in growth parameters. This is in accordance with a similar study done by Kheiri *et al.* (2011b) that involved male broiler chicks. According to the results, the ADG tended to decline with an increase in treatment levels, however, feed efficiency was improved (Kheiri *et al.*, 2011b). Changes in blood biochemical parameters indicated that ractopamine was involved in protein synthesis as demonstrated by reduced blood urea nitrogen and uric acid concentrations. Increased serum cholesterol and TG concentrations were also found, which suggested a possible shift in fat metabolism (Kheiri *et al*., 2011a).

2.8.2 Clenbuterol

Clenbuterol is a β_2-adrenergic agonist, which significantly improves growth rate and feed efficiency in broiler chickens (Fiems, 1987; Litt Miller, 2007). Schiavone *et al*. (2004) studied the effects of supplementing 1 mg clenbuterol/kg on growth performance and muscle composition in broiler chickens. The results revealed an increase in final body weight, carcass, breast and thigh weights, together with an increase in carcass, breast and thigh protein yields (Schiavone *et al*., 2004). Decreased FI and FCR were also observed. This is in accordance with Hamano *et al.* (1998b) and Rehfeldt *et al.* (1997), who also reported improved growth performance and feed efficiency from clenbuterol supplementation.

2.8.3 Cimaterol

Cimaterol is a structural analogue to clenbuterol (β_2-agonist) and is known as an effective bronchodilator. However, the use of cimaterol escalated in the 1990's due to its lean-enhancing properties (Jones *et al*., 1985; Fiems, 1987; Morgan *et al*., 1989; Gwartney *et al*., 1992; Schiavone *et al*., 2004). Improved growth performance and feed efficiency were observed at an inclusion level of 1 mg cimaterol/kg, together with greater carcass weight and protein content in both breast and thigh muscles (Schiavone *et al*., 2004). In contrast, Morgan *et al*. (1989) showed that the size of the thigh muscle increased, but breast muscle composition and size were unaffected by cimaterol treatment. Thigh muscle was also higher in protein and lower in fat when compared to the control birds (Morgan *et al*., 1989). The unequal response between breast and thigh muscle weights proves the differences that exist in tissue affinity for β-agonists, which results in variations in the response between animals.

2.8.4 Terbutaline

Terbutaline is a β_2-agonist used as fast-acting bronchodilators and, more recently, as lean-enhancing agents in animal studies (Boostan *et al*., 2015). Ansari-Pirsaraei *et al.* (2007) studied the effect of terbutaline supplementation on blood parameters and carcass composition in broiler chicks. Three-week-old males and females were assigned to 5, 10, 15 and 20 mg terbutaline per kg diets. Results showed no significant effect on ADG, however, FCR was reduced in males at 5 and 10 mg terbutaline/kg supplementation, when compared to the control group (Ansari-Pirsaraei *et al*., 2007). Results indicate that females had an improved breast to live weight ratio, whereas males had an improved carcass to live weight ratio (Ansari-Pirsaraei *et al*., 2007). The authors concluded that terbutaline is capable of modifying muscle gene expression by increasing protein content and changing protein contractile isoforms, evidenced by an increase in breast and drumstick muscle weight (Ansari-Pirsaraei *et al*., 2007). In a similar study involving Japanese quails, improved carcass composition was observed after the supplementation of different levels of terbutaline (1, 3 and 5 mg terbutaline/kg) (Boostan *et al*., 2015). However, terbutaline treatment had no effect on FI, BW or FCR. Breast, fat and moisture content showed no difference between the treatment groups, however,

higher breast and drumstick protein yields were observed at 3 and 5 mg terbutaline/kg supplementation (Boostan *et al*., 2015). The lack of response in Japanese quails when compared to broilers may be due to a lower receptor density and distribution of β_2-receptors found in muscle.

2.9 The effect of zilpaterol hydrochloride in different species

Currently, only two β-agonists are approved for use in the commercial industry, namely zilpaterol hydrochloride (ZH) and ractopamine (Dilger, 2015). Others have been discarded due to their potential toxicity and reduced meat quality, and therefore, do not confer the same benefits as ractopamine and ZH (Kuiper *et al*., 1998). Zilpaterol hydrochloride is a synthetic β_2-agonist and therefore, not a phenethanolamine like other recognised β_2-agonists (Dikeman, 2007).

Zilpaterol hydrochloride is used for improved weight gain, feed efficiency and carcass leanness (Rogers, 2013). The International Union of Pure and Applied Chemistry‘s (IUPAC) name for zilpaterol is 4,5,6,7-tetrahydro-7-hydroxy-6-(isopropylamino)-imidazo [4,5,1-jk]-1-benzazepin-2(1H)-1 (Stachel et al., 2003). Zilpaterol hydrochloride is a β_2-agonist sold under the tradename Zilmax®. Zilpaterol is approved for use as a feed additive in Mexico, United States of America, Australia, New Zealand, Brazil, Indonesia and South Africa (López-Carlos *et al*., 2010). In South-africa, Zilmax was registered for use in cattle in 1995, however, Zilmax is not yet approved in other production animals such as sheep, pigs and poultry (Webb et al., 2018). Zilpaterol hydrochloride is rapidly eliminated from the body and therefore does not pose the same potential negative effects as other β-agonists, such as salbutamol (Knobel, 2014). Approval studies indicated that the zilpaterol concentration in edible tissues reached a plateau after 12 days and any residues were depleted after 24 hours (Stachel *et al*., 2003). Numerous studies have reported that ZH supplementation offered a distinct advantage in the growth efficiency and carcass yield of cattle and sheep and was found to be more adaptable to certain breeds (Dikeman, 2007; López-Carlos *et al*., 2010). The approved inclusion rate of Zilpaterol hydrochloride is 7.5 mg ZH/kg feed and is supplemented in the finishing phase of animals. In cattle, there is no economic benefit of feeding ZH beyond 20 days, which led to the normal feeding regimen with a three day withdrawal period (Radunz, 2011). Dikeman (2007) reported that meat tenderness was reduced if ZH supplementation period was longer than 30 days. As a result, Dikeman, (2007) suggested that the appropriate electrical stimulation and ageing could be used to reduce the negative effect on tenderness. Similarly, in poultry there is no economic benefit related to feeding ZH for more than 14 days (Rogers, 2013).

The response to ZH is mediated through β-AR binding and activation. Zilpaterol hydrochloride has a strong affinity for β_2-receptors and the supplementation of ZH has been shown to increase the expression of β_2-receptors (Miller *et al*., 2011). Upon activation of receptors by ZH, intracellular actions through cyclic cAMP stimulate key enzymes involved in lipolysis and protein synthesis (Strydom *et al*., 2009). In addition, ZH decreases adipose sensitivity to insulin, which inhibits fat storage and leads to an increase in glucose uptake in muscle (Miller *et al*., 2011).

Therefore, ZH acts as a repartitioning agent that disrupts fat tissue to store nutrients and leads to an increase in the availability of nutrients for muscle hypertrophy (Baxa, 2008). Furthermore, ZH alters the muscle fibre to a faster glycolytic type, which explains the altered protein synthesis and degradation of skeletal muscle (Baxa, 2008).

2.9.1 Cattle and sheep

Supplementation of zilpaterol hydrochloride improved ADG, FCR, carcass weight, and reduced fat deposition of steers, with no effect on FI (Baxa, 2008). The results showed that ZH improved performance and enhanced carcass composition without stimulating FI (Reeds & Mersmann, 1991). This led to an increased efficiency with which nutrients are concentrated into muscle and not fat (Baxa, 2008). A study by Estrada-Angulo *et al*. (2008) in which feedlot lambs were fed increasing levels of ZH for a period of 30 days, resulted in improved dressing percentage, ADG, FCR and reduced carcass fat. This is in contrast to a study by López-Carlos *et al*. (2010), who supplemented ZH for 40 days and observed no significant improvements in growth performance when compared to the control group. The cause of the inconsistencies between these two studies is unclear, however, the dose and length of treatment has a large influence on the magnitude of response to β-agonists. Extended treatment periods and over-exposure to ZH stimulation may have resulted in the downregulation of the β-AR. López-Carlos *et al.* (2010) studied the effect of ZH and ractopamine supplementation in feedlot lambs and observed greater improvements for all growth and carcass characteristics by ZH supplementation compared to ractopamine. The basis for the observed changes is due to the affinity for a specific compound to adrenergic receptors (Smith, 1998). Skeletal muscles consist mainly of β_2-receptors and therefore, have a greater affinity for β_2-agonists such as ZH (Anderson *et al*., 2004). Alternatively, the potency of ZH is greater than that of ractopamine, which resulted in a greater response.

2.9.2 Japanese quails

Zilpaterol hydrochloride improved the performance of Japanese quails in the same manner as in ruminants (Mohammadi-Arekhlo *et al*., 2015). Mohammadi-Arekhlo *et al.* (2015) reported that the supplementation of 0.2 mg ZH/kg to Japanese quails improved growth performance. Zilpaterol hydrochloride was fed for 14 days, either daily or every two days. According to the results, birds fed every two days resulted in greater BW gain (Mohammadi-Arekhlo *et al*., 2015). Supplementing ZH every two days may have caused an upregulation of receptors, which resulted in higher weight gain values (Mohammadi-Arekhlo *et al*., 2015). Furthermore, differences in breast composition were not observed, however, thigh muscle content was higher in protein and lower in fat, when compared to the control group (Mohammadi-Arekhlo *et al*., 2015). The authors speculated that the lack of response in breast muscle to ZH treatment might have been due to the higher concentration of white muscle fibres, which appears to have a lower β-receptor density as compared to red fibre types.

2.9.3 Chickens

Rogers (2013) performed a study to evaluate the response of broiler chickens to ZH supplementation. Zilpaterol hydrochloride was fed at three concentration levels (1, 3 and 5 mg ZH/kg) for 7 or 14 days. The results obtained from the study indicated that ZH significantly improved the growth performance and the carcass characteristics of broilers (Rogers, 2013). Higher BW, ADG and carcass yield were observed at the highest concentration of ZH (5 mg/kg). Based on the large BW increase and ADG, feeding ZH will reduce the number of days by approximately two days to reach its desired market weight. Interestingly, feeding ZH for 14 days increased the performance of treated broilers to a greater degree than feeding ZH for seven days (Rogers, 2013).

2.10 Conclusion

β-adrenergic agonists stimulate a response by binding to specific β-ARs found on target tissues. β-agonists are structurally and functionally similar to natural catecholamines and therefore elicit a similar response in growth and FI. The general response in muscle is an increase in size and an improvement in lean (protein) mass. β-agonists stimulate protein synthesis through increased muscle RNA synthesis and reduced protein degradation by altering muscle proteolytic activity. Furthermore, β-agonist treatment reduces fat deposition by stimulating the adipocyte lipolytic system and simultaneously inhibiting lipogenesis in the liver and adipose tissue. However, the resulting response in fat tissue is less potent as compared to muscle due to the lower density of β-receptors found on adipose cells.

Extrapolation of research results to other species or tissues will pose a risk of over- and/or underestimating response to β-adrenergic agonist supplementation. The variations in response to β-agonist treatment between mammalian and avian species can be explained by the different amounts and distributions of β-adrenergic receptors present on a specific tissue, as well as differences in β-adrenergic receptors structure. The genetic potential for growth in animals also differs, for example, modern broilers are nearing their biological maximum for growth and feed efficiency and therefore show less response to β-agonist treatment. Another reason is the biological variations between birds and mammalians related to absorption and excretion of metabolites that ultimately cause differences in amount and efficacy of β-agonists administered. Some β-agonists may not be as effective in one species compared to another. Variation in response could be due to low affinity for receptor at target tissue, ineffective coupling between agonist and receptor complex or factors affecting delivery of β-agonist to receptors. Lastly, differential receptor downregulation may also explain the variations in response observed between and within species.

The response in FI to β-agonist treatment involves direct (tissue specific) and/or indirect changes in hormonal metabolism. Based on the numerous controversies observed in FI between animal studies, the resulting response might be associated with an indirect effect of β-agonist

treatment related to endocrine function. Leptin and insulin are major hormones involved in feeding behaviour and therefore, the maintenance of BW in animals. Although there is no direct link between β-agonist treatment and leptin secretion, indirect involvement of β-agonists on the size of adipose tissue stores may result in altered feeding behaviour and variation in response between animals. Animal BW and FI are major determinants of a successful production system and it is therefore essential to understand the mechanisms that regulate FI and the possible influence of β-agonist treatment.

The main objective for any successful animal production system is to ensure that more animals are able to reach market weight at an earlier age in order to reduce maintenance and feed costs. β-adrenergic agonists are successful feed supplements that are effective in producing animals with a higher BW that utilise their feed more efficiently. In addition, β-adrenergic agonists improve carcass characteristics by enhancing muscle protein yield and decreasing undesirable fat deposition. As a result, the use of metabolic modifiers not only benefits the producers, but the consumers as well, as they receive healthier meat that is high in protein content and low in fat.

Chapter 3

Materials and Methods

The use of animals was approved by the Animal Ethics Committee (AEC) of the Faculty of Natural and Agricultural Science of the University of Pretoria (approval number EC035-17). The study was conducted on the experimental farm at the University of Pretoria (Hatfield, Pretoria). A total of 2208 day-old Ross chicks were purchased from Eagles Pride Hatchery (Roodeplaat, Pretoria) and reared in an environmentally controlled broiler house for the entire 35-day trial period. Dietary treatment of zilpaterol hydrochloride (ZH) was administered during the finishing phase for 7 days from day 28 to day 35.

3.1 Experimental design

The broiler house consisted of 96 pens of one m^2 in size. A total of 2208 day-old chicks were allocated by sex into each of the 96 pens with 23 birds per pen. The study was conducted using a 2 x 4 factorial arrangement with males and females being reared separately and four levels of zilpaterol hydrochloride inclusion (0 mg/kg, 5 mg/kg, 7 mg/kg and 9 mg/kg). The broiler house was divided into four blocks, which consisted of 24 pens per block. Each block contained two replicates (total of 12 replicates per treatment) that were allocated according to a randomised complete block design.

3.2 House preparation

The house was cleaned and disinfected prior to chick placement. Clean pine shavings were placed into each pen that served as bedding material throughout the trial. The house was pre-heated to approximately 36 ℃ to ensure a litter temperature of 34 ℃ upon chick arrival. Each pen contained five nipple drinkers and one tube feeder. Additional feed trays and bell drinkers were added to each pen for the first seven days of the trial to ensure unlimited feed and water intake.

3.3 Chick placement and management

Male and female chicks were reared separately. Chick sexing was performed at the experimental farm according to the differences in male and female primary feather length. Upon arrival, 23 males and 23 females were randomly selected and placed into a crate to be weighed. Each chick was neck tagged with a coloured (blue for males and pink for females) and numbered tag designated to a specific pen. *Ad libitum* feed and water were available for the entire 35-day trial period. House temperatures were monitored according to the Ross 308 guidelines and gradually reduced until 22 ℃ was reached. During the initial seven days, 23 hours of light and one hour of darkness was applied, followed by 16 hours of light and eight hours of darkness for the remainder of

the trial. Temperature and ventilation were monitored twice daily and were adjusted according to the Ross 308 guidelines.

3.4 Broiler diet and feeding program

A three-phase feeding program was followed during the 35-day trial period; starter (0-14 days), grower (15-28 days) and finisher diet (29-35 days). The three diets consisted of a standard commercial broiler diet. The diets were formulated to meet the minimum nutrient requirements based on commercial feed specifications and mixed at Simple Grow feed mill (Centurion, Pretoria). The feed compositions of the three different phases are shown in Table 3.2. Feed from each diet phase was mixed in one batch and then divided to include the four different ZH concentrations.

Zilpaterol hydrochloride is licensed under the tradename Zilmax®, which contains 4.8% ZH as active ingredient. Dietary treatment of Zilmax® was limited to the finishing phase from day 28 to day 35. For the finisher phase, all four treatments consisted of the same basal diet and differed only in the concentration of ZH added (0 mg, 5 mg, 7 mg and 9 mg zilpaterol/kg feed). The different dietary treatments used are shown in Table 3.1.

Table 3.1 Treatment groups included in the study

Treatment groups	**Zilpaterol hydrochloride inclusion**
Control	No zilpaterol hydrochloride
T_2: 5 mg zilpaterol hydrochloride	5 mg zilpaterol hydrochloride/kg feed
T_3: 7 mg zilpaterol hydrochloride	7 mg zilpaterol hydrochloride/kg feed
$T_{4:}$ 9 mg zilpaterol hydrochloride	9 mg zilpaterol hydrochloride/kg feed

Table 3.2 Feed ingredient and calculated nutrient composition of starter, grower and finisher diets (% inclusion)

Ingredients (%)	**Starter**	**Grower**	**Finisher 1**	**Finisher 2**	**Finisher 3**	**Finisher 4**
Yellow maize	55,3	63,3	64,5	64,5	64,5	64,5
Soya oilcake (46.5%)	23,5	16,6	14,7	14,7	14,7	14,7
Full fat soya	12,0	12,0	12,0	12,0	12,0	12,0
Sunflower oilcake (36%)	4,00	4,00	4,00	4,00	4,00	4,00
Crude soybean oil (degummed)	0,90	0,63	1,74	1,74	1,74	1,74
Lysine (78%)	0,26	0,28	0,27	0,27	0,27	0,27
Methionine (98%)	0,28	0,24	0,22	0,22	0,22	0,22
Threonine (98%)	0,06	0,05	0,05	0,05	0,05	0,05
Feed lime	1,68	1,40	1,28	1,28	1,28	1,28
Monodicalcium phosphate (70%)	1,19	0,66	0,44	0,44	0,44	0,44
Salt	0,14	0,14	0,14	0,14	0,14	0,14
Sodium bicarbonate	0,35	0,36	0,36	0,36	0,36	0,36
Axtra phytase 10000 P	0,01	0,01	0,01	0,01	0,01	0,01
Choline chloride (60%)	0,10	0,10	0,10	0,10	0,10	0,10
Salinomycin (12%)	0,05	0,05	0,05	0,05	0,05	0,05
Zinc bacitracin (15%)	0,05	0,05	0,05	0,05	0,05	0,05
Broiler starter premix	0,15	0,15	-	-	-	-
Broiler grower premix	-	-	0,10	0,10	0,10	0,10
Calculated nutrient values (%)						
Dry matter (DM)	95,5	95,6	95,7	95,7	95,7	95,7
Metabolisable energy (AME)	11,3	11,7	12,1	12,1	12,1	12,1
Crude Protein	21,3	18,8	18,0	18,0	18,0	18,0
Fat	5,40	5,23	6,33	6,33	6,33	6,33
Crude Fibre	4,49	4,39	4,34	4,34	4,34	4,34
Ash	5,97	4,85	4,43	4,43	4,43	4,43
Calcium	1,05	0,84	0,76	0,76	0,76	0,76
Phosphorus	0,65	0,52	0,47	0,47	0,47	0,47
Available phosphorus	0,50	0,40	0,36	0,36	0,36	0,36
Sodium	0,16	0,16	0,16	0,16	0,16	0,16
Chloride	0,20	0,20	0,20	0,20	0,20	0,20
Potassium	0,89	0,77	0,74	0,74	0,74	0,74
Available lysine	1,15	1,00	0,95	0,95	0,95	0,95
Available methionine	0,57	0,51	0,48	0,48	0,48	0,48
Available threonine	0,72	0,63	0,60	0,60	0,60	0,60
Available tryptophan	0,21	0,18	0,17	0,17	0,17	0,17
Available isoleucine	0,79	0,68	0,65	0,65	0,65	0,65
Available arginine	1,28	1,10	1,05	1,05	1,05	1,05
Available histidine	0,50	0,44	0,42	0,42	0,42	0,42
Available leucine	1,59	1,45	1,40	1,40	1,40	1,40
Available valine	0,86	0,76	0,72	0,72	0,72	0,72

3.5 Data collection

3.5.1 Performance measurements

Birds were weighed weekly on a per pen basis on day 0, 7, 14, 21, 28 and 35 to calculate BW. Birds from each pen were placed into a crate and transferred onto a portable scale, and weighed. Bird weights were recorded and birds were placed back into pens. On day 35 each bird was individually placed onto a portable scale and body weights were recorded. Residual feed for each phase was weighed and recorded on day 7, 14, 21, 28 and 35. Average feed intake was calculated and used to determine the FCR values. Dead birds were collected and weighed each day and the weights were used to correct the FCR for mortalities in each pen.

3.5.2 Carcass measurements

On day 35 (day before slaughter), two birds from each pen were selected and marked for sampling. Birds were selected based on its individual BW being closest to the average BW of all birds in the pen. On day 36, the selected birds from each pen were placed into a portable crate and transported to the experimental farm's abattoir. Each bird was individually weighed and live body weights were recorded. Birds were then electrically stunned to render them unconscious and placed into bleeding cones where the carotid artery and external jugular vein were cut. After bleeding, birds were placed into a scalding tank with a temperature of 70ºC for a maximum period of two minutes followed by de-feathering of each carcass. Carcasses were eviscerated on stainless steel tables where their feet, giblets and intestines were removed and discarded. Carcasses and abdominal fat were weighed separately and recorded for each bird. The breast meat, drumsticks, thighs and wings were carefully excised and weighed.

Live body weight (BW) was determined as the average weight of two fasted birds per pen at time of slaughter. Carcass weight was considered the whole bird without head, neck, feet, abdominal fat, organs and intestines, calculated as the average empty carcass weight (ECW) of the two birds. Carcass yield (CY %) was calculated as ECW/BW x 100. Portion yield (PY %), namely the breast, thighs, drumsticks and wings was calculated as Portion weight/ECW x100. Abdominal fat pad yield (ADF %) was calculated as ADF/ECW x 100.

3.6 Feed sampling and analysis

Representative feed samples were taken from each of the diets, and ground through a 1 mm sieve and analysed for their nutritional content at NutriLab (Department of Animal and Wildlife Sciences, University of Pretoria). Samples were analysed for dry matter, ash, crude protein, crude fibre, crude fat and ether extract. Calcium and phosphorus content for each of the four diets were also analysed. The analysed results for each phase are shown in Table 3.3.

Table 3.3 The analysed chemical composition of the starter, grower and finisher diets on an as-is basis (%)

Analysed results	Starter	Grower	Finisher
Dry matter	88,7	87,7	88,3
Crude fibre	3,15	3,29	3,00
Crude protein	22,3	19,4	17,9
Crude fat	5,97	5,44	7,06
Ash	5,73	4,64	4,58
Calcium	0,89	0,62	0,68
Available Phosphorus	0,70	0,53	0,49

Dry matter (DM) was analysed using the AOAC, (2000) Official Method of Analysis 934.01. Duplicate samples were taken to ensure the accuracy of the composition. Feed samples were weighed (2 g) into porcelain crucibles and placed into a 105 °C oven for 12 hours. Oven dried samples were then placed into a desiccator to cool before weighing and recording of data.

Ash content was analysed using the AOAC (2000), Official Method of Analysis 935.13. Oven dried samples (from DM analysis) were weighed and placed into an incinerator for one hour at 200 °C followed by four hours at 600 °C. The crucibles were placed into a desiccator to cool. The samples were weighed and the ash content was determined.

Crude fat (CF) content was analysed using the Buchi Soxtec (2000), Official Method of Analysis 920.39. Feed samples (2 g) were weighed onto a 125 mm Whatman filter paper after which it was folded and placed into marked extraction thimbles. The thimbles were transferred to the Soxtec extraction tubes where 80 mL of petroleum ether was poured into Buchi beakers connected to the thimbles. The water taps was opened and steam generator turned on. Fat extraction with petroleum ether occurred for one hour followed by one hour of rinsing and drying. The evaporated ether were collected and filtered into a waste tube. The Buchi beaker containing the remaining fat was placed into a 70 °C dry oven for one hour. The samples were weighed and mass used to calculate the crude fat. The difference between the flask mass before and after extraction was used to calculate the CF.

Crude protein (CP) was analysed using the AOAC (2000), Official Method of Analysis 968.6 using the Dumas method. Nitrogen (N_2) was freed by pyrolysis and subsequent carbon dioxide (CO_2) combustions. The freed N_2 was then carried by formed CO_2 into a nitrometer where CO_2 was absorbed in potassium hydroxide (KOH) solution. Results were expressed as a percentage of residual N_2 (measured) and CP was calculated using a conversion factor of 6.25.

Calcium (Ca) and phosphorus (P) content were analysed using the AOAC (2000), Official Method of Analysis 935.13. Feed samples were weighed (0.5 g) into digestion tubes and 25 mL nitric acid (HNO_3) was added to each sample. The samples were placed onto a 240 °C heating block and boiled for 15 minutes. Sample was removed and cooled after which 10 mL of perchloric acid ($HClO_4$) were added and transferred to a heat block for 40 minutes. When samples showed orange/yellow colouration, the samples were removed and deionised water was added up to 50 mL mark. The Ca and

P concentration was determined using the absorption reading (at 400 nm) on the Perkin Elmer Atomic Absorption Spectrophotometer.

3.7 Statistical analysis

Data were analysed statistically as a randomised block design with the generalised linear model (GLM) using Statistical Analysis Systems software (SAS, 2017), for the average treatment effects at the end of the study. Repeated Measures Analysis of Variance with the GLM model was used for repeated week or period measures. Means and standard errors were calculated and significance of difference (P <0.05) between means was determined by the Fischers test (Samuels, 1989).

The linear model used is described by the following equation:

Yijk = μ + Ti + Sj + Bk + e

Where Y = variable studied during the period

μ = overall mean of the population

T = effect of the i^{th} treatment

S = effect of the j^{th} sex

B = effect of the k^{th} block

e = error associated with each Y

Chapter 4

Results

4.1 Performance of broilers from day 28 to 35

On day 28 when experimental treatments started, there were no significant differences in body weight, feed intake and feed conversion ratio between the four treatment groups. A summary of the effect of feeding zilpaterol hydrochloride (ZH) on performance of broilers are shown in Table 4.1.

Male birds had significantly higher BW than female birds (2430 g and 2153 g, respectively). The BW of groups that received ZH treatment were lower than the control group (P <0.05). The birds in the control group had a 2% higher BW compared to the birds in the 7mg ZH and 9 mg ZH treated groups (P <0.05). However, there were no significant differences in BW between the control (2323 g) and 5 mg ZH (2291 g) treatment groups. Results indicated a significant treatment x sex interaction between the treatment groups. Male birds in the control group had the highest BW of 2485 g and the difference was significant compared to the males in the ZH treated groups (P <0.05). By contrast, female birds in the 7 mg ZH group had the highest BW, but differences were not statistically significant compared to the control and 5 mg ZH treatment group. However, differences were noted between females birds in the 7 mg ZH (2177 g) and 9 mg ZH (2116 g) treatment groups (P <0.05)

Male birds had numerically lower FCR than female birds, but the difference was not statistically significant. Control group birds had the best average treatment FCR (1.5 points), and the differences were significant compared to the treated groups Results indicated treatment x sex interactions between the treatment groups (P <0.05). Both male and female control birds had the lowest FCR, 1.55 and 1.60 respectively, however, only the male control group showed significant difference from the other ZH treated groups (P <0.05). All male treated groups had lower FCR values than the female treated groups (P < 0.05), however, no significant difference between male (1.554) and female (1.597) control birds were observed.

The males (1363 g) had a significantly higher FI compared to the females (1186 g). The mean treatment feed intakes differed between the control and the ZH treated groups (P <0.05). Control birds had the lowest FI and 5 mg ZH had the highest FI of 1252 g and 1289 g, respectively (P <0.05). A significant treatment and sex interaction was observed on FI response between treatments. The male control birds had the lowest FI of 190.7 g, and the differences were statistically significant compared to 5 mg ZH (197.4 g) and 9 mg ZH (197.6 g). In the female birds, 7 mg ZH had the highest FI of 173.6 g and were significantly different from the control (166.6 g) and 9 mg ZH (166.1 g) female birds (P <0.05).

4.2 Performance of broilers from day 0 to 35

The effects of ZH treatment on the accumulated FI and FCR of broilers for the period 0 to 35 days are summarised in Table 4.1.

Male birds had a lower FCR than the female birds, 1.52 and 1.53 respectively, but the differences were not statistically significant. The mean treatment results indicated that birds that received no ZH treatment had lower FCR compared to the ZH treated birds (P >0.05). Data indicated treatment x sex interactions between male birds (P <0.05). Male control birds had the lowest FCR of 1.49 and the difference was statistically significant from 9 mg ZH with a FCR of 1.54. Similarly, the female control birds had the lowest FCR but the differences were not significant compared to the ZH treated females.

Male birds (3639 g) consumed significantly more feed than the females (3230 g). The average treatment results showed that the 5 mg ZH birds had the highest FI of 3453 g and the control birds had the lowest FI of 3426 g for days 0 to 35 (P >0.05). No treatment x sex interaction was observed on FI response between treatments (P >0.05). The male 7 mg ZH birds had the lowest FI of 3581 g and 9 mg ZH had the highest FI of 3692 g, but the differences were not statistically significant from the control birds which had a FI of 3633 g. In contrast, the female 7 mg ZH birds had the highest FI of 3281 g and the control females had the lowest FI of 3219 g, however, the differences were not significant.

Table 4.1 The effect of feeding different concentrations of zilpaterol hydrochloride (ZH) on performance parameters in male and female broilers

	BW (g) (35 d)	**FCR (28-35 d)**	**FCR (0-35 d)**	**FI (g) (28-35 d)**	**FI (g) (0-35 d)**
Sex					
Male	2430[a]	1,61	1,52	1363[a]	3640
Female	2153[b]	1,63	1,53	1186[b]	3230
SEM	*8,495*	*0,009*	*0,008*	*5,685*	*17,55*
ZH Treatment					
Control	2323[a]	1,58[b]	1,50	1252[b]	3426
5 mg ZH	2291[ab]	1,64[a]	1,53	1289[a]	3453
7 mg ZH	2283[b]	1,62[a]	1,52	1283[a]	3431
9 mg ZH	2269[b]	1,64[a]	1,53	1274[a]	3428
SEM	*12,01*	*0,013*	*0,011*	*8,040*	*24,81*
Treatment x Sex					
Male					
Control	2485[a]	1,55[b]	1,49[b]	1335[b]	3633[ab]
5 mg ZH	2424[b]	1,64[a]	1,52[ab]	1382[a]	3652[ab]
7 mg ZH	2389[b]	1,61[a]	1,51[ab]	1349[b]	3581[b]
9 mg ZH	2421[b]	1,65[a]	1,54[a]	1385[a]	3692[a]
Female					
Control	2161[ab]	1,60	1,51	1168[ac]	3219[ab]
5 mg ZH	2158[ab]	1,63	1,53	1197[a]	3254[ab]
7 mg ZH	2177[a]	1,64	1,53	1217[bd]	3281[a]
9 mg ZH	2116[b]	1,64	1,53	1163[b]	3164[b]
SEM	*16,99*	*0,018*	*0,016*	*11,37*	*35,09*
Significance					
Sex	**	NS	NS	**	**
Treatment	*	**	NS	**	NS
Treatment x Sex	**	NS	NS	**	*

[a,b] Column means with different superscripts differ significantly (P <0.05)
NS = Not significantly different at (P >0.05)
*Significantly different (P <0.05) and ** significantly different (P <0.01)
SEM = Standard error of the mean
BW = Body weight
FCR = Feed conversion ratio
FI = Feed intake
Experimental period from day 28 to day 35

4.3 Carcass characteristics of broilers

The effect of feeding different levels of ZH on live body weight, carcass yield and different portion weights are shown in Table 4.2 and Table 4.3. Portion weights for breast, thighs, drumsticks, wings and abdominal fat pad are expressed as a percentage ECW.

4.3.1 The effect of sex on carcass characteristics of broilers

Female birds had a higher carcass yield (75.37%) than males (74.41%), as well as higher thigh and breast weights, however, only carcass yield was significantly different from males (P <0.05). Male birds had higher wing and drumstick weights, but only male drumstick weights significantly differed from the female birds. Female birds had a higher abdominal fat yield (1.329%) compared to males (1.455%) (P <0.05).

4.3.2 The effect of zilpaterol hydrochloride between different treatments

The birds that received 7 mg ZH had the highest average values for all tissue yields; thighs, drumsticks, wings and breast tissue, however, significant treatment differences were only observed for breast yield (P <0.05). The highest breast yield of 31.14% was obtained in birds that received 7 mg ZH and differed significantly from the other treatment groups and the control. The treatment group with the lowest concentration of ZH (5 mg ZH) had the lowest breast yield of 29.95% (P <0.05). However, birds that received 5 mg ZH had the lowest abdominal fat yield of 1.321% and the difference was statistically significant from birds in the 9 mg ZH group, which had the highest abdominal fat pad yield of 1.469%. The two treatment groups that received the highest concentration of ZH (7 mg ZH and 9 mg ZH) had greatest abdominal fat deposition and differed significantly from the control (8% and 10% respectively).

4.3.3 The interaction between zilpaterol hydrochloride treatment and sex in broilers

Leg yield: Male birds that received 7 mg ZH had the highest thigh yield of 17.37%, with a 7% increase compared to the control birds (16.23%), and the results were significantly different. No significant differences were noted in thigh yields between male and females, except for females in the 9 mg ZH group (P <0.05), which had higher thigh yield (17.22%) than males (16.30%). Both male and female control birds had the lowest thigh yield, however, only the male birds showed significant difference from the treated groups.

Drumstick yield: Male birds in the 7 mg ZH group had the largest drumstick yield of 13.30%, but the difference was not statistically significant from the other males. On the other hand, female birds in the 5 mg ZH group resulted in the highest drumstick yield compared the control birds (P >0.05). There were significant sex and treatment interactions between treatments. Male and female birds in the 5 mg ZH group had similar drumstick yields (P >0.05), however males in the 7 mg ZH, 9 mg ZH and the control group had higher drumstick yields than females (P <0.05).

Wing yield: Male birds in the 7 mg ZH and 9 mg ZH group obtained the highest wing yields, whereas the 5 mg ZH group had the lowest wing yield (P >0.05). By contrast, female birds that received 5 mg ZH had the highest wing yield, however, the differences were not significant from the other female groups.

Abdominal fat yield: The male control birds had the lowest abdominal fat yield of 1.232% and was significantly different from 7 mg ZH (1.421%), which had a 15% greater abdominal fat deposition (P <0.05). In contrast, the female 5 mg ZH group resulted in a 5% lower fat deposition, however, results showed no significant difference to the control. Significant differences were noted between females in the 5 mg ZH group (1.36%) and 9mg ZH groups (1.550%). Significant treatment and sex interactions were observed between the male and female treatment groups. Females deposited significantly more abdominal fat than males in all treatment levels, including the control groups (P <0.05).

Breast yield (without the bone): Male birds that received 7 mg ZH had the highest breast yield (31.20%) and were significantly different from the control (29.51%) and 5mg ZH (29.37%) and 9 mg ZH (29.80%) treatment groups. Similarly, female birds in the 7 mg ZH group had the highest breast yield, however, the difference was not statistically different from the other female groups. No treatment x sex interactions occurred between the male and female treated groups (P >0.05).

4.4 The effect of zilapterol hydrochloride on the mortality rate in broilers

The mortality rate was very low for the entire 35 day period. A total of 12 (0.56%) birds died during the 7 day experimental period (between day 28 and 35). Zilpaterol treatment had no effect on mean mortality rates compared to the control group (P >0.05).

Table 4.2 The effect of zilpaterol hydrochloride (ZH) treatment on live body weight, empty carcass weight and carcass yield

	Body weight (g)	**Empty Carcass Weight (g)**	**Carcass Yield (%)**
Sex			
Male	2543	1892	74,41[b]
Female	2234	1684	75,37[a]
SEM	*10,70*	*10,26*	*0,337*
ZH Treatment			
Control	2418[a]	1802	74,55
5 mg ZH	2387[ab]	1798	75,35
7 mg ZH	2387[ab]	1773	74,37
9 mg ZH	2363[b]	1778	75,29
SEM	*15,14*	*14,52*	*0,477*
Treatment x Sex			
Male			
Control	2594[a]	1924[a]	74,17[ab]
5 mg ZH	2527[b]	1900[a]	75,21[a]
7 mg ZH	2529[b]	1851[b]	73,22[b]
9 mg ZH	2522[b]	1893[ab]	75,05[b]
Female			
Control	2242	1679	74,94
5 mg ZH	2248	1696	75,49
7 mg ZH	2245	1695	75,52
9 mg ZH	2203	1664	75,54
SEM	*21,40*	*20,53*	*0,674*
Significance			
Sex	**	**	*
Treatment	NS	NS	NS
Treatment x Sex	NS	NS	NS

[a,b] Column means with different superscripts differ significantly
NS = Not significantly different at (P >0.05)
*Significantly different (P <0.05) and ** significantly different (P <0.01)
SEM = Standard error *of* the mean

Table 4.3 The effect of zilpaterol hydrochloride (ZH) treatment on relative portion weights of broilers expressed as a percentage of empty carcass weight (%)

	Thighs	**Drumsticks**	**Wings**	**Fat pad**	**Breast (WB)**	**Breast (W/OB)**
Sex						
Male	16,73	13,15[a]	9,747	1,329[b]	34,01	29,97
Female	16,85	12,56[b]	9,648	1,455[a]	34,39	30,60
SEM	*0,157*	*0,105*	*0,087*	*0,033*	*0,233*	*0,230*
ZH Treatment						
Control	16,46[b]	12,82	9,584	1,337[ab]	34,13[ab]	30,03[b]
5 mg ZH	16,88[ab]	12,92	9,682	1,321[b]	33,82[b]	29,95[b]
7 mg ZH	17,06[a]	12,91	9,812	1,440[ab]	34,99[a]	31,14[a]
9 mg ZH	16,76[ab]	12,78	9,711	1,469[a]	33,86[b]	30,02[b]
SEM	*0,223*	*0,149*	*0,123*	*0,047*	*0,330*	*0,324*
Treatment x Sex						
Male						
Control	16,23[b]	13,12	9,604	1,232[b]	33,53[b]	29,51[b]
5 mg ZH	17,01[ab]	13,04	9,583	1,274[ab]	33,43[b]	29,37[b]
7 mg ZH	17,37[a]	13,30	9,887	1,421[a]	35,19[a]	31,20[a]
9 mg ZH	16,30[b]	13,15	9,913	1,388[ab]	33,89[b]	29,80[b]
Female						
Control	16,68	12,52	9,564	1,442[ab]	34,72	30,56
5 mg ZH	16,76	12,80	9,781	1,367[b]	34,21	30,52
7 mg ZH	16,75	12,52	9,737	1,459[ab]	34,78	31,08
9 mg ZH	17,22	12,40	9,510	1,550[a]	33,84	30,23
SEM	*0,315*	*0,210*	*0,174*	*0,066*	*0,466*	*0,459*
Significance						
Sex	NS	*	NS	**	NS	NS
Treatment	NS	NS	NS	NS	*	*
Treatment x Sex	NS	NS	NS	NS	NS	NS

[a,b,] Column means with different superscripts differ significantly
NS = Not significantly different at (P >0.05)
*Significantly different (P <0.05) and ** significantly different (P <0.01)
SEM = Standard error of the mean
WB = breast weight with bone and W/OB = breast weight without bone

Chapter 5

Discussion

Zilpaterol hydrochloride was supplemented for seven days from day 28 to day 35 to determine the effect on growth performance and carcass characteristics of broilers. β-adrenergic agonists have been used as lean-enhancing agents in livestock, which proved to be effective in enhancing growth, feed efficiency and carcass characteristics in cattle, sheep, pigs, turkeys and broilers. However, the supplementation of β-agonists in broilers has yielded diverse and conflicting results. Performance, measured as BW, FI and FCR plays a major role in the efficiency and success of any animal production operation. The ultimate goal for any animal production system is for animals to reach market weight at a younger age in order to reduce feed and management costs. The response to β-adrenergic agonist treatment is mainly determined by dose, duration and type of agonist used, which may have a great impact on the variability of results obtained between and within species.

5.1 Broiler performance

5.1.1 The effect of zilpaterol hydrochloride on body weight and feed conversion ratio

Results indicated that the birds that received no ZH treatment significantly outperformed the ZH treated groups. The higher BW and lower FCR obtained in the control group are in agreement with studies done by Morgan *et al.* (1989) and Kheiri *et al.* (2011), which demonstrated a negative effect on performance parameters after ractopamine and cimaterol treatment, respectively. On the other hand, Buyse *et al.* (1991) and Hamano *et al.* (1998b) supplemented clenbuterol to broilers and found no significant effect on BW, FI or FCR. Numerous other studies, including Gwartney *et al.* (1991), Rehfeldt *et al.* (1997) and Tahmasbi *et al*. (2006) have reported a positive influence on broiler performance from β-agonist treatment as evidenced by improved BW, FI and FCR. The conflicting data obtained from broiler studies indicate the large impact the type of agonist and potency of the molecule has on animal response to treatment. For example, Schiavone *et al.* (2004) administered both clenbuterol and cimaterol to broilers and found that clenbuterol improved BW and FCR, whereas cimaterol had a negative effect on BW. However, other studies have shown a positive response with cimaterol treatment (Dalrymple *et al*., 1984; Gwartney *et al*., 1991). Similarly, Mohammadi-Arekhlo *et al.* (2015) supplemented ZH at concentrations of 0.2, 0.225 and 0.25 mg ZH/kg LW to Japanese quails. Results showed no significant effect on BW or FI, but FCR was improved. By contrast, Zare Shahneh *et al.* (2012) supplemented salbutamol to Japanese quails and results indicated a significant and positive response in BW, FI and FCR. Therefore, the type of agonist used has a significant influence on the variation and magnitude of response, even in the same species of animals.

Another contributing factor that explains the variability in response is the dose, as well as the duration of the β-agonist treatment. According to Dalrymple *et al.* (1984), there is a clear reduction in

performance and the repartitioning effects of β-agonists at high dosage levels. Dalrymple *et al.* (1984) supplemented 0.25, 0.5, 1, and 2 mg cimaterol/kg to broilers and found that performance was improved at lower concentrations of cimaterol, specifically at 0.25 mg/kg and 0.5 mg/kg inclusion levels. At higher concentration levels of cimaterol (1 mg/kg), broiler BW started to decline due to lower feed intakes. However, the group that received 1 mg cimaterol/kg still had improved growth and feed efficiency compared to the control. On the other hand, when cimaterol was fed at a concentration of 2 mg/kg, performance was significantly reduced compared to the control. The lower body weights obtained by Dalrymple *et al.* (1984) when high concentrations were fed, agree with the findings from the present study. The control birds had significantly higher body weights (2323 g) compared to the birds that received 7 mg ZH (2283 g) and 9 mg ZH (2269 g), the highest ZH concentrations. However, no significant difference was observed between the control birds and the birds that received the lowest concentration of 5 mg ZH (2291 g). The results therefore indicate that the inclusion levels were too high, which had a negative impact on growth performance.

A similar study by Rogers (2013), in which various concentrations of ZH (1, 3, 5, and 7 mg ZH/kg feed) were supplemented to broilers for 7 and 14 days from the age of 21 and 28, produced similar results compared to the present study. The treatment groups that received ZH treatment at a concentration of 5 mg ZH/kg resulted in the best performance values when supplemented for 14 days (Rogers, 2013). Based on our data, FCR and FI were better at the lowest inclusion of ZH (5 mg/kg) compared to the higher ZH treatment groups, despite being lower than the control. The highest ZH inclusion level (7 mg/kg and 9 mg/kg) had the greatest negative impact on BW, FI and FCR. Similarly, Duquette *et al.* (1986) supplemented different levels of L-640,033 (β_2-agonist) for 7, 14, 21 and 28 days and found that the highest inclusion level (4 mg/kg) decreased weight gain and feed efficiency. The authors concluded that this concentration level was too excessive for optimal production. In addition, Duquette *et al.* (1986) also proved that β-agonist treatment for more than 28 days had no significant advantage in performance compared to 21 days, however, 7 day treatment period was inadequate to stimulate a growth response. In contrast, Gwartney *et al.* (1991) showed that supplementing 1 mg clenbuterol/kg only improved final BW after 28 days of treatment. The results indicated that the final BW of broilers supplemented with clenbuterol were lower compared to the control when treatment period was only 14 and 21 days (Gwartney *et al.*, 1991). These inconsistencies found in research studies confirms the importance of dose and duration on the efficacy of the molecule. Based on these findings and the negative results obtained from the present study, we concluded that the concentration levels used were too high and the treatment period was possibly too short. For that reason, a concentration of 3 mg ZH/kg for 14 days might have produced more desirable results.

5.1.2 The effect of zilpaterol hydrochloride on feed intake

Response in FI to β-agonist treatment involves direct (tissue specific) and indirect (endocrine) changes related to fat and muscle metabolism (Mersmann, 1998). When β-agonists are used to alter growth rates in animals, it is natural to assume that a higher FI is required to stimulate the increased growth rates. However, β-agonists generally have an appetite depressing effect, which allows the repartitioning effects on carcass composition responsible for improved feed efficiency of animals (Schiavone *et al*., 2004). The response in FI to β-agonist supplementation in broilers have yielded diverse and contrasting results (Buyse *et al.*, 1991; López-Carlos *et al.*, 2010; Kheiri *et al.*, 2011). According to Hagemann *et al.* (1998), the variations in response seen between poultry species are thought to be the result of increased selection for fast growing birds, causing differences in catecholamine circuitry in the hypothalamus. Research has shown that β_1 agonists stimulate FI in poultry species (Denbow *et al*., 1986), whereas β_2 agonists stimulate FI in broilers and layers specifically (Bungo *et al.*, 1999; Tachibana *et al.*, 2009).

β-agonists are structurally and functionally similar to naturally occurring catecholamines, epinephrine (E) and norepinephrine (NE). These hormones are released in response to stress and have shown to be involved in the feeding behaviour of animals (Buttery & Dawson, 1987). Research done by Baghbanzadeh *et al.* (2015), proved the involvement of β_2-adrenergic receptors located in the brain on food and water intake in broilers. Therefore, β-adrenergic agonists can have a central effect on FI as it can cross the blood brain barrier more easily and is generally supplemented at higher doses than E and NE (Howes & Forbes, 1987). According to Baghbanzadeh *et al.* (2015), hypothalamic micro-injections of a mixed β-agonist, isoproterenol (ISOP), increased feed intake in broilers with increasing ISOP concentration. In contrast, micro-injections of E resulted in reduced FI and body temperature of turkeys (Denbow, 1983; Denbow & Sheppard, 1993). Based on our results, ZH treatment increased FI by 3%. It is possible that ZH provoked a stress stimulus similar to the effects of endogenous catecholamines, which resulted in the increased FI in broilers treated with ZH. Rogers (2013) demonstrated that ZH improved performance of broilers when ZH was supplemented for 14 days compared to when ZH was supplemented for 7 days. Based on these findings, it is possible that the extended period of administration (14 days) allowed the birds to adapt to ZH treatment, which removed the initial stress effect on FI, resulting in improved BW and FCR. Alternatively, β-agonists may have a hepatic action on FI via β_2 receptors found in the liver (Howes & Forbes, 1987). Howes & Forbes (1987) injected different concentrations of salbutamol (β_2 agonist) into the portal vein of chickens and the results showed a linear decrease in FI with increasing β-agonist concentration. Results from the present study disagree with the findings of Howes & Forbes (1987). The groups that received ZH treatment had significantly higher feed intakes compared to the control group (P <0.05). The present findings agree with Kheiri *et al.* (2011) and Zare Shahneh *et al.* (2012), however, differs from Gwartney *et al.* (1991) and Schiavone *et al.* (2004) who also found a dose-related reduction in

feed intake. According to our present findings, FI decreased with increasing ZH concentration, however, FI was still greater than the control (P <0.05).

Based on the numerous controversies observed in feeding behaviour between animal studies, the resulting response from β-agonists treatment might be indirectly related to endocrine function. Metabolic pathways are integrated with neuroendocrine pathways via the CNS to regulate feed intake in animals (Richards, 2003). Therefore, the change in feeding behaviour from β-agonist treatment might be due to an indirect response to circulating hormone levels, such as insulin and leptin. Insulin, a hormone secreted in response to circulating levels of glucose, has a significant influence on feeding behaviour. High plasma glucose concentrations stimulate the release of insulin, which in turn signals the brain to reduce feed intake. Administration of β-agonists have been reported to decrease (Beerman, 1987), increase (Yang & McElligott, 1989) or have no effect (Fiems, 1987) on insulin concentrations in animals. Shiraishi *et al.* (2008) performed a study on insulin control in chicks and concluded that central insulin injections supressed FI via melanocortins, similar to mammals. In situations where a β-agonist does cause a reduction in blood insulin levels, it is possible that animals will increase their FI to sustain their metabolic needs. Similarly, leptin is a signalling hormone released from adipose tissue in response to the size of available energy stores. In horses, adipose-specific cytokines leptin and adiponectin are released from adipose tissue and are highly correlated to change in fat mass and appetite (Kearns & McKeever, 2009). Animals that have an inherently lower adipose tissue mass will have a lower concentration of circulating leptin compared to animals that are obese. A study by Kearns & McKeever (2009) showed that the administration of clenbuterol increased adiponectin and decreased leptin concentrations in horses. However, the function of leptin seem to be different in avian species (Tachibana & Tsutsui, 2016). To date, no scientific data has proven a direct link between β-agonist treatment and leptin secretion on feed intake in animals. Based on the findings of Kearns & McKeever (2009), β-agonists might have an indirect response on FI through leptin secretion. Our present data shows that ZH treatment resulted in reduced abdominal fat deposition. Consequently, the lower available adipose tissue reduced plasma leptin concentration which in turn stimulated the increased FI observed in our study. According to our results, the 5 mg ZH group had the lowest abdominal fat yield of 1.321% and the highest FI of 1289 g. Likewise, the highest ZH treatment group (9 mg ZH) had the highest abdominal fat yield of 1.469% and the lowest FI of 1274 g. Based on the function of hormones in regulating BW and energy homeostasis in animals, it is possible that β-agonists might have an indirect response on FI through reduced adipose stores and leptin secretion. The present study did not measure insulin and leptin concentrations however, based on the regulatory role of these hormones on feed intake, future research should include the measurements of these hormones in broilers studies.

5.2 Carcass characteristics

The supplementation of ZH resulted in an overall positive effect on carcass composition. Male birds that received 7 mg ZH had the highest thigh, drumstick, wing and breast yield, however, only thigh and breast yield showed significant difference from the control. On the other hand, female birds that received 5 mg ZH resulted in the highest tissue weights for drumstick, wings and abdominal fat (P >0.05). The highest ZH (9 mg ZH) inclusion level had a negative effect on the different portion weights in female birds. In contrast, male birds that received the lowest ZH (5 mg ZH) concentration, obtained the lowest portion weights, however, was still greater than the control (P <0.05). The birds that received 5 mg ZH deposited the least amount of abdominal fat in both male and female treatment groups (P <0.05). The groups that received the highest ZH concentrations resulted in the highest abdominal fat deposition of 13% and 7% for males and females, respectively. The control group had on average a 2% higher live weight and empty carcass weight compared to the ZH treated groups.

5.2.1 The effect of zilpaterol hydrochloride on breast and thigh muscle yield

β-agonists are considered to induce true muscle hypertrophy through increasing muscle size instead of muscle number. This explains the moderate improvement in carcass yield observed in broiler studies compared to mammals, due to their smaller muscle size. In the present study, ZH treatment had a positive influence on carcass composition with a maximum response at 7 mg ZH inclusion level. Interestingly, the control group had the highest live body weight (P <0.05), however, the carcass yield, thigh, drumstick and wing yields were the lowest when compared to the ZH treated birds. According to the results, the group that received 7 mg ZH had the highest portion yield for breast, thigh, drumstick and wing weights, however only the breast weight showed significant difference from the control group. Our findings agree with Gwartney *et al.* (1992), Rehfeldt *et al.* (1997), Kheiri *et al.* (2011), Zare Shahneh *et al.* (2012) and Boostan *et al.* (2015). In contrast, Morgan *et al*. (1989) and Mohammadi-Arekhlo *et al.* (2015) reported no improvements in carcass composition after β-agonist treatment. Hamano *et al.* (1998a) performed a study on broilers where clenbuterol (1 mg/kg) was supplemented to a diet containing various concentrations of protein (220, 240 and 260 g protein/kg feed). Clenbuterol had no significant effect on performance or carcass yield, however, clenbuterol distinctly increased thigh muscle weight. These findings agree with Morgan *et al*. (1989), which found a lower degree of response in breast muscle weight compared to thigh muscle after cimaterol treatment. According to Morgan *et al*. (1989), receptor density is a function of muscle type, with red fibres having a larger receptor density than white fibres. Red fibres are found in the thigh and wing muscles, whereas white fibres are mainly located in the breast muscle. Morgan *et al*. (1989) therefore concluded that the lower response observed in breast muscle weight compared to leg and wing weight could be due to a lower β-receptor density in white fibres. Our present data indicated that ZH at a concentration of 7 mg ZH improved both thigh and breast yield significantly (4%) over the control group (P <0.05).

β-agonists increase the metabolic rate of animals by increasing their heart rate and arterial blood flow, which increases nutrient flow to muscle for protein synthesis (Mersmann, 1998). In addition, β-agonists reduce the sensitivity of adipose tissue to insulin stimulation, which decreases glucose uptake from fat tissue and consequently improves muscle glucose uptake (Sejresen & Vestergaard Jensen, 1987). Improved carcass yield and portion yield in the ZH treated groups in the present study proved this mechanism of action. The supplementation of ZH increased blood flow and nutrient supply, which resulted in higher breast and thigh yields compared to the control. Interestingly, birds within the control group had higher final body weights despite having lower carcass and portion weights. Studies have shown that β-agonists decrease the weights of certain organs, such as the liver, kidney and heart (Reeds *et al*., 1986), while other studies showed no effect on organ weight (Dunshea *et al*., 1993). Organ weights were not measured in the this study however, it is possible that ZH treatment reduced the weight of certain organs, such as the heart, liver and kidneys, which explains the differences in final body weight between the control and treated groups.

5.2.2 The effect of zilpaterol hydrochloride on abdominal fat deposition

Chicken adipocytes are generally insensitive to β-agonist treatment, unlike mammals, and are therefore less effective in reducing fat deposition. The variation in response between mammals and birds are related to differences in metabolic pathways associated with fat synthesis and storage. The response to β-agonist treatment on adipose tissue in chickens has been variable and inconsistent, which suggest that β-agonist may have an indirect influence on carcass composition in poultry. In chickens, the liver is the major site for triacylglycerole (TAG) synthesis and therefore any change in hepatic lipid synthesis reflects the entire body lipogenesis (Harris *et al*., 1988). Harris *et al.* (1988) performed a study using isolated chicken hepatocytes to determine the adrenergic influence on liver lipogenesis. According to the results, stimulation of β_2-receptors inhibited lipogenesis with the greatest inhibition observed at the lowest concentrations (Harris *et al*., 1988). These findings agree with the present results in which the abdominal fat deposition was reduced by 1% at the lowest ZH inclusion level, whereas fat deposition increased with increasing ZH concentration. The birds that received 5 mg ZH had the lowest abdominal fat yield of 1.321%, but was not significantly different from the control (1.337%; $P > 0.05$). However, the results did show a significant difference to the highest ZH treatment group (9 mg ZH), which had the highest abdominal fat yield of 1.469%. Our present findings disagree with work done by Dalrymple *et al.* (1984) who observed no significant effect on abdominal fat pad size after cimaterol treatment. However, Dalrymple *et al.* (1984) did find that total carcass fat was significantly reduced. Dalrymple *et al.* (1984) suggested that abdominal fat is less sensitive to β-agonist stimulation compared to total carcass adipose tissue. In the present study, ZH treatment at a concentration of 5 mg ZH had a positive influence on adipose fat deposition. Therefore, ZH may have altered adipose tissue deposition indirectly by inhibiting hepatic lipogenesis which favoured lean growth over fat deposition (Harris *et al*., 1988).

A clear sex effect on the magnitude of response from ZH treatment was observed in the present study. According to the results the female birds responded better to ZH treatment compared to male birds evidenced by their higher carcass yield (P <0.05). Our findings agree with Dalrymple *et al.* (1984), which also observed a greater response in females treated with cimaterol. Similarly, Rehfeldt *et al.* (1997) demonstrated that clenbuterol improved carcass composition of females to a greater extent than males, mainly due the fact that females deposit more fat than males. Even though females deposited more fat than males in our present study, the difference in female abdominal fat yield compared to the control was significantly lower than males. The females had a 5% lower fat yield compared to the control, whereas the males had 10% higher fat yield compared to the control. Therefore, ZH had a positive influence on fat deposition in female birds, but not in male birds.

Chapter 6

Conclusion

Synthetic β-adrenergic mimetics are chemically closely related to endogenous catecholamines, more specifically norepinephrine, a hormone released in response to stress. β_2-adrenergic agonists are regarded as potent metabolic modifiers as they are able to cross the blood brain barrier due to their lipophilic chemical structure. In addition, β-agonists are supplemented at higher effective doses, which further increase their biological activity and subsequent response. Studies have shown that catecholamines have a central effect on feeding behaviour, which induce feed intake in stressed situations. In broilers, β-agonists generally have no effect or reduce feed intake. In the present study, the supplementation of ZH resulted in a marked increase in feed intake compared to the control. The reason for the increase in feed intake is unclear. However, based on previous research studies, it is possible that ZH provoked a stress stimulus similar to endogenous catecholamines, which resulted in an increased feeding behaviour. Interestingly, the increased FI did not result in higher body weights. According to our findings, the ZH treated birds had significantly lower body weights compared to the control birds. Consequently, the increased feed intakes and lower body weights obtained in birds treated with ZH resulted in higher FCR values.

In contrast, supplementation of ZH had a positive effect on carcass characteristics. The control group had higher live body weights and empty carcass weights, however resulted in the lowest portion weights compared to the ZH treated groups. Birds that received 5 mg ZH and 7 mg ZH resulted in higher carcass yields, as well as thigh and breast yields. Therefore, it can be assumed that ZH treatment increased blood and nutrient flow to muscle tissues, which resulted in enhanced protein synthesis, evidenced by the improved thigh and breast muscle yields obtained in the present study. Alternatively, β-agonists alter the proteolytic enzyme activity in muscle, which cause a reduction in muscle protein degradation. In a rested or active state of muscle activity, muscle protein breakdown is always faster than muscle protein synthesis. It is therefore possible that ZH treatment reduced the rate of muscle protein breakdown resulting in larger muscle weights. Accordingly, the lower portion yields obtained in the control groups could be due to the higher rates of protein degradation.

The supplementation of ZH had a positive effect on fat deposition, especially in the female birds. Birds that received ZH treatment at a concentration of 5 mg ZH resulted in the lowest abdominal fat yield compared to the control and other ZH treatment groups. The improved carcass characteristics observed in the present study illustrates the repartitioning effects of β-agonists. Zilpaterol hydrochloride supplementation allowed more energy to become available for protein accretion, resulting in less abdominal fat deposition. In contrast, ZH supplementation had a negative influence on male fat deposition. These differences in response between males and females prove the sexual dimorphic effect of β-agonists observed in poultry species. The greater response seen in females is mainly due to the inherent ability of females to deposit more fat than males. On the other

hand, higher inclusion levels of ZH had a negative effect on abdominal fat deposition. This may be due, in part, to the greater feed intakes from ZH treatment which consequently led to excess energy stored in adipose tissue.

When comparing the Zilpaterol treated groups only, the highest ZH concentration of 9 mg ZH had the greatest negative impact on performance, as well as carcass characteristics. According to the results, the lowest inclusion level of 5 mg ZH had better performance values when compared to the two higher ZH concentration levels. These findings suggest that the ZH inclusion levels were possibly too high, and therefore, did not produce the desired response. In addition, the lowest ZH inclusion (5 mg ZH) level resulted in the lowest abdominal fat deposition compared the higher ZH treatment groups and the control. On the other hand, 7 mg ZH had a positive influence on carcass yield and portion yield. The highest dosage level of 9 mg ZH might have over-stimulated muscle receptors, which resulted in receptor down regulation and consequently, a diminished response.

This study revealed the significant impact of dose on the degree of response to β-agonist supplementation. Based on the results and previous literature, ZH is a highly potent and effective β_2-agonist compared to other β-agonists, and therefore only requires small dosage levels to stimulate a response. However, the supplementation of ZH at a concentration of 7 mg ZH per kg feed proved to be effective in improving carcass composition of broilers, despite the negative performance values obtained. Due to the contrasting results obtained in the present study, information related to the physiological activity of broiler response to ZH supplementation is shortcoming.

Critical review and recommendations

Literature clearly shows the significant impact of the type of β-adrenergic agonist used on the degree of response in animals. β-agonists have multiple physiological pathways through which it can alter the pattern of muscle growth and fat deposition in different species. Therefore, a standard growth production study is insufficient to depict the exact mechanism of action. Muscle protease enzymes, namely calpain and calpastatin concentrations, are used as indicators of muscle proteolytic activity. These enzymes' concentrations help identify whether β-agonists stimulate muscle accretion through increased protein synthesis or through decreased protein degradation. In addition, blood protein concentrations, such as blood urea nitrogen (BUN), albumin, globulin and uric acid are also indicators of protein metabolism. Studies showed increased concentrations of blood proteins in broilers supplemented with ractopamine (β_1-agonist). Furthermore, blood concentrations, such as glucose, cholesterol and triglyceroles (TG) also serve as indicators of β_2-agonist activity. High blood cholesterol and TG levels will confirm the effect of β-agonists on fat metabolism, by inhibiting liver lipogenesis and increasing lipolysis in adipose tissue. In order to fully understand the mechanism by which ZH induce muscle hypertrophy in broilers, I would recommend that growth production trials should be combined with serum analysis, which will allow more accurate conclusions to be drawn from the results.

β-agonists are structurally and functionally similar to endogenous stress hormones and therefore, elicit a similar response related to growth and feeding behaviour. Based on the negative effect on FI observed in the present study, we hypothesise that ZH elicited a stress response similar to E and NE. Studies have shown that 7 days is insufficient to produce a maximum response from β-agonists treatment in animals. For future application, I would recommend that a longer administration period should be applied to allow the birds to first adapt to the given supplement in order to achieve the desired response. Furthermore, based on the negative results obtained in the study, we concluded that the ZH levels were too high, which resulted in possible receptor down regulation and diminished response. I would recommend a lower inclusion level of between 1 mg/kg ZH and 3 mg/kg ZH in order to minimise the effect on FI and subsequent growth and carcass characteristics.

Studies have shown that β-agonists decrease the weights of certain organs, such as the liver, kidney and heart. According to the results, the control birds had the highest live body weights, however, their relative portion weights were significantly lower than the treated groups. The weights of the organs were not measured in the present study, which make it difficult to determine whether the differences in final body weight between the control and treated groups are related to organ weight. I would recommend for future studies that every aspect of the carcass to be weighed, including the head, feet, organs and intestines to minimise speculation and variation in results.

Chickens are less responsive to β-agonists stimulation compared to mammals, specifically related to fat deposition. Multiple causes for the variation in response exist, however, the density and affinity of β-receptors on adipose tissue is the major reason. Research has indicated that abdominal adipose tissue is less sensitive to β-agonists treatment compared to total carcass adipose tissue. Studies on the effect of β-agonists supplementation on fat deposition in broilers rely greatly on the abdominal fat portion, due to the ease of obtaining sample weights. However, by only determining the weight of the abdominal fat pad could result in over-estimation or under-estimation of the response. Carcass fat plays a crucial role in meat processing and consumer acceptance. In addition, fat plays a vital role in the nutritional quality and flavour of poultry meat. The supplementation of β-agonists might reduce the abdominal fat deposition, but could have detrimental effects on the flavour of the meat due to lower intra-muscular fat content. It is therefore essential to obtain results from broiler studies that reflect the entire carcass fat composition and not only the abdominal fat.

Lastly, supplementation of β-agonists in combination with minerals or hormones has shown to improve the response to β-agonists. The combination of trace minerals, such as zinc methionine (ZnMet), functions to up regulate and modulate receptor activity, thereby enhancing the effect of β-agonists on muscle growth. Similarly, supplementing β-agonists and hormones, such as glucocorticoids, improves β-agonists activity indirectly by stimulating metabolic pathways that facilitate the response to β-agonists. Research studies on finding methods to improve β-agonist activity will be beneficial to increase broiler performance to treatment.

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