EFFECTS OF DIETARY SALBUTAMOL ON GROWTH AND CARCASS COMPOSITION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) (WALBAUM)

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Abstract

Salbutamol, a β_2 adrenergic agonist, has been shown to reduce carcass fat and increase muscle mass and improve feed conversion efficiency in pigs. In the present study, the effects of dietary salbutamol at 20 ppm on growth, feed conversion efficiency, carcass recovery, visceral organ weight, and whole carcass composition of rainbow trout (*Oncorhynchus mykiss*) were studied. Rainbow trout (eighteen months old; average initial weight 324.0±0.4 g) were fed either the control or control + 20 ppm salbutamol diet for four weeks in a completely randomized design. Fish were weighed at the start and termination of the study, and records of feed intake were maintained. Carcasses were analyzed for protein, fat and ash at the start and completion of the four weeks feeding period. Dietary salbutamol had no adverse effect on fish mortality, health or feed intake. Dietary salbutamol had no effect ($p>0.10$) on growth, feed intake or feed conversion efficiency of rainbow trout. Internal organ weights such as liver, heart, gonads and viscero-somatic index and hepato-somatic index were also not affected ($p>0.10$) by dietary salbutamol. Interestingly, kidney weight was significantly ($p<0.01$) increased by salbutamol. However, it is unlikely that salbutamol directly increased the kidney weight. Increased metabolic load on kidney and blood flow to the kidney could be reasons for increased kidney weight. Although the final weight and the growth rate were not affected by salbutamol, the carcass recovery was significantly higher ($p<0.01$) in salbutamol treated trout. Whole carcass protein content of both treated and control fish showed no significant differences and clearly reflected the normal allometric growth and body composition. It was concluded that dietary salbutamol at 20 ppm level had no repartitioning effect in growing rainbow trout. The effects of salbutamol at various doses in more mature rainbow trout need to be studied in future studies.

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1. INTRODUCTION

Fish as food makes a very significant contribution to human nutrition and health. Although relatively unimportant as a source of energy, fish is a palatable, convenient, and still moderately priced source of high quality protein, vitamins, minerals, micro-nutrients and essential fatty acids (EFA). Particularly, the low fat nature of fish and the presence of EFA eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexonoic acid (DHA,C22:6n-3) which are essential to human health (Bjerve 1987) make it a highly desirable food. The essentiality of n-3 unsaturated fatty acids found in fish and consumers search for diversity and improved nutritional quality of food have led to an increased demand for seafood.

International trade in fish is increasing, driven by employment and the need to earn foreign exchange. In 1992, a total of 17 million tons of fish and fish products, valued at US$40.3 billion entered the international market. The share of developing countries has risen rapidly and currently exceeds 50% of the world catch. Either directly or indirectly fisheries, support about 200 million people, mostly in developing countries.

Demand for seafood is steadily increasing, but wild fisheries have already reached their maximum exploitation (FAO 1993). Aquaculture provides a promising alternative to this ever rising world demand for fish. During the last two decades aquaculture has grown rapidly and FAO (1999) predicted that by the year 2000, aquaculture will account for approximately 25% of world fisheries production. More than 220 aquatic species are farmed, ranging from giant clams which obtain most of nutrients from symbiotic algae, mussels which filter plankton, herbivorous carps to carnivorous salmon (FAO 1999).

Aquaculture on world-wide basis is now a profitable but competitive animal production industry (Lovell 1991). High growth rates and low recurrent costs determine the productivity and the profitability of the operation. In intensive aquaculture systems such as salmon and trout farming, feed costs can be as high as 60% of the recurrent cost.

Therefore, in aquaculture, high growth rate and efficient feed utilisation by fish is of paramount importance in increasing productivity and profitability.

Efficient feed conversion indirectly helps to reduce the depletion of wild fisheries stocks used in feeds for the aquaculture industry. Production of a kilogram of rainbow trout uses about 2.46 kg of wild fish as fish meal or fish oil (Naylor *et al.*, 2000). In 1997 more than 10,000 metric tones of wild fish were used to feed the most commonly farmed fish. Hunter and Roberts (2000) predicted that 20-25% of the total fish meal production will be used in the aquaculture industry in 2000. Therefore ways of improving feed conversion efficiency are important to reduce the use of fish meal and fish oil for aquaculture feeds.

Aquaculture industries are under increasing pressure to reduce the level of solid and dissolved wastes discharged to the environment (Mayer and McLean, 1995). Increased feed conversion efficiency helps to reduce the waste disposal by reducing level of phosphorous and nitrogenous compounds released to the environment. Increasing environmental concerns by consumers has made it imperative to produce fish sustainably.

Various technologies have been used to increase the growth rate, feed efficiency and carcass composition mainly in terrestrial farm animals. Use of anabolic steroids, although proven to be successful, has already been banned in almost every country. Breeding and selection for the above parameters will be slow for already highly selected breeds and may only be possible with unselected wild stocks. Although the prospects of improvement through genetic engineering is promising, apart from the associated high cost, increasing public concern over genetic modification justifies the search for alternative approaches to improve production. Somatotropins modify the carcass composition by increasing protein content and reducing fat content while increasing the feed conversion efficiency. The major draw-back of somatotropins is that they have to be administrated by injection or by implantation devices. Immersion techniques have not been successfully used in commercial situations.

During the last 15 years β adrenergic agonists have been extensively studied as potential candidates for manipulating growth and carcass composition mainly in terrestrial animals. It is generally accepted that some β agonists change the carcass composition by increasing the skeletal muscle protein content while reducing fat content. In some cases, the growth and feed conversion efficiency have also improved. Since these compounds redirect the nutrients away from adipose tissues towards skeletal muscle hypertrophy, they are generally termed as repartitioning agents. The major advantage of β adrenergic agonists is that, compared to somatotropins, β adrenergic agonists are orally active and therefore can be given with feeds. A variety of β adrenergic agonists such as cimaterol, clenbuterol, ractopamine, L644,969 and salbutamol have been studied in various terrestrial farm animals and laboratory animals. Until recently use of β agonists for food animal production had been banned. Now two β agonists, namely ractopamine and zilpaterol, have been cleared for food animal industries in several countries, including the USA.

Although β agonists have been extensively studied in terrestrial animals, they have been less extensively studied in fish. The first such study was reported in 1992. Two β agonists, namely, ractopamine and L644, 969 have been studied in rainbow trout and channel catfish. Available literature suggests that dietary β agonists are not as effective in fish as in terrestrial animals. Of the fish species studied, channel catfish have found to be more sensitive to dietary β agonists. Salbutamol is a selective β_2 agonist and has found to be effective in pigs. The present study investigated the effects of feeding salbutamol at 20 ppm on the growth performance, body composition and the organ weight in young rainbow trout (*Oncorhynchus mykiss*).

2. LITERATURE REVIEW

The effects of dietary β agonists on growth and carcass composition as well as their possible modes of actions have been subjected to extensive studies during the last 15 years. Consequently, the general effects of dietary β agonists on skeletal muscle protein accumulation and adipose tissues of terrestrial animals are well established. Although a significant body of knowledge has been accumulated on the way β agonists affect animal growth and carcass composition, the exact mechanisms of their actions are yet to be understood. The aim of this chapter is to summarise the available information of the effects and possible modes of actions of β agonists on animals.

Fewer than six papers, describe the effects of β agonists on fish growth and carcass composition. Consequently, the following review draws heavily from the studies on terrestrial animals. In the light of differences which exist between those animals and fish, and the great degree of variation of the responses in animals given β agonists, caution must be exercised in extrapolating terrestrial animals findings to fish.

2.1. Manipulation of growth in farm animals

Anabolic steroid and antimicrobial growth promoters are regarded as the first generation of farm animal growth promoters or performance enhancers. Anabolic agents increase the protein deposition and therefore the edible meat yield, particularly in ruminants. Use of antimicrobial agents has been found to increase the live weight gain particularly in poultry and pigs. The physiological activity, efficiency and safety of these two groups of compounds in terrestrial animals have been reviewed by several authors (Meissoneir and Mitchell-Vigneron, 1983; Armstrong 1986; Lamming 1986). Donaldson *et al.* (1979) and Higgs *et al.* (1982) extensively reviewed the application of steroid hormones as anabolic agents in fish. Steroids and thyroid hormones are not current candidates for use in commercial production systems due to regulatory and economic reasons, respectively (Donaldson and Devlin, 1996).

Lamming and Peters (1987) reviewed the techniques in farm animal growth manipulation under four broad categories; a) somatotropins and related techniques, b) immunological techniques, c) the β-adrenergic agonists and d) direct gene manipulation. Of these techniques somatotropins and β adrenergic agonists act on the animal's metabolism and can be referred to as metabolic modifiers.

2.1.1. Somatotropins and related techniques

It is well established that exogenous somatotropins modify carcass composition of a variety of species by increasing protein and reducing fat content, enhance the feed conversion efficiency, growth and milk yield (NRC 1994). Gill *et al.* (1985) first reported that recombinant avian and bovine growth hormone increased the growth rate of juvenile pacific salmon. Many studies particularly with young fish have shown that mammalian and piscine recombinant growth hormones were effective in enhancing growth rate in salmon (Reviewed by Down and Donaldson, 1991; Sumpter 1992; Le Bail *et al.*, 1993; Donaldson and Down, 1993; Mayer and McLean, 1995). Somatotropins must be administrated by daily injection or by sustained release implants or by immersion, in the case of fish (Down *et al.*, 1989). Immersion however, is not a very effective means of growth hormone administration (Agellon *et al.*, 1988; Schulte *et al.*, 1989). Most of the studies have been conducted with young pre-smolt or smolting salmon and consequently the effects of these compounds in grow-out fish are not available.

Administration of growth hormone releasing factors and somatomedins (insulin like growth factors; IGF) and production of transgenic animals with additional genetic materials for growth hormone production have been investigated as alternative approaches of somatotropin administration techniques of growth regulation. Although growth-hormone releasing factors has been isolated and characterised from salmonids, there are no reports of their use in *in-vivo* growth studies (Donaldson and Devlin, 1996). Slow-release administration of IGF-1 via osmotic pump resulted in increased growth in coho salmon (McCromick *et al.*, 1992). The role of somatostatin in fish growth regulation appears to still be under investigation (Diez *et al.*, 1992).

2.1.2. Immunological techniques

The rationale for immunisation against somatostatin is to reduce its inhibiting effects on growth hormone release. But the results obtained so far have been unconvincing. Spencer and Williamson (1981) and Spencer *et al* (1983a,b) observed higher growth hormone, somatomedin levels and rates of growth in lambs immunised against somatostatin but Varner *et al.* (1980) did not. Using chinook salmon as a model, Mayer *et al.* (1994) suggested that it may be possible to block the inhibitory effects of somatostatin on growth hormone release and thus stimulate the growth by administrating monoclonal antibody against somatostatin. The positive responses of immunisation against somatostatin were more pronounced with plesiomorphic breeds compared to with improved breeds (NRC 1994). This could indicate that selection for superior growth automatically selected strains with low levels of plasma somatostatin. Furthermore, like other immunological techniques for controlling endocrine regulation, there is a variable response between individuals. Active immunisation of lambs against GH-RH resulted in increased lean content in carcass (Keeling and Crighton, 1983). The effects achieved so far are not sufficiently encouraging for commercial operations.

2.1.3. Direct genetic manipulation

Genetic selection is slow to affect changes in body composition in farm animals and varies with species. Transgenic salmonids including rainbow trout with increased growth rates were first reported by Chourrout *et al.* (1986) and later by several authors (Fletcher *et al.*, 1988; Guyomard *et al.*, 1989; Du *et al.*, 1992; Devlin *et al.*, 1994). Although the impact of biotechnology in animal growth manipulation seems to be promising, issues such as ecological consequences, product safety and above all consumer acceptance of genetically modified animals have to be taken in to account.

2.2. β-adrenergic agonists: Structure, classification and functions

Compounds which can be fed with diet, and which reliably and favorably alter carcass composition are of great interest to the livestock and meat industries including aquaculture. During the early 1980's interest and active investigation into the influence of synthetic β-adrenergic agonists on tissue growth and carcass composition was rekindled. Research by

Asato *et al.* (1984), Baker *et al.* (1984), Dalrymple *et al.* (1984a,b) and Ricks *et al.* (1984) demonstrated the potential of these compounds in reducing carcass fat while increasing muscle mass in various species.

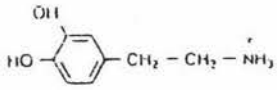
The terminology used for description of synthetic β adrenergic agonists is based on their similarity in structure and function with the naturally occurring catecholamines (CA); dopamine, epinephrine and norepinephrine (Beermann 1993) and octopamine (Fontana *et al.*, 2000). The three endogenous catecholamines are related in structure, function, biosynthesis, metabolism, and their adrenergic control of metabolism, and have been comprehensively reviewed by several authors (Martin 1985; Norman and Litwack, 1987; Timmermann, 1987; Mersmann 1989a; Weiner and Molinoff, 1989; August 1993). Both catecholamines and β adrenergic agonists have properties both of neurotransmitters of the sympathetic nervous system and hormones. Dopamine and norepinephrine, however, act primarily as neurotransmitters in the central and parasympathetic nervous system. The chemical structures of three catecholamines and β-adrenergic agonists that have proven to be effective in altering the carcass composition in farm animals are shown in Fig. 1.

2.2.1. Receptor types

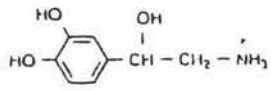
Response to these endogenous CA and synthetic agonists or antagonists in a specific tissue requires the presence of a receptor, specific for the compound and the functional presence of a signal transduction pathway within the cells. The sometimes differential and sometimes even opposite effects of epinephrine and norepinephrine in tissues led to the concept that different receptors (α and β) and associated transduction pathways were present for each catecholamine. The relative potency or specificity of β agonists could be determined by the relative numbers and affinities of these receptors in different organs (Beermann 1993). Studies with fish erythrocytes (Reid *et al.*, 1991, 1993) and hepatocytes (Reid *et al.*, 1992) found that the magnitude of CA stimulated physiological responses are directly related to the number of receptors in the target cells. Based on radioligand labelling, and molecular sequencing techniques the adrenergic agonists are now classified into; α_1(A, B, C); A_2 (A, B, C and D); β_1; β_2 and β_3. β adrenergic receptors have >400 amino acids in a continuous chain. Models for β receptors indicate that seven relatively

hydrophobic transmembrane domains anchor the receptor in the plasma membrane (Mersmann 1998).

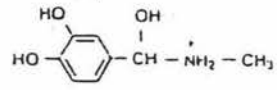
Dopamine

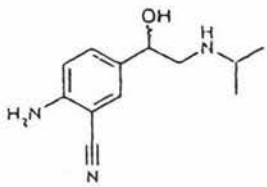


Norepinephrine

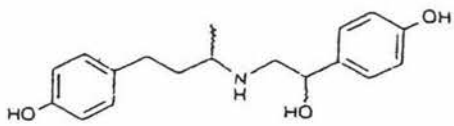


Epinephrine

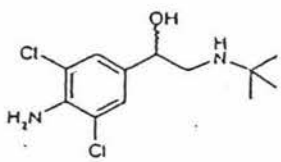




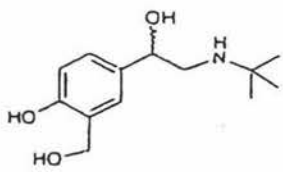
Cimaterol (β_2)



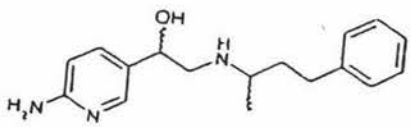
Ractopamine (β_1)



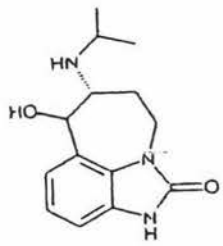
Clenbuterol (β_2)



Salbutamol (β_2)



L 644,969 (β_2)



Zilpaterol (β_2)

Figure 1. Chemical structures of the endogenous catecholamines and β adrenergic agonists that can alter carcass composition in farm animals (Moody *et al.,* 2000).

2.2.1.1. Receptor types in fish

Details regarding non-mammalian adrenergic receptors are scarce. Fabbri *et al.* (1992) found that adrenoreceptor subtypes present in fish liver are different from those of mammals. Studies with eel and bullhead (Zhang *et al.*, 1992), catfish (Fabbri *et al.*, 1994), channel catfish (Garcia-Sainz *et al.*, 1995) and in rainbow trout (Michelson and Sheridan, 1990) indicated that both α and β receptor types were present in fish liver. However, in rainbow trout Fabbri *et al.* (1995) could not find an intra-cellular signal transduction mechanism associated with α receptors although the α and β receptors were present. Fabbri *et al.* (1992, 1998) found that the β receptor mediated pathway is prominent in rainbow trout liver. Receptor types in the skeletal muscles of the fish have not been studied. The predominant receptor sub-type in mammalian skeletal muscle is β_2 (Liggett *et al.*, 1988). Trout heart was found to be exclusively of the β_2 type (Ask *et al.*, 1980, 1981; Gamperl *et al.*, 1994). The presence of red blood cell β adrenergic receptors has been reported in eel (Bennett and Rankin, 1985) and rainbow trout (Reid *et al.*, 1991). Bennett and Rankin, (1985) confirmed that they were not type 1 but the exact sub-type is yet to be established.

Adrenergic receptor types in the adipose tissues of fish have not been studied. Generally, it is believed that β_2 type is predominant in vertebrate adipocyte (Timmermann 1987). The stimulation of lipolysis by CA is activated through β receptors while through α_2 receptors lipolysis is inhibited (Fain and Garcia-Sainz, 1983). Recent studies (Lafontan and Berlan, 1993; Carpene *et al.*, 1994; Fontana *et al.*, 2000) have shown that β_3 receptors were also involved with lipolysis in mammals.

Generalisation of receptor types and their effects in other terrestrial vertebrates to fish should be done with care. Although the fish heart is primarily a β_2 type organ, mammalian heart is a β_1 type organ (Timermann 1987). Many or even most tissues or organs do not contain a pure population of α or β receptors. Rather a mixture of subtypes is usually present, with wide variation in proportion of each found between species, tissues, or even

cell types within a tissue. For example β_1 receptor concentration in heart may vary from 100% in guinea pig to 65 % in human (Beermann 1993).

2.3. Signal Transduction pathway of adrenergic agonist

All β-adrenoreceptor sub-types share a common signal transduction pathway whereas α_1 and α_2-adrenergic receptors do not (Fabbri *et al.*, 1998). A diagram showing the general view of the signal trasduction pathways of CA binding to α or β adrenoreceptors is given in Fig. 2. α_1 receptors are coupled to G-protein (G_q) and through phospholipase C, activate the intra cellular second messengers inositol 1,4,5-triphosphate and diacylglycerol. These second messenger systems, in turn increase the intra-cellular Ca^{++} level and protein kinase C, respectively. α_2 receptors are coupled with G_i protein and inhibit the adenosine cyclase. The β receptors are coupled to protein G_s and then the α sub-unit of the G_s protein activates the adenyl cyclase second messenger system (Mersmann 1998). Adenyl cyclase in turn stimulates cAMP production. Cyclic AMP subsequently binds to the regulatory sub -unit of a protein kinase and causes dissociation of the ligand-receptor complex and the release of the active enzyme to phosphorylate the target protein. This phosphorylisation results in either activation or inactivation of enzyme.

2.4. Catecholamines and their functions in fish

Internal factors such as non-cholinergic neurotransmitters, and/or neuromodulators, and direct blood borne factors including arterial oxygen (Po_2), carbon dioxide tensions and external factors (hypoxia, pollutants) are involved in catecholamine release. Plasma CA concentrations in fish are similar to those of mammals (Fabbri *et al.*, 1998). The resting plasma CA level ranges from 1 to 10 nM, which can be elevated 100 fold under acute stress (Colletti and Olson, 1988; Gilmour *et al.*, 1994).

As in other animals, CA are known to affect carbohydrate and lipid metabolism in fish. CA activate glycogen phosphorylase and inhibit pyruvate kinase leading to increased glycogenolysis and gluconeogenesis (Perry *et al.*, 1988; Mommsen *et al.*, 1988; Wright *et al.*, 1989; Reid *et al.*, 1992). Consequently exogenous CA induce hyperglycaemia in rainbow trout (Morata *et al.*, 1982; Mommsen *et al.*, 1988; Wright *et al.*, 1989; Reid *et al.*,

1992). CA induced hyperglycaemia is associated with simultaneous fall of liver glycogen level and subsequent recovery after 12-24hrs (Ottolenghi *et al.*, 1982). This suggests that CA directly induce hepatic glycogenolysis. Hyperglycaemic conditions induced by CA can be blocked by propranolol, a β receptor antagonist, (Wright *et al.*, 1989) but not by the α receptor antagonist phentolamine (Mommsen *et al.*, 1988) indicating the involvement of β receptors. However, using radioligand techniques, Fabbri *et al.* (1992) established the presence of α receptors in rainbow trout liver. As noted earlier, despite the presence of α receptors in the liver, only β receptor mediated pathway is metabolically active (Fabbri *et al.*, 1992,1998).

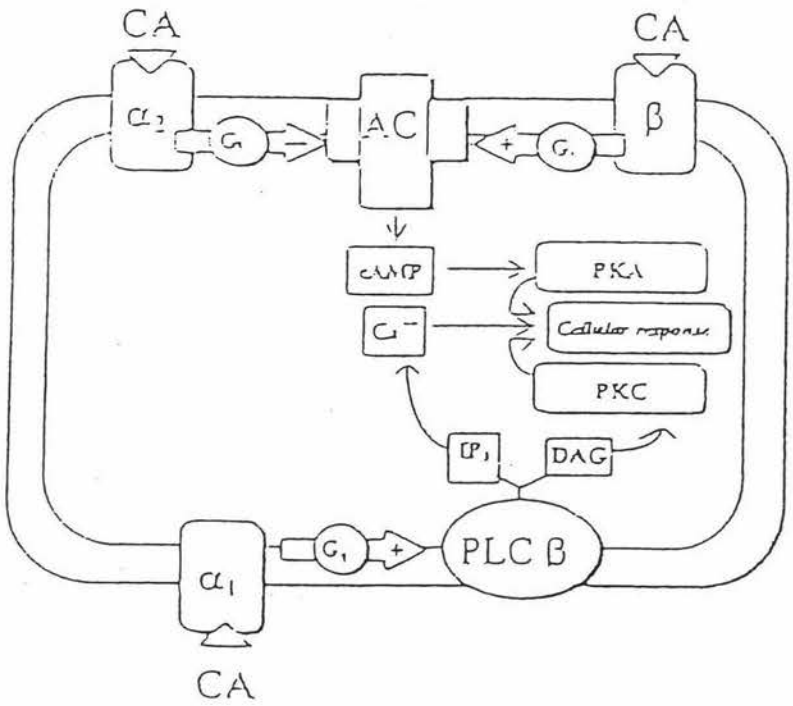


Figure 2. A schematic diagram showing the general view of the signal transduction systems of CA binding to α or β adrenoreceptors on a liver cell. Arrows indicate the direction of the signalling pathway, with – and + refering to a negative or a positive effect on the subsequent step. Abbrevations: G refers to G-protein type; AC, adenylyl cyclase; PLG β, phospholipase β; IP_3 , inositol 1,4,5-triphosphate; DAG, diacyl-glycerol; PKA and PKC, respective protein kinase enzymes (Fabbri *et al.*, 1998).

In rainbow trout, insulin inhibits the hepatic gluconeogesis from lactate (Peterson *et al.*, 1987) and from alanine (Cowey *et al.*, 1977). Although β agonists acutely increased pancreatic insulin synthesis or release (Yang and MacElligott, 1989), β agonists chronically reduced the plasma insulin level in pigs (Beermann *et al.*, 1987; Mills *et al.*, 1990). Vandenberg *et al.* (1998) observed an increase in blood glucose level in rainbow trout fed ractopamine and suggested that chronic catecholamine treatment in fish depressed insulin concentration and promoted hepatic gluconeogenesis.

Cornish and Moon (1985) found that the production of glucose from lactate accounted for less than 1 % of the total glucose production in American eel. They suggested that amino acids, rather than lactate functions as the main precursor for gluconeogenesis in fish. Vandenberg *et al.* (1998) observed a positive correlation between the blood glucose level and the weight (protein mass) of fish chronically fed dietary β agonist ractopamine. It can be assumed that the major input for gluconeogensis is amino acids in fish, since gluconeogenic capacity increases as the fish grow.

Literature on the effects of catecholamines on lipid metabolism in fish is conflicting. In general CA mobilise lipid stores by activating triglycerol lipase through a cAMP-dependent protein kinase, resulting in glycerol and free fatty acid release (Fain and Garcia-Sainz, 1983; Sheridan 1987). Findings of Sheridan (1987) and Sheridan and Muir (1988) that lipolysis was stimulated by noradrenaline in salmon liver support this proposition. In contrast, adrenaline had no effect on non-esterified fatty acid (NEFA) concentration in rainbow trout *in-vivo* or *in-vitro* (Murat *et al.*, 1985). Vandenberg and co workers (1992, 1998) found that in rainbow trout, lipolytic response (particularly in liver) to ractopamine was relatively low compared to other animals. Vandenberg *et al.* (1998) found that chronic administration of the β agonist ractopamine reduced the plasma NEFA level in a time related manner. Similar responses have been reported in sheep (Beermann *et al.*, 1987) and in mice (Eisen *et al.*, 1988). Plisetskaya (1980) and Vandenberg *et al.* (1998) suggested that catecholamine-induced hyperglyceamia influences the effects of catecholamines on lipid metabolism in salmonids by stimulating the re-esterification of NEFA.

2.5. Effects of β-adrenergic agonists on growth and body composition in terrestrial animals

The major effects of β agonists are (1) to alter the normal allometric growth by increasing protein accretion while reducing lipid deposition, (2) to increase the carcass weight relative to the live weight, (3) to enhance the feed conversion efficiency and (4) in some cases to increase the growth rate (Reviewed by Hanrahan *et al.,* 1986; Moloney *et al.*, 1992; Beermann 1989, 1993; NRC 1994; Mersmann 1998; Moody *et al.*, 2000). Ricks *et al.* (1984) termed these effective β adrenergic agonists as repartitioning agents because of their ability to redirect nutrients away from adipose tissues toward the muscles.

The β adrenergic agonists that have been reported to repartition the dietary nutrients elicit differing response patterns in laboratory animals (rat) and farm animals including poultry and fish. Responses are not consistent even within a species. The seven most extensively used β agonists are clenbuterol, cimaterol, ractopamine, L644,969, zilpaterol, salbutamol and L655,871. All of them are phenethanolamines. Clenbuterol, salbutamol, zilpaterol and L644,969 are selective β_2-agonists while ractopamine is primarily a β_1 type agonist (Moody *et al.*, 2000; see Fig 1).

2.5.1. Ruminants and swine

Generalisation of the effects of β agonists is difficult due to differences in agonists and dosages used, species, their age and physiological stage, treatment period and nutritional differences of the diets given. Within mammals, ruminants exhibit greater compositional and growth performance responses to β adrenergic agonists than swine, and responses are least in poultry and fish. In many studies with ruminants, (Hanrahan *et al.*, 1987; O'Connor *et al.*, 1988; Moloney *et al.*, 1990; Anderson *et al.*, 1989) average daily weight gain was increased by 20%-30%, and feed conversion efficiency was improved 14%-30%. Pigs show almost half of these responses; daily weight gain of 9% (Anderson *et al.*, 1991) and feed efficiency of 12% (Bracher-Jacob and Blum, 1990). The 20-40% increase in skeletal muscle mass commonly observed in growing ruminants was much higher than the 8-22% muscle mass increment observed in pigs (Adeola *et al.*, 1990). Likewise, the 20-40% reduction in carcass fat level seen in ruminants is approximately twice that seen in pigs.

2.5.2. Poultry

Poultry exhibit only modest improvement in growth performance and carcass composition in response to dietary β adrenergic agonist. Generally, daily gain, feed efficiency, and protein accretion increased by 4, 5 and 6 %, respectively, while fat level was reduced by 4-8% (NRC 1994). However, as large as a 10% increment in growth rate has been reported in turkeys by Wellenreiter and Tonkinson (1990).

These species differences may be attributed to their genetic potential to grow (Mersmann 1998). Species such as poultry have been extensively selected for growth and thus may have a growth rate close to the biological maximum with little room for response. Other species such as sheep have not been so intensively selected for growth and therefore have more potential to grow in response to dietary β agonists. Differences in agonist receptor selectivity, density and their signal transduction pathways in different species may also be responsible for the differential responses.

β_2 adrenergic agonists have found to be more effective in sheep and cattle but less effective in swine. Ractopamine, a β_1 type of agonist is less effective in ruminants, particularly in sheep but more effective in swine (Moody *et al.*, 2000). Compared to clenbuterol, cimaterol and ractopamine, salbutamol has been tested less extensively as a repartitioning agent. All the studies reported in the literature have used pigs. When effects of salbutamol in pigs, as reported by Cole *et al.* (1987), are compared with the effects of ractopamine as reported by Anderson *et al.* (1991), it can be inferred that ractopamine is more effective in relation to growth, feed efficiency and protein accretion while salbutamol is more effective in reducing carcass fat than ractopamine. However, it must be noted that the methodological differences between these two experiments render this comparison superficial.

2.6. Effects of β agonists in fish

Fish have lower energy requirements and respond to diets with a higher protein:energy ratio than birds and mammals (NRC 1993). Therefore it can be speculated that repartitioning agents might have different effects in fish.

While there are numerous reports on the effects of β agonists on terrestrial livestock, there are only a few which deal with fish (Table 1). Mustin and Lovell (1993) conducted the first such study with fish and reported that channel catfish (*Ictalurus punctatus*) fed with a diet containing 20 ppm ractopamine gained 17% more weight, 24% more muscle protein and 18% less mesenteric fat than those fed control diet. Muscle water content increased with decreased fat content. Dressing percentage was significantly reduced in the treated fish. They argued that decreased muscle fat content might be the reason. In their study Mustin and Lovell (1993) fed the channel catfish with a diet containing 36% crude protein, a higher level than usually used in commercial catfish production. Webster *et al.*, (1995) found no significant difference in weight gain in channel catfish fed with dietary L644, 969 in a low protein diet (27%). In a later study, Mustin and Lovell, (1995) fed channel catfish to satiation with diet containing three levels of dietary protein (240 g kg^{-1}, 300g kg^{-1} or 360g kg^{-1}) with 0 or 20 ppm ractopamine and at a restricted diets containing 240 g kg^{-1} or 360 g kg^{-1} dietary protein with 0 or 20 ppm ractopamine. They found that weight gain response to ractopamine was dependent on the dietary protein concentration and ration size. However, compaed to growth parameters, body compositional parameters were found to be less dependent upon protein concentration of the diet and the ration size.

Webster *et al.* (1995) studied the effects of L644,969 on the body composition and growth in blue catfish fed with two dietary protein levels. They did not find a significant effect of this β_2 agonist on growth parameters at either of the protein levels. However, treated catfish had higher fillet dressing percentage and lower abdominal fat than control fish. Furthermore, feeding L644,969 increased the fillet protein 15% and reduced the fat 28%.

Recently, Vandenberg and Moccia (1998) studied the effect of four levels (5, 10, 20 and 40 ppm) of the β_1 agonist ractopamine on rainbow trout. They reported higher thermal growth co-efficient (= Final $weight^{0.333}$-initial $weight^{0.333}$/ mean daily temperature * time; see Cho 1992) in fish fed 10 ppm ractopamine between weeks 1 and 4. During the first four weeks of feeding, at 10 ppm daily feed intake was significantly reduced while during the same time period at 20 ppm the feed intake was significantly increased.

Table 1 . Effect of ractopamine and L644,969 on the performances and body composition in different fish species.

Species	Temperature ^0C	Initial wgt(g)	β Agonist		Feeding time (weeks)	Proportional changes in relation to control					Source
			Name	Dose ppm		Weight gain %	FCE % (gain /feed)	Dressing yield %	Carcass		
									Protein	Fat	
Channel Catfish	30	156	Ractopamine	20	4	+ 17*	ND	-1.3*	+ 17*	- 24*	Mustin and Lovell (1993)[1]
Channel catfish	29	48	Ractopamine	20	8[2]	-12.1	- 1.0	ND	+ 0.8**,[3]	-1.6**,[3]	Mustin and Lovell (1995)[1]
					8[4]	- 2.5	0.0	ND	+0.7**,[3]	-1.1*	Mustin and Lovell (1995)[1]
					8[5]	-8.8	-1	ND	+0.8**,[3]	-0.6[3]	Mustin and Lovell (1995)[1]
Blue Catfish	24	110	L644,969	3	13	-9.8	+1	-0.8	+ 9.4*	- 9.2*	Webster *et al* (1995)[6]
Rainbow trout	11	196	Ractopamine	20	4	+3.45	+ 2.0	-0.75	-0.39	- 2.05	Vandenberg *et al* (1998)[7]

[1] 36% protein. [2] 24% protein. [3] Muscle and fat content. [4] 24% protein, restricted diet. [5] 36% protein, restricted diet. [6] 36% protein, satiation feeding.
[7] 45% protein satiation feeding. 7 ND. Not determined. * Significantly different p<0/05 * Significantly different p<0.01

The feed conversion efficiency was also higher during first four weeks in fish fed with 10 ppm ractopamine. When 10 ppm ractopamine was fed, carcass protein content was significantly increased with reduced carcass fat content during the 4-8 week period. At 5 ppm ractopamine whole carcass protein content was higher after the first four weeks. However, the carcass fat content was not significantly reduced. Neither of these effects were seen after the 8^{th} week.

Although the growth rate data do not agree with the observations of Mustin and Lovell (1993 and 1995), later studies by Webster *et al.* (1995), Vandenberg and Moccia, (1998) found an increased carcass protein content and decreased carcass fat content in fish fed β agonists compared to control fish, indicating that β agonists have repartitioning effects in fish, particularly in catfish. Furthermore, as in the case of other terrestrial animals, the β agonist used, the dose, dietary nutrients, length of the treatment, physiological stage of the fish seem to be the determinants of the effects of β agonists in fish

2.7 Interaction between dietary β agonists and experimental conditions

2.7.1. Nutrition

The repartitioning effect of dietary β agonists have been reported in both adequate and restricted feeding conditions in lambs (Kim *et al.*, 1989) and pigs (Bracher-Jacob and Blum, 1990; Bracher-Jacob *et al.*, 1990; Dunshea *et al.*, 1993; Mitchel *et al.*, 1990; Oksbjerg *et al.*, 1994). However, significant increases in growth rate have occurred only in well-fed animals. MacRae *et al.* (1988) observed increased protein deposition and reduced fat deposition in lambs given clenbuterol fed at 1.3 or 2 times maintenance energy supply. Mustin and Lovell (1995) found that in channel catfish, the growth promoting effect of ractopamine was greatly diminished when dietary protein level was reduced.

Generally animals fed β agonists require increased dietary protein to accommodate the increased muscle deposition rate. Increased maintenance energy and reduced energetic efficiency increase the energy requirement (Reeds and Mersmann, 1991). Furthermore, although the growth is enhanced by β agonists, in many cases, daily feed intake does not change or in some cases reduces, at least initially. Therefore, in order to achieve the full potential of the beneficial effects of dietary β agonists, it is essential to increase the nutrient density including energy of the diet.

2.7.2. Age of the animal

The magnitude of the effects of dietary β agonists is related to the animal's potential to deposit fat and skeletal muscles. Thus, the effects are more prominent in heavier fattening animals compared to pre-weaning and young rapidly growing animals. In contrast to consistent effects in heavier finishing animals, the effects of β agonists on carcass composition and growth of young pigs (Mersmann *et al.*,1987), calves (Williams *et al.*, 1987b) and in lambs (Williams *et al.*, 1989) were not significant. However, Oksbjerg *et al.* (1994) found that salbutamol could increase the muscle growth in growing pigs if the level of dietary protein was raised by 10 % above the recommended level.

The lack of response in young animals may be due to the low receptor numbers in these animals, lower affinity, or more rapid desensitisation of receptors to β agonists.

2.7.3. Treatment time

In general, the magnitude of differences is greatest within first 1-3 weeks of treatment and declines with continued administration (Beermann *et al.*, 1986; Wallace *et al.*, 1987; Kim *et al.*, 1989; Moloney *et al.*, 1990). The treatment time required to plateau the daily gain is higher for cattle compared to sheep and swine. In cattle given β agonists, the daily gain plateaued after 10-16 weeks (Fiems *et al.*, 1990), 5 weeks (Sillence *et al.*, 1993), and 8 weeks (Barash *et al.*, 1994) after treatment. In swine, ractopamine stimulated daily gain plateaued after 3 weeks of treatment, but carcass composition changes continued as ractopamine treatment duration increased (Anderson *et al.*, 1987). It is not surprising that time required for the growth responses to plateau reflects the general species differences in growth. Chronic administration causes down regulation of receptors leading to an attenuation of the responses. Kim and Sainz (1990) measured the β adrenergic agonists receptors in rat skeletal muscle at various times during cimaterol treatment and found that reduction in receptor density correlated with the attenuation of muscle weight gain. Reduced response can be restored by withdrawing the drug or by increasing the dose (Kim *et al.*, 1995).

2.7.4. Sex differences

Sex differences among species that respond to the dietary β agonists are minimal or non-existent in ruminants (Hanrahan *et al.*, 1987; NRC 1994) but have been reported in poultry (Dalrymple *et al.*, 1984b; Dalrymple and Ingle, 1987) and in swine (Uttaro *et al.*, 1993). Treatment of broilers

with cimaterol reduced the carcass fat of the order of 10% in female birds but only approximately 5% in male birds (Dalrymple and Ingle, 1987). This sexual dimorphic effect may be due to the tendency of female birds to deposit more fat than male birds (NRC 1994). Uttaro *et al.* (1993) found that ractopamine reduced the carcass cholesterol content in barrows, but not in gilts.

2.7.5. Genetic differences

Reports on the effects of genetic make up on the responses in animals given dietary β agonists are conflicting. Although animals having a greater potential to accrete lean might be expected to have a greater response to dietary β agonists, this is not always the case. When cimaterol was fed to obese and lean pigs (Yen *et al.*, 1990a), ractopamine to genetically obese or lean pigs (Yen *et al* 1990b) or to pure or cross bred pigs (Yen *et al.*, 1991) no differential responses were detected. Hanrahan *et al.* (1987) observed no breed differences in lambs in response to cimaterol. Eisen *et al.* (1988) fed cimaterol to two mice lines (genetically selected for higher growth and unselected control group) and found a similar response in growth and fat reduction in both lines. In contrast, several studies have shown that significant breed differences in dietary β agonists induced responses. Gu *et al.* (1991a,b) observed a greater muscle mass response in ractopamine fed pigs of leaner genotype. Bark *et al.* (1992) found that ractopamine increased the muscle accretion and reduced the fat deposition to a greater degree in pigs with a high genetic capacity for lean tissue growth than those with a low capacity. Salbutamol increased the carcass protein and reduced the fat in both traditional and meat type pigs. The reduction in fatness was however less pronounced in the leaner meat genotype (Warris *et al.*, 1990a).

The rate of muscle protein synthesis is directly associated with muscle DNA concentration. In pigs, muscle DNA concentration is greater in breeds with high genetic capacity to deposit more lean (Lundstrom *et al.*,1983; Hausman and Campion, 1986). The different responses may be due to differences in number and/or density of β receptors in the tissues. In support of this argument, Bocklen *et al.* (1986) found that an extremely muscular breed of pig (Pietrain) had higher densities of β adrenergic receptors in skeletal muscles and adipose tissues than in the same tissues of pigs of a less muscular breed (Large White).

Hence, it can be inferred that β adrenergic agonists are effective in a wide range of genotypes, but a greater response can be obtained when genetically superior genotypes that have higher lean

tissue accretion are used. One possible reason for this is that genetic selection for higher lean growth simultaneously may result in establishing genotypes with higher receptor density or efficient underlying metabolic pathways involving β agonist actions.

2.8. Effects on other organs

β agonists target skeletal muscle for protein deposition (Reeds *et al.*, 1986; Williams *et al.*, 1987b; Forsberg and Wehr, 1990; Moloney *et al.*, 1990). The weight of most visceral organs, including liver, kidneys, gastrointestinal tract, skin, and bone is not altered or may be decreased. Decreased liver weight has been reported in dietary β agonists given to pigs (Moser *et al.*, 1986; Mersmann *et al.*, 1987), rats (Emery *et al.*, 1984) and chickens (Hamano *et al.*, 1999). Reduced kidney weight has been reported in pigs (Moser *et al.*, 1986) and in rats (Emery *et al.*, 1984). Bracher-Jacob and Blum (1990) has reported decreased heart weight in pigs treated with repartitioning agent RO-16-8714. In contrast, increased protein deposition in heart has been reported in rats (Reeds *et al.*, 1986) and in calves (Williams *et al.*, 1987b).

2.9. Muscle redistribution effect of dietary β agonists

Several studies have shown that β agonists have a muscle redistribution effect in terrestrial animals. Dose dependent increases in proportion of muscle mass associated with the hind quarters and decrease in proportion associated with fore quarters have been observed in steers (Moloney *et al.*, 1990; Wheeler and Koohmaraie, 1992) sheep (Beermann *et al.*, 1986; Cleays *et al.*, 1989; Higgins *et al.*, 1988) and in pigs (Moser *et al.*, 1986; Warris *et al.*, 1990a) given various β agonists. In broiler chicken, cimaterol increased the weight of the leg muscle (Morgan *et al.*, 1989; Gwartney *et al.*, 1991). Bark *et al.* (1992) suggested that differential responses in different body areas may reflect the different number and/or density of the receptors.

2.10. Effects of β agonists on muscle fibre types

Dietary β agonists cause muscle hypertrophy rather than hyperplasia (Martin 1985; Beermann *et al.*, 1987; Kim *et al.*, 1987). Several authors (Humby *et al.*, 1986; Kim *et al.*, 1987; Beermann *et al.*, 1987; Miller *et al.*, 1988; Vestergaard *et al.*, 1990; Koohmaraie *et al.*, 1991a) have found that

responses of type II muscle fibres were greater than type I muscle fibres. Dietary salbutamol in pigs (Oksbjerg *et al.*, 1994) and cimaterol in bulls (Vestergaard *et al.*, 1990) decreased the proportion of fast type a (FTa) while increasing fast type b (FTb).

Protein turnover varies in different skeletal muscles due to the differences in fibre type composition. Proteolytic capacity varies in different muscle types and in the same muscle from different species (Quali and Talmant, 1990; Koohmaraie *et al.*, 1991b). For example, pig red muscles have low calpastatin activity compared to white muscles and the activity in the longissimus muscle is lower than that found in the same muscles of cattle and sheep (Koohmaraie *et al.*, 1991b).

Differences in relative muscle fibre types, their metabolism, proteolytic activity in different species and even between the different muscles within a species and their interactions may play a role in the relative efficiency of agonists in different species, and muscle area distribution within a species

2.11.1. Mode of action of β adrenergic agonists on skeletal muscles

How β adrenergic agonists affect skeletal muscle metabolism is unclear. Clenbuterol induced muscle hypertrophy was blocked by propranolol, a non-selective β_1 and β_2 antagonist (MacLennan and Edwards, 1989) or by β_2 specific antagonist ICI-118-551, (Choo *et al.*, 1992). Though these findings confirm the involvement of β adrenergic receptor mediated pathway of action, the primary site of action is controversial. Evidence has been reported for both direct increased skeletal muscle protein synthesis and reduced protein degradation. Other indirect mechanisms include altered plasma hormones and metabolites and increased blood flow.

2.11.1.1. Increased blood flow

β agonists stimulate blood flow to muscle of the hind limbs of cattle (Eismann *et al.*, 1988; Eismann and Huntington, 1993), sheep (Beermann *et al.*, 1987; Aurousseau *et al.*, 1993) and pigs (Mersmann 1989a, b). An increase in blood flow to the skeletal muscle may enhance the process of hypertrophy by delivery of increased amounts of substrate and energy sources for protein synthesis. Likewise, increased blood flow to adipose tissue may be important to carry the NEFA away from the adipose tissue to facilitate the efficient lipolysis (Mersmann 1998). Recently, Byrem *et al.* (1998) measured the blood flow to the contralateral hind-limbs of steers

surgically catheretized to infuse cimaterol or saline and could not find significant increase in blood flow to the cimaterol infused hind-limb.

2.11.1.2. Hormones

Metabolic hormones such as insulin, growth hormone, and the thyroxine play a key role in the regulation of protein, lipid and carbohydrates metabolism. Therefore, β agonists may exert their effects via these hormones.

Growth hormone

In general the reported literature on the effects of β adrenergic agonists on plasma hormone profile of treated animals are inconsistent across the studies. *In-vivo* studies with sheep fed dietary L 644,969 (Zhang *et al.*, 1995) or cimaterol (Beermann *et al.*,1987) have shown that dietary β agonists increased the plasma growth hormone (GH) levels. On the other hand salbutamol (Hansen *et al.*, 1997), ractopamine (Dunshea and King, 1994) and clenbuterol (Miller *et al.*, 1988) did not change the GH level and plasma insulin like growth factors-1 (IGF-1) (Dunshea and King, 1994).

Additive effects of a combination of growth hormones with clenbuterol in cattle (Maltin *et al.*, 1990), salbutamol in pigs (Hansen *et al.*, 1997) and ractopamine in pigs (Jones *et al.*, 1989) have been reported. This implies that growth hormones and β agonists act through two distinct pathways. The receptor structure and intra-cellular pathways of GH and β adrenergic agonists are quite different. GH has generalised hypertrophic effect on almost all the organs while the effect of β agonists is mainly confined to skeletal muscles. GH has a profound effect on feed intake whereas the effect of β agonists on feed intake is minimal. (Mersmann 1998). The absence of consistent effects on GH level and the above mentioned differences between GH and β agonists provide strong evidence against the possible role of GH in the mode of action of β agonists.

Insulin

Although cimaterol induced an acute increase in insulin in sheep (Beermann *et al.*, 1986; O'Connor *et al.*, 1988) and cattle (NRC 1994), chronically decreased insulin levels have been reported in dietary β agonist treated pigs (Mills *et al.*, 1990) and sheep (Beermann *et al.*, 1987). Furthermore, clenbuterol has increased the muscle weight in control and diabetic induced rats as well as diabetic induced rats given daily insulin replacement (McElligott *et al.*, 1987).

Increased insulin sensitivity or responsiveness in the skeletal muscles of dietary β agonists treated cattle (Eismann *et al.,* 1988) and rats (Budohoski *et al.*, 1987) has been reported. Meanwhile, adipocytes of dietary β agonist fed rats (Hausman *et al.*, 1989) and pigs (Liu and Mills, 1990) showed a decreased sensitivity to insulin. Anderson *et al.* (1991) suggested that the repartitioning effect of β agonists are due in part to the opposing effect of β agonists on insulin sensitivity in skeletal muscle and adipose tissues. In contrast, an *in-vitro* study by Orcutt *et al.* (1989) and *in-vivo* studies by Dubrovin *et al.*, (1990) and Eismann and Bristol, (1998) did not find significant differences in insulin sensitivity of the adipocytes between β agonist fed or control animals. In conclusion, it is unlikely that insulin directly or indirectly plays a significant role in the mechanism of β agonist action.

β agonists induced skeletal muscle growth has been observed in diabetic (Eisemann and Bristol, 1998), hypophysectomized (Thiel *et al.*, 1987), castrated and adrenalectomized (Rothwell and Stock, 1988), denervated (Zeman *et al.*, 1987), genetically somatotropin deficient (Bates and Pell, 1991) and hyper and hypothyroid (Forsberg and Wehr, 1990) animal models. Recent work by Byrem *et al.* (1998) confirmed that the effect of β agonists on skeletal muscle is direct and any indirect effect via hormones and increased blood flow is insignificant. No chronic increase in blood flow, or changes in endocrine status was found in cimaterol infused hindlimb of sheeps. However, the net protein synthesis was increased in the cimaterol infused hind-limb confirming that the effect of cimaterol on skeletal muscle was not mediated through increased blood flow or changed endocrine status.

2.11.1.3. Increased protein synthesis or decrease degradation or both?

Increase in muscle protein deposition can be brought about either by increased protein deposition or by decreased degradation or by both. Evidence for all three possibilities is available in the literature.

Increased protein synthesis rates have been reported in response to clenbuterol in rats (Emery *et al.*, 1984), and in sheep (Cleays *et al.*1989; Inkster *et al.*, 1989). On the other hand, Reeds *et al.* (1986) and Bohorov *et al.* (1987) found that clenbuterol did not increase the fractional rate of protein synthesis but reduced the protein degradation rates in rats and sheep, respectively. Reduced methylated histidine excretion (Williams *et al.* 1987b) and decreased plasma NH_2 level

(Eismann *et al.*, 1988) have been reported in clenbuterol fed calves. Similarly, Wilson *et al.* (1988) and Morgan *et al.* (1989) found that cimaterol was effective in reducing protein degradation rate. Reduced activity of the proteolytic enzyme calpain or increased anti-proteolytic calpastatin has been observed in several species treated with β adrenergic agonists (Higgins *et al.*, 1988; Wang and Beermann, 1988; Koohmarie *et al.*, 1991a,b) and supports the argument that reduced protein degradation plays major role.

Anderson *et al.* (1991) proposed a diphasic mechanism of action of β agonists in skeletal muscle; initial reduction in overall muscle protein degradation is followed by increased protein synthesis. In support of this hypothesis, Wheeler and Koohmarie (1992) found that the reduced protein degradation effect of L644,969 started in the first week of treatment and was maintained until the third week and by the sixth week the degradation rates were similar between treated and control groups. Similar effects of β agonists on skeletal muscle protein metabolism has been reported by Reeds *et al.* (1986), Eismann *et al.* (1988), Eadara *et al.* (1989), Yang and McElligott, (1989) and Koohmarie *et al* (1991a). Collectively these findings support the diphasic mechanism proposed by Anderson *et al.* (1991) of an initial reduction in protein degradation followed by an increased rate of protein synthesis.

Interestingly, all the β adrenergic agonists that have been shown to be effective through increased protein synthesis and/or reduced degradation are β_2 type agonist. In all but one example (Dunshea and King, 1994), ractopamine, a β_1 agonist has shown to be effective through increased protein synthesis. *In vitro* studies with pigs (Helferich *et al.*, 1990) also confirmed that ractopamine acts primarily through increasing protein synthesis. Collectively, these findings support the hypothesis that β_2 agonists act by increasing protein synthesis rate and/or decreasing protein degradation rate while β_1 agonists increase the protein synthesis rate.

2.11.2. Mode of action in adipose tissue

Reduced lipid level can be achieved either by reducing lipogenesis or by increasing lipolysis. It is generally accepted that β agonists cause activation of hormone sensitive lipase and inactivation of lipogenic enzymes involved in *de-novo* synthesis of tryglycerides (Fain and Garcia-Sainze, 1983). Acutely increased plasma NEFA levels have been reported in pigs (Mersmann *et al.*, 1987; Adeola *et al.*, 1992), in cattle (Blum and Flueckiger, 1988) and in fish (Vandenberg *et al.*, 1998) given various β agonists, suggesting a lipolytic pathway. However,

in-vitro studies have not yielded consistent results (Duquette and Muir, 1985; Rule *et al.*, 1987; Miller *et al.*, 1988; Mills and Liu, 1990; Peterla and Scanse, 1990). Different experimental conditions, particularly incubation conditions, may be the reason for inconsistent *in-vitro* results. Liu *et al.*, (1989) found that β agonists reduced the lipogenesis and increased lipolysis in pigs. However, lipolytic responses were more pronounced than the anti-lipogenic responses. Determination of lipogenesis is not straightforward and involves the measurements of lipogenic enzyme activities. Nevertheless, *in-vitro* studies with chicken tissues (Rosenberg and Steel, 1995) and pigs adipose tissue (William *et al.*,1987a) have shown that β agonists depress lipogenesis as well.

2.11.2.1. Increased energy expenditure

Reeds and Mersmann (1991) presented evidence to suggest that decreased rate of fat deposition was at least partly due to the diversion of amino acid carbon chains from energy producing metabolic pathways and partly due to increased rate of energy expenditure in β agonist treated animals. Increased skeletal muscle protein synthesis uses a large proportion of available amino acids and thus makes them unavailable for energy production. Consequently more and more fats are utilised for energy production. Reviewing the literature, Reeds and Mersmann (1991) concluded that up to 60% of the reduction of fat deposition could be attributed to the non-availability of amino acid carbon as an energy source and the use of fat in place of them. The remaining reduced fat deposition is largely associated with increased energy expenditure. In support of this argument, increased energy expenditure in β agonists treated calves (Williams *et al.*, 1987b), sheep (MacRae *et al.*, 1988) and rats (Rothwell and Stock, 1988; Emery *et al.* 1984) has been reported. Energy expenditure increased due to several reasons. Kim *et al.* (1989) found that cimaterol increased the fasting heat production and metabolisable energy requirement for maintenance in sheep. Since energy cost of protein deposition is higher than that of fat (20 kj/g vs 13 kj/g) increased protein deposition demands more energy. Reeds *et al.* (1982) estimated that synthesis of protein contributed a minimum of 17% daily energy expenditure. The energetic efficiency of fat storage is reduced by producing a condition in which considerably more fatty acids are synthesised *de-novo* than are deposited. Therefore, increased energy requirement arises from maintenance, protein deposition as well as for lipid metabolism (Reeds and Mersmann, 1991). According to this hypothesis, increased protein deposition, at least in part, is a prerequisite for reduced fat deposition.

2.12. β agonists and meat quality

Dietary β agonists influence the meat quality both favourably as well as unfavourably. Effects on meat quality are dependent on the agonist used, its dosage and the species (Moody *et al.*, 2000).

Reduced meat tenderness has been reported in β agonist treated broiler chickens (Morgan *et al.*, 1989; Gwartney *et al.*, 1991), lambs (Higgins *et al.*, 1988; Krestchmar *et al.*, 1990) and pigs (Koohmaraie *et al.*, 1991b). Since reduced fat content can also reduce the meat tenderness, the reduced meat tenderness observed in β agonist treated animals may be partly due to reduced fat content.

Several authors have shown that the effects of ractopamine (Stites *et al.*, 1994; Jeramiah *et al.*, 1994) and zilpaterol (Casey *et al.*, 1997) on meat tenderness in sheep and cattle was lower than other agonists such as cimaterol, clenbuterol and L 644,969. Reduced meat tenderness can also result from increased calpastatin activity which reduces the protein degradation in the skeletal muscles. It has been shown that ractopamine functions mainly to increase muscle protein synthesis rate without any effect on protein degradation whereas others function through increased protein synthesis and/or reduced protein degradation. Warris *et al.* (1990b) found that salbutamol improved the lean meat yield but slightly increased the potential to produce dark, firm, dry meat and reduced the meat tenderness. Uttaro *et al.*(1993) reported paler muscles in pigs fed ractopamine.

Favourable effects of β agonists on meat quality have also been reported. Although increased leanness is usually associated with reduced juiciness, short term administration of β agonists during finishing period reduces the fat content without affecting meat juiciness (Moody *et al.*, 2000). Reduced cholesterol in ractopamine fed pigs has been reported by Uttro *et al.*(1993).

2.13. The pharmacokinetics, metabolism and tissue residues of β agonists

To achieve beneficial effects of β agonists, it is necessary to use doses that are 10 times therapeutic doses (Miller *et al.*, 1988). In rats, absorption of dietary salbutamol was found to be

56% (Smith 1998). When cattle are given dietary salbutamol at 78 μg/kg, the peak plasma salbutamol level (4.0-4.8 ng/ml) was detected 3-4 hrs after administration. The half-life of salbutamol in humans is around 3-9 hrs (Morgan *et al.*, 1986). The bioavailability of dietary β agonists varies greatly between species. For example, salbutamol has high bioavailability in cattle but has low bioavailability in other species (Smith 1998). The lower bioavailability of ractopamine may explain the high dose of ractopamine required to elicit an effect. Low bioavailability of some β agonists limit the clinical effectiveness in human (Morgan 1990) and perhaps other species (Mersmann 1995).

Malucelli *et al.* (1994) studied the residual accumulation of β agonists in chicken tissues after 19 days of feeding. Salbutamol was most accumulated in feathers, liver, eye and kidney and undetectable in edible tissues after 2 weeks of withdrawal. They recommended at least 2 weeks of withdrawal to permit complete elimination of residues.

2.14. Limitations for the use of β agonists for meat animals.

Despite numerous advantages of β agonists in meat animals, several disadvantages have also been reported. To be used for food animal industries, a withdrawal period is required to clear the β agonists residues from the edible tissues. However, 7-14 days withdrawal of β agonists from chicken (Gwartney *et al.*, 1991), and from swine (Jones *et al.*,1985; Prince *et al.*,1985; Dunshea and King, 1994) reverses the fat reduction effect in all cases and growth performance effect in some cases.

3. MATERIALS AND METHODS

Diet Composition

A basal (control) diet based on hoki meal (*Macruronus novaezelandiae*) and fishmeal was formulated to contain 50% crude protein. The ingredients and nutrients compositions of this diet are presented in Table 2. The diet contained 4215 kcal/kg gross energy. The test compound (salbutamol) was incorporated at a rate of 20 ppm into the control diet according to the standard operating procedure (Appendix 1) of the Massey University Monogastric Research Centre. Racemic salbutamol was supplied by Bridge Pharma Inc, Sarasota, Florida, USA, as Salbutamol sulfate. Following mixing, the diets were cold pelleted (65 °C).

Animal and Experimental Design

A total of 144 Rainbow trout (*Oncorhynchus mykiss* Walbaum), 18 months of age and of indeterminate sex were used in this study. The experiment was carried out during winter (May-July). Visibly healthy fish with a live weight of 324.0 ± 0.4 g (mean ± SE) were selected from the outdoor rearing pond of the hatchery. In the rearing pond, fish had been hand-fed on commercial trout ration, twice daily (1000 and 1530hr).

Fish were housed in four concrete tanks (3.7m x 1.0m x 1.0m) at the National Trout Centre, Turangi, New Zealand. Each tank had one water inlet and one outlet and was supplied with high quality oxygen saturated water at a constant rate of 80 litres/min and the water depth maintained at 0.7 m. The fish were subjected to 12:12 h light:dark cycle using fluorescent lights and the water temperature was between 9 – 11 °C (mean 10.3 °C). The temperature was recorded twice a day at 0830h and 1600h. Each tank was partitioned across the middle (using wire mesh) to make a total of 8 sub-tanks (Fig. 3). The experiment followed a completely randomised design with the two treatments (control diet or control diet + salbutamol) randomly assigned to four larger tanks and each treatment was replicated in a single tank, giving a total of four replicates per treatment.

Table 2. Ingredients and nutrients composition of the control diet (Vickie Seager NRM Feeds. Personal communication)

Ingredient	%
Hoki meal	28.6
Fish meal	24.5
Pollard	19.0
Meat protein	14.1
Skim milk powder	5.4
Wheat flour	5.4
Blood meal	2.2
Betaine anhydrous	0.3
Fish mineral mix	0.3
Vitamin mix	0.2
Calculated Analysis	
Moisture	8.4
Crude protein	50.2
Fat	6.5
Crude fibre	2.5
Nitrogen-free extract	18.7
Ash	14.1
Calcium	3.9
Total phosphorus	2.4
Gross energy (Kcal kg^{-1})	4215

The fish were sorted into the treatment groups four weeks prior to the experiment such that the density variation between the sub-tanks was minimized. Fish were captured from the rearing pond by a net and kept in an indoor holding tank prior to the sorting. Fish were lightly anaesthetised with Aqui-S (Fish Transport System, Lower Hutt, New Zealand), weighed and

those between 290 and 350g were selected and the fork length measured. Sorting and allocation to groups were carried out so there was a similar weight range of fish within each sub-tank. Although individual fish were measured, fish were not individually identified thus whole sub-tank weights were calculated. No sex selection was performed at the beginning of the experiment. The initial stocking density was 4.50 kgm^{-3}.

All fish were fed the control diet and maintained on a restricted intake (1.5% of body weight) for a 28-day acclimation period during which they were hand-fed twice a day (0830 and 1600hrs). For the first two weeks of the acclimation period the fish were acclimatized to the tank and environment (they fed little during this time) and in the second two weeks they acclimatized to the control diets.

On day 29 of the acclimation period the fish were not fed for 24 hours following which three fish were randomly sampled from each of the eight sub-tank. These fish were euthanased with an overdose of benzocaine, (Sigma Chemicals, St Louis, USA) for 5 minutes at a concentration of 100 ppm, weighed, dissected and the carcasses were frozen for subsequent analysis (baseline data).

At the end of the acclimation period and after the baseline sampling, the water supply to one tank was mistakenly disconnected, resulting in the death of 30 fish in two sub-tanks. Due to the fact that there were two tanks (4 sub-tanks) of treatment fish and only 1 tank (2 sub-tanks) of control fish remaining, the fish from one of the treatment group sub-tanks were transferred into one of the empty sub-tanks and reassigned to the control group (T0R1). This gave a total of three replicates per treatment with 15 fish per replicate for the experiment. The experimental layout of these six sub-tanks is shown in Fig. 3.

All remaining fish (n=90) were lightly anaesthetised with Aqui-S (Fish Transport System Limited, Lower Hutt, New Zealand), individually weighed (initial live weight; LW) and the fork length measured. These 90 trout were fed either the control diet or the control + salbutamol (20ppm) for a period of 28-days. On day 29 of the trial all animals were not fed for 24 hours and on day 30 were euthanased before measurements were made.

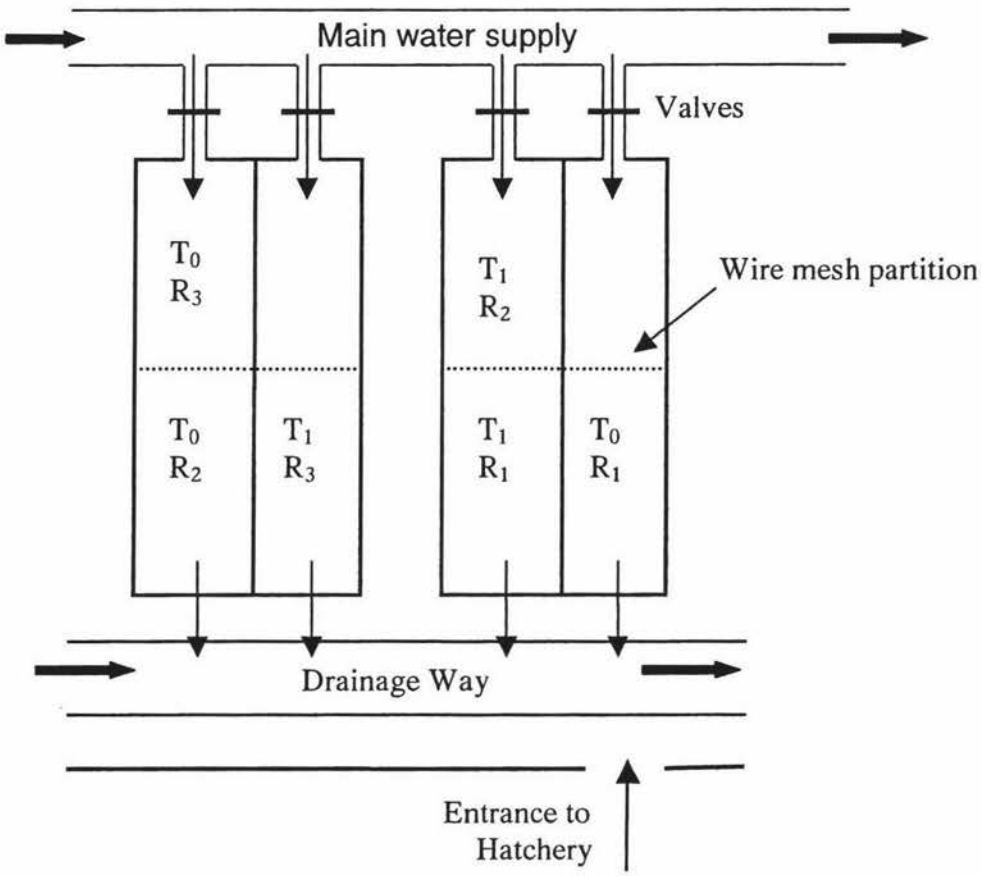


Figure 3. Experimental tank layout in the hatchery (T= Treatment, R=replicate).

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Feeding method

Weekly feed allowance was calculated as 3% of initial body weight of a treatment group, and the allowance divided into two equal parts (morning and afternoon). Before each feeding, tanks were cleaned by siphoning the bottom of the tank. At 0830h and 1600h the fish were hand fed to satiation. Fish were fed until they stopped actively seeking the feed which took about 10 minutes. Refused feed was collected using a gauze cloth attached to the outlet of a manual siphon. The feed refusals were dried (75 ^{0}C for 24 hours) and weighed. Fresh feed samples were also dried and the dry weight calculated. From these data the weight of the uneaten feed was calculated. Weekly feed intakes were calculated per sub-tank as follows:

Weekly feed intake (g) = feed allowance (g) - refused feed (g)

Measurements

Individual body weights were determined on day 0 of the trial (after a 28-day acclimation period) and on day 30 of the trial. On day 0 of the trial three fish per sub-tank (i.e. a total of 18 fish) were randomly selected and used for the determination of organ weights, carcass recovery and carcass composition (baseline data). On day 30 of the trial the remaining fish were euthanased, weighed and fork length measured. All the fish were exsanguinated and sex was determined. The following tissues/ organs were removed from the fish and individually weighed: liver, kidney, heart, gonads, gastrointestinal tract and eviscerated carcass. The carcasses were frozen (-20 ^{0}C) for further analysis.

Carcass analysis

Carcasses were bulked together within sub-tank for both before and after the treatment period (i.e. for each sub-tank there was one baseline sample of 3 fish and one after-treatment sample of 3 fish). Frozen carcasses were first cut into small pieces using a knife and ground twice in a Hobard meat grinder (Model E232) using a 10-mm die followed by twice through a 6-mm die to a homogeneous mass. The ground material was then freeze-dried, ground and analysed for dry matter (DM), organic matter (OM), assh, nitrogen and fat. Dry matter was determined by oven drying at 105°C while ash was determined by heating the sample to 550 °C for 16 h. Nitrogen was determined by the Kjeldahl technique (AOAC, 1994) and lipid analysed by soxhlet extraction (with anhydrous diethyl ether) (AOAC, 1994).

Calculations

From the data, the following calculations were made:

Body content of protein was calculated as nitrogen % x 6.25.

Feed conversion ratio ($g.g^{-1}$) = feed consumed (g) / body weight gain (g)

Carcass recovery (%) = (carcass weight (g)/live weight (g) x 100

Hepato-somatic index (HSI) = (liver weight (g)/live weight (g) x 100

Viscero-somatic index (VSI) = (viscera weight* (g)/live weight (g) x 100

* Visceera= liver, kidney, heart, gastro intestinal trtact and gonands

As the fish were not individually identified the individual body weight gains could not be calculated so the average fish weight per sub-tank was used in the analyses. The control and treatment groups were significantly different in live weight at the beginning of the treatment period due to the variability in weight gained during the acclimation period. For this reason weight gain was expressed as a percentage of initial body weight.

Statistical analysis

All the data were subjected to Bartlett's and Shapiro-Wilk tests for homogeneity of variance and normality of the distribution, respectively. All the ratio and percentage data were subjected to Arcsin transformation prior to the statistical analysis.

Statistical analyses of data were performed using general lenear GLM procedures of SAS (1996). The growth performance data were subjected to covariate analysis. Fish weights of the each sub-tank at the initial sampling were used as the covariate of the final fish weights of respective sub-tanks. Organ weights and carcass recovery at initial sampling (3 fish per replicate) and final sampling (15 fish per replicate) were subjected to two separate GLM procedure analyses. Feed intake data of four weeks were analysed in 2*4 factorial design. Carcass composition data (three trout per sub-tank) were analyzed on an individual basis. Differences were considered significant at $P < 0.05$, although probability values up to $P < 0.10$ are shown in the text if the data suggest a trend.

Power analysis

A power analysis was performed to confirm whether the experiment as it was actually carried out was statistically powerful enough to detect differences between treatments. Ideally we had hoped to use four replicates with 22 fish per replicate (a total of 176 fish). However only 144 fish of suitable size were available for the study. Because of the death of 30 fish at the end of the acclimatisation period, three replicates of 18 fish were actually used. Considerable variation in growth between fish as well as between different tanks was observed in the experiment. To make the most realistic evaluation, values measured in the experiment (standard error in growth rate) have been used for the power analysis, instead of relying on previously reported values.

The weight increase in treated and control fish were 28.0% and 26.9%, respectively. For both original and actually executed protocol, the level of the smallest difference in growth rate which could be significant between treatments was far higher than the actual growth rate difference between treatment and control fish. Therefore, the changes in protocol (reduction of replicate number from 4 to 3 and number of fish from 22 to 18) could not have affected the conclusions from the analyses of the results.

4. RESULTS

Mortality

Except for the problem with the water supply during the acclimatisation, no fish died during the trial. All fish were visibly healthy.

Growth data

The influence of dietary salbutamol on performance parameters is summarised in Table 3. Dietary inclusion of salbutamol (20ppm) had no effect on growth rate (as a % of initial body weight). The control and treatment groups were significantly different in live weight at the beginning of the treatment period, due to a large between-tank variation in weight gained during acclimatisation period. The percentage increase in live weight of the control group over the trial period was 26.9 ± 2.4 % and for the salbutamol treated group, 28.0 ± 0.5 %.

Inclusion of salbutamol had no effect on the final weights of treated fish. Both control and treated fish exhibited a significant weight gain ($P<0.01$) over the 28-day treatment period. The standard errors of the final weight of both control and treated fish were higher than those values at the beginning of the experiment.

In contrast to the live weight, carcass weight at the end of the trial was significantly higher ($p<0.01$) in treated fish compared with control fish.

Table 3. Influence of salbutamol (20ppm) on body weight gain, feed intake, feed conversion ratio (FCR) and carcass recovery (mean ± SEM)

Treatment Group	Day of Trial	Body weight (g) [1]	Carcass weight (g) [2]	Weight gain (%)[1]	Feed intake (% of LW) [1]	FCR (g/g) [1]
Control	0	318.6 ± 5.0	302.8 ± 16.2			
	30	404.4 ± 8.0	342.3 ± 12.0	26.9 ± 2.4	1.5 ± 0.3	1.70 ± 0.5
20 ppm Salbutamol	0	329.3 ± 5.3	297.9 ± 11.8			
	30	421.6 ± 8.2	373.3 ± 11.5	28.0 ± 0.5	1.7 ± 0.3	1.72 ± 0.12
Probability, P =						
Salbutamol	0	*	ns	ns	ns	ns
	30	ns	*	ns	ns	ns

[1] Average of three sub-tanks of fifteen fish each.

[2] Average of three sub-tanks of three fish each (day 0) or fifteen fish each (day 30)

* $P<0.05$

ns not significant

Feed conversion ratio

Feed intake as % of body weight increased over the 28-day feeding period from 1.49 % of the initial live weight at the end of the first week to 1.85 % at the end of the fourth week (Figure 4). The intake during third and fourth weeks was significantly ($p<.05$) higher than during the first and second weeks. However, feed intake was not significantly different between control and treated fish except during the third week in which treated fish had significantly higher ($P<0.05$) feed intake. When averaged over four weeks, treated fish had higher feed intake than control fish (1.69 % initial LW vs 1.54%) although this difference was not significant ($P=0.10$). The feed intake in the morning and afternoon was also not significantly different between treatment groups (data are not shown).

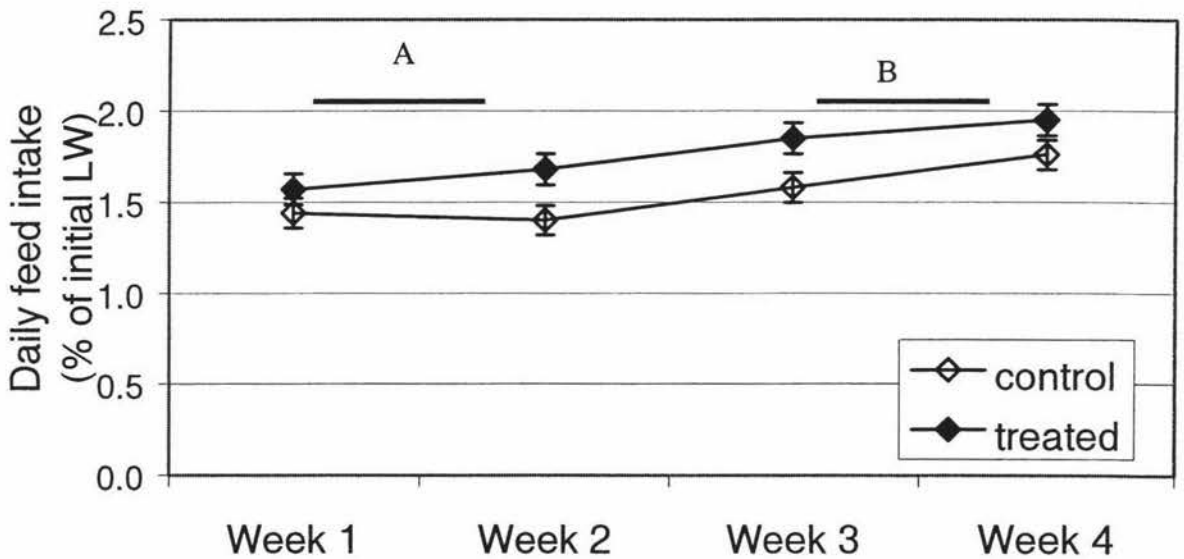


Figure 4. Daily feed intake (as a % of initial LW; mean± SE) in Rainbow trout fed either control or control + salbutamol (20 ppm) for four weeks. Each error bar represents three replicates per treatment with 15 fish per replicate. Overall feed intake of fish in weeks 1 and 2 (A) was significantly ($p<0.05$) different from feed intake in fish in weeks 3 and 4 (B).

Feed conversion ratio (FCR); feed/gain was not significantly different between treated and control fish ($P=0.55$; Table 1). However, when the FCR was analysed on a tank basis, the

fish in the tank near the entrance (T0R1) had a significantly lower ($p<0.05$) FCR than fish in the other three tanks.

Carcass recovery and composition

The influence of Salbutamol on carcass recovery and composition data are summarised in Table 4. Salbutamol (20ppm) had a statistically significant ($p<0.01$) effect on carcass recovery percentage. Dry matter, organic matter and fat content of the whole carcass were increased significantly ($P<0.01$) over four weeks of feeding. Moisture, ash and crude protein percentages of the whole carcasses were significantly ($p<0.01$) reduced during the trial. The dry matter content of treated fish at the end of the experiment tended to be higher compared to control fish ($P=0.08$). Carcass crude protein content was not significnatly different between treated and control fish either on fresh weight or dry matter basis. The fat percentage was also not significantly different between treatment groups irrespective of the basis of expression.

Organ weights

The influence of salbutamol on organ weights is presented in Tables 5 and 6. Fish fed the diet containing salbutamol had heavier kidneys ($P<0.01$). Salbutamol did not affect the sexual maturation as indicated by the gonadal weight of control and treated fish. In both control and treated fish, total visceral organ weight increased over four weeks. Control and treated fish had statistically similar liver, heart and visceral organ weights and HSI and VSI.

TABLE 4 Influence of Salbutamol (20ppm) on the carcass recovery and composition parameters of Rainbow trout. Each figure represents mean ± SE for 3 fish from each tank at the beginning (day 0) and three fish from each tank at the end (day 30) of the experiment.

	Day of Trial	Carcass recovery	Carcass moisture	Carcass protein	Carcass fat	Carcass ash	Carcass protein	Carcass fat
					(%)		(%)	
				---------- Fresh weight ----------			------Dry matter basis --------	
Control	0	87.4 ± 1.6	74.72 ± 0.56	18.3 ± 0.3	4.5 ± 0.5	10.13 ± 0.3	72.3 ± 0.6	17.8 ± 1.5
	30	87.5 ± 0.2	72.58 ± 0.54	18.3 ± 0.1	6.6 ± 0.5	8.82 ± 0.07	66.9 ± 1.1	23.9 ± 0.2
20ppm Salbutamol	0	86.4 ± 1.4	73.60 ± 0.44	18.8 ± 0.2	5.0 ± 0.2	9.93 ± 0.07	71.4 ± 0.5	19.0 ± 0.4
	30	90.6 ± 0.3	71.86 ± 0.28	18.7 ± 0.3	6.8 ± 0.3	8.96 ± 0.12	66.4 ± 0.9	24.1 ± 0.9
Probability1, P =								
Salbutamol	0	ns	ns	ns	ns	ns	ns	ns
	30	**	ns	ns	ns	ns	ns	ns

* Significantly different at $p<0.01$

TABLE 5. Influence of Salbutamol (20ppm) on the tissue and organ weights of Rainbow trout (mean ± SE). Each figure represents the mean ± SE of 3 or 15 fish at the beginning (day 0) and the end (day 30) of the experiment, respectively.

	Day of Trial1	Liver	Heart	Kidneys	Gonads	Viscera
				(g)		
Control	0	5.22 ± 1.50	0.40 ± 0.07	2.88 ± 0.22	ND	32.62 ± 3.35
	30	5.97 ± 1.18	0.58 ± 0.01	2.29 ± 0.1	1.09 ± 0.45	37.3 ± 1.16
20ppm Salbutamol	0	4.61 ± 0.82	0.41 ± 0.51	3.11 ± 0.31	ND	25.26 ± 2.35
	30	6.21 ± 1.18	0.57 ± 0.57	3.02 ± 0.13	0.95 ± 0.30	39.37 ± 1.28
Probability1, P =						
Salbutamol	0	ns	ns	ns	ns	ns
	30	ns	ns	**	ns	ns

1 Day 0 = average of three sub-tanks of 3 fish; Day 30 = average of three sub-tanks of 15 fish.

* Significantly different at $p<0.01$

ND not detected (too small to be accurately removed and weighed).

Table 6. Influence of salbutamol (20ppm) on the hepato-somatic index (HSI) and viscero-somatic index (VSI) in Rainbow trout. Each figure represents mean ± SE of 3 and 15 fish at the beginning (Day 0) and end of the trial (Day 30), respectively.

	Day of Trial	HSI	VSI
		(%)	
Control	0	1.45 ± 0.48	9.27 ± 0.65
	30	1.46 ±0.03	9.2 ± 0.16
20ppm Salbutamol	0	1.31 ± 0.43	7.24 ± 0.52
	30	1.50 ± 0.04	9.32 ± 0.17
Level of significance	0	ns	*
	30	ns	ns

* Significantly different $P<0.05$

ns- Not significant

5. DISCUSSION

Growth parameters

Although the fish used for the experiment were wild stock and unselected for superior growth, the growth rate was comparable with farm raised rainbow trout. The 26.9 average weight gain percentage of the control group is higher than the growth rate increment of 23.0% reported by Vandenberg and Moccia (1998) for farm raised rainbow trout of same weight and at the same temperature. Given that the experiment was conducted during winter, the growth of the fish is high. The higher growth rate may be due to the higher nutritive value, particularly higher protein content of the diet used in the present study.

The results of the present study indicate that dietary administration of 20 ppm salbutamol had no effect on the growth of rainbow trout. Since β agonists mainly function as repartitioning agents, it is not surprising that the growth rate was not influenced. The effect of β agonists on the growth rate of terrestrial animals has been reported to be mixed (Beerman 1993). In general, the effects of β agonists on the growth performances in fish are not as large as for terrestrial livestock. Mustin and Lovell (1993) reported a 17% higher weight gain in channel catfish fed with diets containing ractopamine. The growth rate improvement reported by these workers was higher than the 8% growth rate improvement observed in pigs (Watkins *et al.*, 1990), and comparable with 18 % improvement in cattle and 14 % in turkeys (Anderson *et al.*, 1991). Later, Mustin and Lovell (1995) found that catfish weight gain was significantly higher only at satiation feeding at high protein level (36%), but not at low protein levels (24%) or with restricted ration. In contrast, Webster *et al.* (1995) found no effect of

L644, 969 on the weight gain of blue catfish. In fact they reported lower weight gains in L644, 969 fed fish. Recently, Vandenberg and Moccia (1998) found a significantly higher thermal growth coefficient in rainbow trout fed 10 ppm ractopamine only at the fourth week, but not at any other treatment time*ractopamine level combination.

The percentage weight gain over initial weight in the treatment group was marginally and non-significantly higher (28.0 % in treatment group vs 26.9 % in control group) than that of the control group. This may be due to significantly higher initial weight of the treatment group. Vandenberg and Moccia (1998) observed similar marginal (3.7 %) weight gain improvement after feeding 20 ppm ractopamine to rainbow trout for four weeks. Lower levels of salbutamol (2-8 ppm) given to pigs increased growth rate (Warris *et al.*, 1990b; Hansen *et al.*, 1997). On the other hand above 20 ppm ractopamine reduced the weight gain and feed efficiency in pigs (Watkins *et al.*, 1990). Therefore, it is important to study the effects of lower levels of salbutamol in trout.

Feed conversion efficiency

The overall feed intake as a percentage of initial body weight increased over the 28-day feeding period from 1.49% of the initial body weight at the end of the first week to 1.85% at the end of the fourth week. Feed intake we observed is comparable with normal adequate daily feed intake of rainbow trout reported in literature (Austreng *et al.*, 1987; Storebakken and Austreng, 1987; Storebakken *et al.*, 1991; Vandenberg and Moccia, 1998) and thus indicates the high palatability of the feed as well as the good health and condition of the fish (Peter 1979).

Reduced feed intake in terrestrial animals as a result of β agonists treatment has been reported by some authors (Ricks *et al.*, 1984; Jones *et al.*, 1985; Adeola *et al.*, 1990), whereas others (Dalrymple *et al.*, 1984a; Moser *et al.*, 1986; Anderson *et al.*, 1987; Veenhuizen *et al.*, 1987; Watkins *et al.*, 1990) have reported a less pronounced effect on feed intake in terrestrial species. Throughout the four week feeding trial, the treated trout had a higher feed intake compared to control fish and the intake during the third week was significantly higher than that of the control group. Since the intake is expressed as a percentage of initial live weight, this increment may reflect the increased actual intake due to increased fish weight of the treatment group. Webster *et al.* (1995) found no significant difference in feed intake between channel catfish fed with L644, 969 and control fish. Interestingly in the study of Vandenberg and Moccia (1998), rainbow trout given 10 ppm ractopamine for four weeks showed a significantly lower feed intake whereas at 20 ppm level, treated fish had significantly higher intake. It is likely that the effect of beta agonists on feed intake depends on the species, agonist used and the dose and the duration of treatment. Ricks *et al.* (1984) suggested that β agonists reduced the feed intake via the interactions with the appetite control centers in the central nervous system. Given that variability in the bioavailability of salbutamol in different species (Smith 1998), it can be speculated that the dose we used may not be effective in depressing appetite. Whatever the mechanism of action, results clearly demonstrate that salbutamol at 20 ppm in rainbow trout has no negative impact on feed intake.

The effect of dietary salbutamol on the feed conversion efficiency (FCE) (weight gain/feed intake) was also insignificant in the present study. These findings are in agreement with the results reported by Webster *et al.* (1995) for blue catfish given L644,969 and Mustin and Lovell (1995) for channel catfish given ractopamine. In contrast, Vandenberg and Moccia (1998) reported significantly higher feed conversion efficieny in rainbow trout fed with ractopamine at 5 ppm during 5-8 weeks and 10 ppm during the first 4 weeks. In both periods, increased efficiency was associated with both increased growth and reduced feed intake. Dietary salbutamol, on the other hand did not reduce the feed intake at any time in the present study. Since the weight difference between control and treated fish at the end of experiment was about 17g, it is unlikely that fed conversion efficiency would have been different at any time during the experiment.

The FCE of the fish of the present study is much lower than that reported (Storebakken and Austreng, 1987; Storebakken *et al.*, 1991; Vandenberg and Moccia, 1998) for farm raised rainbow trout. The protein efficiency ratio (PER) (=weight gain/weight of protein fed) of the rainbow trout of the present study is 1.77 and is comparable with the PER of 1.2-2.2 reported for farm raised rainbow trout fed commercial diets by Pfeffer (1982). At the higher end the better FCE may be due to the genetic superiority of the fish they used. Nonetheless, our findings demonstrate that genetic superiority in relation to growth of farm raised fish arises not from the rapid growth, nor from the feed intake but from the efficiency with which feed is converted.

Carcass Recovery

In contrast to other growth related parameters the carcass weight and carcass recovery was significantly higher ($P<0.01$) in treated fish. The average carcass recovery of the control group at the day 0 and at day 30 and treated group at day 0 was 86.1% and is similar to the value reported by Storebakken *et al.* (1991) for 391 g rainbow trout. The carcass recovery was increased by 3.9 % in salbutamol fed fish. In channel catfish, dietary ractopamine significantly reduced the carcass recovery by 1.3%. The increased carcass recovery may partly be attributed to the higher dry matter of the treated fish. Alternatively, it can be assumed that other organs not measured but which comprised the whole carcass such as gills, skin and eyes could be heavier in treated fish. There is, however, no evidence to suggest this this may be the case.

Effect on organ weight

The literature generally reports that the liver, kidney and intestinal weights are unaffected or sometimes reduced in animals given various dietary β agonists. In the present study the weights of the liver, heart, gonads and total viscera were not significantly affected by the dietary inclusion of salbutamol and agree with those reported by Webster *et al.* (1995). Both in Webster *et al.* (1995) and our experiment total viscera weights and liver weights were numerically higher in β agonist treated fish. Warris *et al.* (1990a) reported that dietary salbutamol reduced the liver weight in pigs as a result of glycogen mobilisation. In rainbow trout, liver glycogen mobilisation was not affected by the dietary ractopamine (Vandenberg and Moccia, 1998). Sota *et al.* (1995) found that liver, heart and lung weights were increased in pigs given 2 ppm salbutamol for 38 days. It is clear from our results that salbutamol at 20 ppm does not have any

effect on the liver, heart and total viscera weight or on VSI and HSI. Liver and heart weights were increased as trout weight increased. Body weight dependent increase VSI and HSI in rainbow trout has been reported by Storebbaken *et al.* (1991). Increased visceral weight (heart and liver) in both treatment and control groups probably represents normal allometric growth of the fish.

The VSI of the present study is similar to the VSI reported by Vendenberg and Moccia, (1998) but lower than the value of 11.5 % reported by Storebakken *et al.* (1991) for similar weight farm raised rainbow trout. The visceral weight of the fish in this study is also lower than those reported by Storebakken *et al.* (1991). Carcass fat content in the fish was higher in Strobakken and co-worker's study compared to the same size of fish in the present study. Although the weight of the visceral fat was not measured in the present study, it is reasonable to assume that visceral fat content of the fish in the present study was low compared to farm raised rainbow trout.

The gonadal weights were not affected by the dietary salbutamol. The weights of the gonads were low compared to similar sized trout reported by Storebakken *et al.* (1991), indicating that the fish in our study were sexually immature. The fish of the Lake Taupo population are sexually mature at 2.5 years, around 2.0 kg (Thorarensen *et al.*, 1996).

To our knowledge this is the first study in which the effect of dietary β agonist on kidney weight in fish has been measured. Interestingly, after 28-days on 20 ppm dietary salbutamol, the kidney weight was significantly higher. Similar effects of dietary salbutamol on kidney weight have been reported in pigs by Sota *et al.* (1995). Since

salbutamol is a selective β_2 agonist and vertebrate kidney has a predominance of β_1 receptors (Timmerman 1987), this observation is surprising and it is unlikely that it is due to a direct effect of salbutamol on the kidney. Liver, kidneys and intestine play an important role in the process of elimination of β agonists residues (Smith 1998). Increased heart rate has been observed in many species given dietary β agonists (Eismann *et al.*, 1988; Herbert *et al.*, 1985; Beermann *et al.*, 1986) and Mersmann (1989a,b) suggested that increased heart rate resulted in increased blood flow to many organs. Since trout miocardium is exclusively of a β_2 type organ (Ask *et al.*, 1980; Ask *et al.*, 1981; Gamperl *et al.*, 1994) it can be assumed that increased kidney weight observed in salbutamol fed fish may also have been due to increased blood flow to the kidneys. The reason why other organ weights were unaffected by increased blood flow is not clear.

Carcass composition.

It is well established that in terrestrial animals, dietary β agonists reduce the carcass fat content while increasing protein content. However, in the present study the dietary salbutamol at 20 ppm did not change the whole carcass protein and fat contents in rainbow trout. In both treatment and control groups carcass fat content increased while protein content decreased with the increasing fish weight. The whole carcass protein content of the fish in the present study is more or less similar to the values reported for similar size rainbow trout by Storebakken *et al.* (1991) and Vandenberg and Moccia, (1998). However, the carcass fat content of the fish in our study was remarkedly low compared to farm raised rainbow trout. The reduced fat level may be partly attributable

to the low level of dietary fat (6.5%) used in the present study compared to the 19% by Storebakken *et al.* (1991) and 12.5% by Vandenberg and Moccia (1998).

Mustin and Lovell (1993, 1995) and Webster *et al.*, (1995) reported significantly increased muscle protein and reduced fat content in catfish fed with ractopamine and L644, 969, respectively. But the effects of ractopamine in rainbow trout were not as pronounced as in catfish (Vandenberg and Moccia, 1998). When rainbow trout were fed with ractopamine, the carcass protein content increased significantly during the 5-8 trial weeks period (Vandenberg and Moccia, 1998). The fat content also increased during the same time period, though not significantly. It should be noted that in both Vandenberg and Moccia (1998) and in our study the whole carcass composition was analysed rather than fillet (muscle) composition. Therefore it may be worthwhile checking whether salbutamol affects the fillet muscle protein and fat content rather than the whole carcass composition.

In fish the sites of lipid storage are different from mammals and birds. Mesenteric fat, liver and dark muscles are the main storage sites for lipids in fish, compared to the subcutaneous depot particularly in mammals, and to a lesser extent in birds (Sheridan 1988,1994). In salmonids pyloric cecae also function as a major site of lipid storage. In standard carcass analytical procedures the pyloric cecae, mesenteries and liver are excluded. In fish, these organs may be more responsive to β agonist induced lipolysis. Therefore it is important to analyse the pyloric cecae, mesenteries and liver for fat separately, along with fillets and whole carcass.

The carcass ash content and moisture content were unaffected by the dietary inclusion of salbutamol. In contrast Webster *et al.* (1995) reported significantly higher fillet ash content in blue catfish fed with L644,969 while Mustin and Lovell (1993) reported significantly higher moisture content in ractopamine fed channel catfish.

Low responsiveness of fish to dietary β-adrenergic agonists

Small and non-significant responses in growth parameters of trout, indicate that salbutamol induced responses in fish are low compared to other terrestrial animals. On the other hand for a given β agonist, responses vary even within a species with experimental factors such as dose of the drug, age, sex and the genetic make up of the animals, the plane of nutrition and treatment time. The following discussion examines the possible explanations for the low responses in trout and whether experimental conditions employed may have affected the results and thus the conclusions of the present study.

Reeds and Mersmann (1991) suggested that 60% of the reduced fat deposition in β agonist treated animals is an indirect effect of the use of fat for energy production in place of amino acids which have been diverted to muscle protein synthesis. Energy requirement is increased due to increased maintenance requirement and reduced energetic efficiency of fat and protein metabolism. Consequently, more lipids are used for energy production and metabolism. The daily maintenance energy requirement of rainbow trout at 15 °C is about 61 KJ/kg body weight/day (Cho and Slinger, 1980) and is about 10-20 % that of homeotherms of the same body weight (Talbot 1993). On the other hand a large portion of dietary protein is used as an energy source in fish. Two important conclusions can be drawn from those observations. If the mechanism

proposed by Reeds and Mersmann (1991) exists in fish as well, they should show greater lipolysis in response to the dietary β agonists. Since maintenance energy requirement is low, the β agonist induced lipolytic response depends largely on the degree to which amino acids have been utilised for the protein synthesis. Reeds and Mersmann (1991) further suggested that reduced energetic efficiency of protein deposition compared to that of fat may also increase the energy requirement of β agonists fed animals and thus demands more lipids to be used for energy production. However, in fish the energy requirement per unit protein gain was 2-20 fold lower than in chickens, pigs and cattle (Smith *et al.*, 1978). Therefore, the magnitude of lipolytic response from this mechanism is low in fish compared to other terrestrial animals.

Development of fish musculature is different from that of mammals and birds. Three of these differences can directly influence the β agonist induced muscle protein deposition and, indirectly, the fat reduction. Muscle tissue forms a large part of the body mass of the fish relative to other vertebrates (Johnson 1982). In salmonids, muscle comprises 56-66% of the total body mass (Satchel 1991). This may possibly set a biological constraint to further increases in muscle mass and thus reduce the direct skeletal muscle response to β agonists. As a consequence, fat reduction will be affected as it is dependent on the level to which protein synthesis has increased. In contrast to mammals and birds where muscle hyperplasia is virtually absent in adults, in large fast growing fish species like rainbow trout, hyperplasia persists into adulthood. When it stops and further growth is only by hypertrophy of existing fibres, the fish have generally reached about 40% of their mature size (Koumans and Akster, 1995). In juvenile and adult fish every length class of a species has a characteristic pattern of fibre

diameter frequencies which is unaffected by even major differences in growth rate as caused by nutrition, temperature, experimental situations or even by injection of growth hormones. In terrestrial animals, β agonists cause skeletal muscle hypertrophy but not hyperplasia. The fish used in our study had an average weight of 324 g and represent 15 % of the mature weight suggesting that new muscle fibres are being generated. In our study and all the other fish β agonists studies, young growing fish have been used and may be the reason for less pronounced effects of β agonists on growth parameters. Other growth promotants such as steroids and iodine which have improved fish growth, particularly the muscle protein content have been studied in young fish where hyperplasia is still predominant over hypertrophy. Therefore, in order to confirm this hypothesis, it is important to undertake studies with mature fish where muscle hypertrophy is prominent. These fish however, are of limited interest to commercial production.

To meet the increased amino acid demand of skeletal muscles in response to β agonists and to carry away the free fatty acids a well developed vasculature and blood flow are essential. Beermann (1993) suggested that β agonists induced increased blood flow to the skeletal muscle and adipose tissue played an important role in increased muscle growth and reduced lipid levels in β agonists treated animals. In fish, the white muscle makes up a greater proportion of total musculature and is poorly vascularised and functions anaerobically (Barton 1996). Since a larger proportion of fish musculature is poorly vascularised it may also hinder the muscle response in fish to dietary β agonists.

In terrestrial animals the magnitude of response to dietary β agonists varies greatly with species. Any such comparison with fish should be done with care for two reasons. Only five such studies involving three fish species have been reported in the literature. The experimental conditions such as agonist used, dose, age of the fish, treatment time, nutrition and temperature vary greatly between experiments. Although the experimental conditions are different and comparison between species is somewhat simplistic, the general trend is that of the three fish species studied, channel catfish seem to be more responsive to dietary β agonists than rainbow trout. This generalisation derives from the comparison between the effects of ractopamine in rainbow trout with the effects of ractopamine and L644,969 in channel and blue catfish, respectively.

The reasons for these species differences are not clear. These differences may be attributed to the biological differences between these two species. From a receptor point of view, the species differences may be related to a number of factors such as the efficiency of receptor-effector coupling, the different efficiencies in intra-cellular transduction pathways and the affinity and the density of the receptors (Fabbri *et al.*, 1995). The magnitude of CA induced physiological responses is directly related to the number of receptors on the target cells (Reid *et al.*, 1991, 1993). For example, the β-adrenergic binding sites in trout hepatic membranes as measured by Fabbri *et al.* (1992) were one fifth of that in catfish as measured by Fabbri *et al.* (1995). If this trend is present in other tissues such as the skeletal muscle and adipose tissues of the rainbow trout, the low magnitude of responses reported in rainbow trout given dietary β agonists may be related to the relatively low numbers of binding sites in the former species. Therefore it is important to determine if the differences are related to the differences in

receptor numbers in two species. Alternatively differences may be due to different experimental conditions such as drug used, dosage, age of the animals used, treatment time and nutrition.

In case of salmonids including rainbow trout, the red muscle which makes up only 5-6% of total musculature (Satchel 1991) contains more fat compared to white muscle (Sheridan 1988). Red muscles are used for steady cruising and are primarily fuelled by lipids with inputs from amino acids and carbohydrates. If fat reduction is an indirect effect of the increased skeletal muscle accretion as proposed by Reeds and Mersmann (1991), relative distribution of these two types of muscles and to what degree they are used may be the reason for species differences found in response to dietary β agonists. For instance, if β agonists act more on red muscle compared to white muscles or the fish use them more, the fat reduction effect would be more pronounced. On the other hand, absolute number of β-receptors in white muscle should be higher since white muscle comprises a larger proportion of total musculature. Therefore, it is important to determine whether β agonists have differential effects on these two types of muscles.

Do the experimental factors have an effect on the results ?

The argument that responsiveness is inversely related to the genetic superiority and thus growth potential is rejected by our data. Genetically unselected fish which might be expected to have a greater potential to growth did not respond to the dietary salbutamol. However, their growth rate was comparable with farm raised rainbow trout. Therefore the absence of effect of dietary salbutamol cannot be related to the poor growth rate of the fish we used in the present study.

Providing adequate supplies of dietary amino acids and energy is prerequisite to optimize the growth and feed efficiency in normal management conditions and may be particularly important if protein deposition rate is augmented by β-adrenergic agonists. Feeding guides for rainbow trout, prepared by Cho (1990) estimated 12.7 Kcal of digestible energy /day for 320 g fish. Daily energy intake (=feed intake * energy concentration) indicates that our trout ingested 14.7 Kcal of digestible energy per day, exceeding the estimated requirements. Several authors have found that energetic efficiency of β agonist treated animals were reduced and the total energy expenditure increased (Reeds and Mersmann, 1991). Therefore, in order to meet the possible increment of energy requirement, the energetic value of the diet was slightly increased.

The dietary crude protein level recommended by NRC (1993) for rainbow trout is 38 %. The effects of dietary β agonists have found to be more pronounced in animals given higher protein levels than adequate or inadequate levels (see Webster *et al.*, 1995; Mustin and Lovell, 1995; Dunshea *et al.*, 1993). Fish depend largely on amino acids for energy production (Pfeffer 1982). These two reasons led us to use higher dietary protein level. Since both dietary protein and energy requirements have been adequately met, and neither carcass protein level, a prerequisite for carcass fat reduction nor the carcass fat responded to the dietary salbutamol, the lack of response to dietary salbutamol in rainbow trout cannot be related to nutritional factors of the present study.

Male:female ratio as determined at the end of the trial was close to 1. Thorarensen *et al.*, (1996) could not find significant sex difference in weight of the Lake Taupo rainbow

trout population. Therefore, although sexing was not performed at the beginning of the experiment, the initial weights of the male and female fish can be assumed to be equal. The interaction between the weight at the final sampling and the sex was also not significnat. Therefore, sex differences could not have been responsible for unresponsiveness to dietary salbutomol.

In many cases, the effects of dietary β agonists are more pronounced during first 4 weeks and diminished after prolonged administration (Mersmann 1998). Since the feeding period of the present study lasted for four weeks, the length of the treatment time was adequate enough to see the effects had they been there. The differences between growth and carcass parameters at the end of the experiment were small. It is unlikely that these parameters would have been significantly different during any time of the four weeks of feeding period or afterwards.

Dietary β agonists have been found to be more effective in mature finishing animals compared to young animals. However, the fish we used were not sexually mature and were 18 months old. These fish mature at around 2-2.5 years. As discussed earlier, these fish are still in the hyperplastic state of their muscular development. The reduced carcass fat content may reflect the immaturity of the fish. Therefore, investigation of the effects of salbutamol on more mature rainbow trout is warranted.

The bioavailability of a β agonist varies greatly between species and influences the effective dose of the drug in humans (Morgan 1990) as well as in other species (Mersmann 1995). In terrestrial animals the effective dose has been reported to be between 2.7 ppm (Hansen *et al.*, 1997; Oksbjerg *et al.*, 1994; Warris *et al.*, 1990b; Sota

et al., 1996) to 8 ppm (Cole *et al.*, 1987). The dose we used produced minimal effects and may not be the effective dose of salbutamol in fish. Further research are needed to determine an effective dose, if any.

The normal allometric patterns of growth from birth to maturity are characterised by decreasing rate of protein deposition and increasing rate of lipid accretion (Beermann 1993). The carcass composition of both treated and control fish showed no significant differences and reflects the normal allometric growth and body composition changes, confirming the dietary salbutamol at 20 ppm has no effect on growth and carcass composition in young rainbow trout.

The findings of the present study do not completely exclude the possible effects of salbutamol on growth and body composition of rainbow trout at different dose levels or at different development stages of fish. Both qualitative and quantitative effects of dietary β agonists on growth and carcass composition differ between species. And even within a species the effects have shown to be dependent on the dose of the drug, age of the animal, genetic make up, treatment duration and nutrition status (NRC 1994). Therefore, further research, using different doses and more mature fish is needed in order to confirm the findings of the present study.

6. Conclusions

- Inclusion of dietary salbutamol at the rate of 20 ppm has no adverse effect on health and mortality of the hatchery reared young rainbow trout.
- 20 ppm dietary salbutamol has no effect on the growth or feed intake.
- The feed conversion efficiency of wild rainbow trout was poor compared to literature vallues for farm raised rainbow trout, even under good feeding conditions.
- The liver and heart weights and HSI and VSI were not affected by dietary salbutamol.
- As an indirect effect, the kidney weight was increased by salbutamol.
- The carcass recovery was significantly increased by dietary salbutamol.
- Whole carcass dry matter, protein and fat contents were not altered by dietary salbutamol.
- The effects of dietary β_2 adrenergic agonist salbutamol at 20 ppm on growth and body composition were found to be minimal.
- Further research involving different doses and fish weights is needed in order to confirm the findings of the present study.

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8. Appendix 1

Standard operating procedure for mixing drugs/additives into diets

1. Accurately weigh the drug
2. Prepare a 50:50 mixture of ground corn and limestone (Filler).
3. Initially hand mix the drug with the filler at a pre-detrtemined ratio using the 'quartering ' technique. In this technique, the filler will be divided into four quarters. The drug will be first mixed with one portion. This will be then mixed with the next portion and so on. The resulting mix will be blended in a laboratory for four minutes ('drug/filler mix').
4. During feed mixing, the drug/filler mix will be first hand-mixed with vitamin-mineral premix using the 'quartering' technique described above. Thi will be then added to major ingredients and mixed in a Horbart mixer ('Diet').
5. The diet will be then pelleted and stored in containers until use.
6. Sampling: Take three random samples each from top, middle and bottom of the containers (Total of nine samples). At each sampl weiugh approximately 250 grams and grind the pellets in a laboratory grinder. From this, take a representative sample (of 50 grams) and store in a airtight container for laboratory analysis.