Chapter 1

LITERATURE REVIEW

1.1 Fat in the Chicken Meat Industry

1.1.1 Problems associated with excess fat

Chicken has enjoyed a reputation as a low fat, low cholesterol and highly nutritious meat. The poultry industry has increased dramatically since the 1940's due to the competitive price and to the wide variety of forms served to the consumer. Today chicken is the second most popular meat in Australia (Fairbrother, 1987).

People are now more sensitive to the potential health hazard associated with animal fat in their diet, especially after two reports which have linked excess intake of dietary lipid with an increased incidence of coronary heart disease in the human population (National Advisory Committee on Nutrition Education, 1983; Dept. of Health and Social Security, 1984). Due mainly to this public concern, considerable and increasing attention has been paid to the animal's carcass composition and all of the meat industries have come under pressure to reduce fat to acceptable levels since approximately 33% of the fat consumed in the United Kingdom is derived from meat (Jones, 1985), and meat and its products contribute 37% of the fat to the average diet in Australia (Fantini and MacDonald, 1987).

In the chicken meat industry excess fat in meat-type broilers is now considered to be the biggest problem. Carcass fat in broiler chickens is responsible for a considerable loss to

the poultry industry and to the consumer. The combined loss due to discarding of body or carcass fat has exceeded $250 million annually in the US (Bertram, 1986). Though fat is the most variable body component, it is often up to 20% of body weight (Leenstra, 1986) in the chicken. In modern commercial broiler stock, the abdominal fat pad can be 4% of body weight and 20% of total body lipid (Whitehead and Griffin, 1984). This implies that roughly 16,500 tons of abdominal fat were produced annually from 1981 to 1986 in Australia assuming an average slaughter body weight of 1.8 kg, which, at the current price, would represent millions of dollars of loss. This readily removed fat is a loss to both processor and consumer.

The abdominal fat pad lies between the abdominal muscles and the intestines and extends within the ischium to surround the cloaca and bursa of Fabricius. It sometimes also includes the fat surrounding the gizzard. This cavity part of body fat is the largest discrete fat depot and is more variable than in other sites.

For broiler chickens, feed is estimated to be 70% of the production costs and, since the amount of energy needed to deposit 1 gram fat or 1 gram of protein is about the same (Pullar and Webster, 1977) and since each gram of protein deposited is associated with three grams of water, a reduction in fat content should give an improvement in feed conversion efficiency and therefore benefit the industry.

1.1.2 The origin of the excess fat problem

It is a widely held view that the rapid growth of commercial broiler chickens is the consequence of over 30 generations of intense selection for increased body weight (McCarthy and Siegel, 1983). It is also suggested that the modern broiler is too fat because selection for increased body weight or growth rate has increased the birds' appetite resulting in an excess consumption of energy leading to excess fat deposition (Hays and McCarthy, 1976; Lin, 1981). Sutherland *et al.* (1973) reviewed research on mice into the consequences of selection for growth rate and concluded that selection for increased growth rate is mandatorily accompanied by increased food consumption.

Pym and Solvyns (1979) in a selection program for increased body weight gain (line

W), food consumption (line F), decreased food consumption (line E) and a random control (line C), found that line F had the highest amount of fat and that there was a discrepancy between line F and line W suggesting that selection for weight gain did not operate wholly on variation caused by difference in the rate of food consumption as suggested by Hays and McCarthy in 1976. The correlation between body weight and abdominal fat content is quite low (Nir, 1984; Cahaner, 1986). Fisher (1985) reviewed the changes in body weight and feed conversion ratio (FCR) and pointed out that the relatively small change in FCR which accompanied the large change in growth rate may be seen as indirect evidence of increased fat deposition due to the selection for increased growth rate. Direct evidence is equivocal as most of the information has been collected on strains which are considerably smaller than commercial birds. Selection for growth rate has sometimes been shown to reduce fat at a given liveweight as observed by Pym (1985a).

One factor that has led to some confusion about the consequences of past selection practices is the positive phenotypic correlation frequently observed between fatness and body weight (an average of 0.24 for total fat content and 0.28 for abdominal fat content), but these correlations must be interpreted with caution since they all measure the relationship between body weight and fatness at a given age and not between growth rate up to, and fatness at, a given body weight (Pym, 1987). Pym (1987) further explained that since fat forms an increasing proportion of the body as growth proceeds, this relationship at a given age will automatically be positive; but the correlation at a given body weight is considerably more important because the associated genetic correlation allows a prediction of change in fatness due to selection for growth rate.

In an earlier review, Pym (1979) suggested that the fat problem is not necessarily due solely to genetic changes. There are other contributing factors such as a greater awareness on the part of the consumer towards dietary fat, a greater proportion of chicken being sold as dissected pieces thus exposing the fat and there is little critical information to assess whether birds today at the same weight and grown on the same diet under the same conditions are any fatter than they were two decades ago. Some studies have shown that the increase in body fat is associated with an increase in dietary nutrient density (Farrell,

1974a), a decrease in dietary protein or lysine level (Kubena *et al.*, 1972), or an increase in the energy : protein ratio (Griffiths *et al.*, 1977). Ewart (1988) also pointed out that the current trend is to utilize diets with a high nutrient profile overall and in this situation even lines selected for low fat content will exhibit high levels of carcass fat.

Though it remains uncertain whether fatness is an inevitable consequence of the genetic improvement in growth rate, Fisher (1985) stated clearly in his review that 'the modern broiler chicken is fatter than its forebears at a given age simply because it grows faster, but is not fatter at a given weight or stage of maturity'.

1.2 Lipid Metabolism and its Control in Chickens

It has been shown that in spite of the similarities in the major pathways of fat metabolism and in the nature and composition of tissue lipids between mammals and birds, there are important differences in several respects in lipid metabolism in the bird.

1.2.1 Lipid digestion, absorption and transport

The processes of digestion and absorption in chickens are similar to those in mammals (Annison, 1971); but, in contrast to mammals where lipid is almost exclusively transported from the intestine as chylomicrons via the lymphatic system, in the chicken it has been shown that at least 90% of absorbed fat entered the portal system as very low density lipoprotein (VLDL). This occurs because the intestinal lymphatic system in the chicken is less well developed and the villi do not possess central lacteals (Noyan *et al.*, 1964).

1.2.2 Fat synthesis in liver

The studies of Goodridge and Ball (1966; 1967) showed that pigeon adipose tissue was much less active in *de novo* lipogenesis than that of the rat and that the liver was the main site of fatty acid synthesis in contrast to the dominant role of adipose tissue in mammalian lipogenesis. Using ^{14}C-labelled glucose and acetate, chicken adipose tissue was observed to incorporate both glucose and acetate into triglycerides, but neither substrate was an

important source of fatty acids which were largely derived from circulating triglycerides (O'Hea and Leveille, 1968). The rate of lipogenesis was much greater in chicken liver than in adipose tissue (Goodridge, 1968a and b). It was calculated that the lipogentic contribution of the liver was between 90 and 95% in the chicken (Goodridge, 1968c; O'Hea and Leveille, 1969). The liver of the embryonic chick has only slight lipogenic activity; but on hatching the diet changes from the high-fat diet of the embryo to a high-carbohydrate cereal mash or crumbles and hepatic lipogenesis develops rapidly thereafter (Goodridge, 1968a). The dominant role of the liver in lipogenesis is maintained throughout the life of the bird (Annison, 1971).

Factors which stimulate or inhibit the enzymes that are active in chicken lipid synthesis have been intensively studied in efforts to provide an enzymic basis for the regulation of lipogenesis (Annison, 1971). The low activity of ATP-citrate lyase in chicken adipose is in consistent with the minor role of this tissue in lipogenesis (Goodridge and Ball, 1966). The pentose phosphate pathway, which is an important source of NADPH in fatty acid biosynthesis in mammals, was found to be of little importance in providing reducing equivalents in chickens (O'Hea and Leveille, 1968; Pearce, 1980). The activity of malic enzyme in chicken liver was found to be much higher than that in adipose tissue implying that this enzyme may serve an important function in the production of NADPH for fatty acid synthesis in liver via the 'malate transhydrogenation cycle' (O'Hea and Leveille, 1968).

1.2.3 Fat storage in adipose tissue

Chicken adipose tissue has little capacity for fatty acid synthesis as stated previously, but it is able to take up fatty acids transported from the liver as lipoproteins (Annison, 1971). These circulatory lipoproteins have been separated into high-density (HDL), low-density (LDL) and very-low-density (VLDL) lipoprotein fractions and with VLDL being richest in lipid (Griffin *et al.*, 1982). In adipose tissue, the triglycerides of the lipoproteins are acted upon by the enzyme lipoprotein lipase and then the fatty acids are moved into the adipocytes for reconversion to triglycerides for storage (Cherry, 1987).

The accumulation of adipose tissue can result from the filling of existing fat cells (hypertrophy) or through the formation of new fat cells (hyperplasia) with the relative contribution of these two processes varying at different stages of development. Hood (1982) has shown in birds that fat cell numbers increase up to about 14 weeks of age and thereafter any increase in fat deposition is due to cell hypertrophy (Ballam and March, 1979). It would be generally expected that cellularity is of less importance in birds than in animals in which the adipose tissue is the site of lipogenesis (Fisher, 1985). This was confirmed by Hood and Pym (1982), who showed that the differences between the lines in fatness were due to adipose cell volume and not to cell number.

1.2.4 Fat mobilization and its control

Energy is stored in adipose tissue when food is readily available, and mobilized from adipose tissue when food sources are limited. Because of the sporadic energy intake, the periodicity of feeding and the time required for digestion and assimilation of ingested food, body fat is continuously being synthesized and degraded. Even in the animal in energy balance, a considerable fraction of the depot lipid is mobilized daily, to enter the blood stream and be delivered to the various organs. The regulation of lipolysis in fat cells of adipose tissue appears to be the key step in the utilization of stored energy during starvation and other conditions.

Fat cells contain adenylate cyclase activity that can be activated by all hormones that are capable of increasing lipolysis (Fain, 1980). The cascade of reactions stimulated by different hormone-receptor complexes is shown in Figure 1.1. Briefly, hormones react with their specific receptors on the adipose membrane. The formation of the hormone receptor complex leads to activation of the membrane bound adenylate cyclase system. The accumulation of cyclic AMP (cAMP) then activates fat cell protein kinase which phosphorylates inactive to active triglyceride lipase. The fatty acids liberated by lipolysis enter the circulation as non-esterified fatty acids (NEFA). Adipocytes also contain active cyclic nucleotide phosphodiesterase which hydrolyses cAMP to AMP.

The mechanisms by which 10 different kinds of lipolytic agents activate lipolysis have

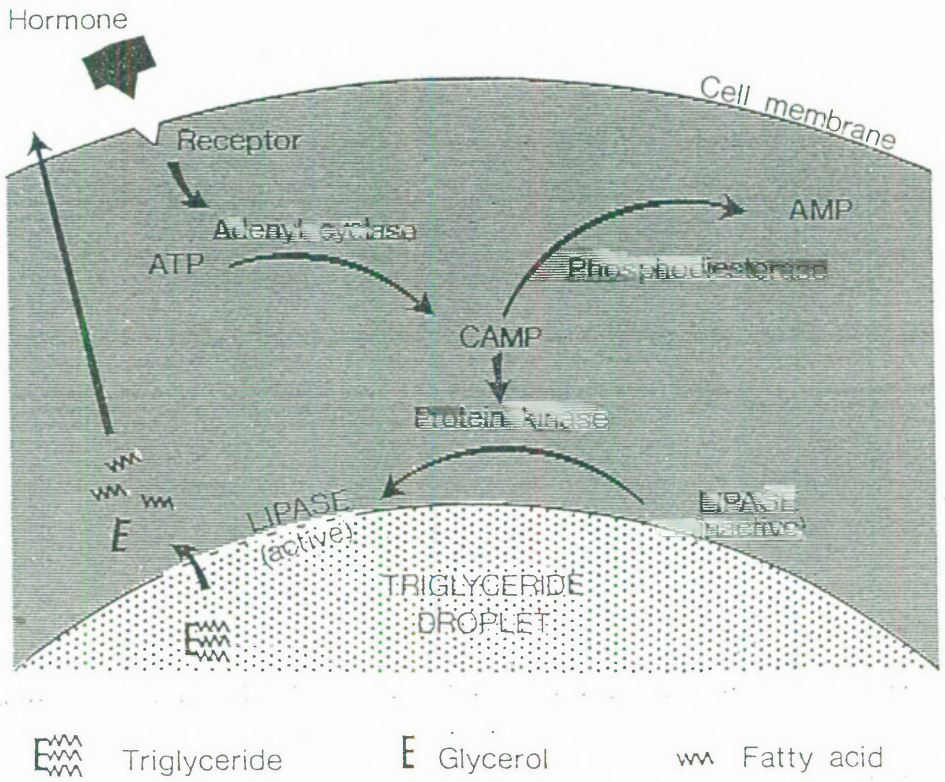

Figure 1.1: Representation of the regulation of hormone-sensitive lipase in the adipocyte (Evans, 1977)

been reviewed by Fain (1980), but most of the information and results are from studies on the rat. There is considerable species variation with respect to both the minimum dose and the actual hormones which elicit a lipolytic response from adipose tissue. Many fundamental studies have been undertaken to determine which hormones are responsible for lipolysis in birds and to compare the systems operating in mammals.

1.2.4.1 Endocrine pancreas

Following the classic observation on the dog after pancreatectomy, it was discovered (1893) that removal of the pancreas in ducks and geese failed to produce signs of *diabetes mellitus* (Annison, 1971). These observations, later confirmed by many workers, suggested that birds and mammals differed considerably in their regulation of carbohydrate and lipid metabolism and the normal plasma glucose concentration of fowls is 2–3 times higher than

mammals though there appears to be no major differences in the pathways by which glucose and NEFA are utilized by birds and mammals (Annison, 1971).

A. Insulin

Insulin is probably the most important physiological regulator of lipogenesis in mammals. A clear antilipolytic action of insulin on intact adipose tissue from fasted and refed rats and on isolated rat adipocytes has been noted (Fain *et al.*, 1966). In contrast, in the chicken, insulin is not antilipolytic (Heald *et al.*, 1965). In the rat, insulin stimulates lipogenesis in both the adipose tissue and liver whereas avian adipose tissue is poorly sensitive or insensitive to the lipogenic action of insulin, which was thought to be related to the fact that avian adipose tissue is not the main site of lipogenesis. The activities of liver lipogenic enzymes have been shown to be stimulated in the fowl but this was only seen with a relatively large dose of insulin (Goodridge, 1973). Insulin inhibited basal lipolysis and the increased lipolysis due to growth hormone plus glucocorticoid, ACTH, and theophylline in rats but not the lipolytic action of glucagon (Fain *et al.*, 1966). Inhibition studies of lipolysis in chickens *in vitro* have also shown that neither ox nor fowl insulin antagonized glucagon-stimulated lipolysis in fowl adipose tissue pieces or in the isolated fat cells (Goodridge, 1968a; Langslow and Hales, 1969). Insulin has actually been found to potentiate the lipolytic action of glucagon but in fowl high concentrations are required (Fain, 1980). Since insulin alone was not lipolytic, this finding was somewhat unexpected and it was suggested that insulin has some physiological significance in regulating lipolysis (Goodridge, 1968a).

The amino acid sequence of chicken insulin has been shown to vary in six positions from that of bovine hormone and this might be be a reason for the resistance and insensitivity of fowl to mammalian insulin (Langslow and Hales, 1971). Chicken insulin has also been found to have greater binding affinity and, consequently, high biological activity (Simon *et al.* 1974) and lower dissociation rate of the insulin receptor complex (Simon *et al.* 1977). However, the number of insulin binding sites is approximately five-fold lower in chicken cells than that in rat tissues and this phenomenon might be only partially compensated for by the higher activity of chicken insulin and explain the hyperglycemia in birds (Simon *et al.* 1977).

B. Glucagon

Glucagon has been shown to be much more effective than insulin in birds and is the main lipolytic hormone while catecholamines fulfil this role in mammals (Langslow and Hales, 1971; Langslow, 1972).

In their study on lipolysis in chicken adipose tissue, Langslow and Hales (1969) found marked difference in hormonal sensitivity between chicken and mammalian adipose tissue. Among the lipolytic agents which are active in mammals, only glucagon stimulated lipolysis at physiological levels and this *in vitro* result correlated well with the *in vivo* data of Heald *et al.* (1965) who found that the intravenous injection of glucagon caused a large and immediate rise in plasma NEFA concentration. This extreme sensitivity to glucagon in chicken adipose tissue has been observed in a wide variety of avian species (Pearce, 1980).

Chicken glucagon has the same amino acids sequence as the mammalian hormone except that serine replaces asparagine at position 28; and the pancreas of the birds was found to contain 10–20 times more glucagon than other vertebrates studied (Cramb and Langslow, 1984). It remains unclear whether these differences alter its biological potency.

C. Avian Pancreatic Polypeptide (APP)

This hormone, a single polypeptide chain of 36 amino acids, was first extracted as a contaminating protein during the isolation of chicken insulin (Kimmel *et al.*, 1968). Both *in vivo* (Hazelwood *et al.*, 1973) and *in vitro* (McCumbee and Hazelwood, 1978) studies have shown its antilipolytic effect, i.e., antagonistic effect on glucagon-stimulated lipolysis. APP injection significantly lowered circulating glucagon concentrations but had no effect on insulin levels (Cramb and Langslow, 1984). Whether APP has other effects in birds awaits further study.

1.2.4.2 Adrenocorticotrophic hormone (ACTH) and growth hormone (GH)

Early experiments on lipolysis showed that fowl adipose tissue responded either weakly or not at all to ACTH and significant effects needed high concentrations (Langslow and Hales, 1969). Using a lower level of porcine ACTH in chicken adipose tissue pieces, Carlson *et al.* (1964) observed no response.

Both *in vivo* and *in vitro* studies from hypophysectomized cockerels has shown impaired lipid mobilization and this, rather than increased lipid synthesis, was supposed to be involved in the development of obesity following hypophysectomy (Gibson and Nalbandov, 1966). Langslow and Hales (1969) found that crude chicken growth hormone was lipolytic alone, though this was only significant after 4 hours incubation, and the effect was enhanced by hydrocortisone sodium succinate.

1.2.4.3 Catecholamines

Fain (1980) summarized the effects of fast-acting lipolytic hormones and he concluded that there is no evidence that any of those hormones, except catecholamines, are physiological regulators of lipolysis. But avian species were singled out as unresponsive to catecholamines. This unresponsiveness was first reported by Carlson *et al.* (1964) with norepinephrine on 8-week old chickens and later confirmed by Langslow and Hales (1969) and Langslow (1972). Adrenalin and noradrenalin did stimulate lipolysis in birds but at relatively high concentrations for a significant effect and maximum stimulation was always much less than that for glucagon. They seem unlikely therefore to be physiologically significant. Gibson and Nalbandov (1966) argued that their results were different from those of Carlson *et al.* (1964) in that they showed epinephrine increased NEFA release by adipose tissue and reduced incorporation of glucose into lipids *in vitro* in adipose tissue from hypophysectomized cockerels. But they ignored the difference in concentrations that had been used. In the study of Carlson's group, only 0.5 and 0.8 μg/ml were used from which no increase in glycerol and NEFA was obtained. The experiment of Langslow and Hales (1969) showed that lipolysis was only stimulated at very high concentrations of catecholamines, 9 μg/ml to obtain a rise in glycerol release and 10 μg/ml, to obtain an increase in NEFA release. However, in the first test of Gibson and Nalbandov, 100 μg/ml was used and NEFA release was only influenced in adipose tissue from hypophysectomized birds and not in that from intact birds; glycerol release was not influenced. In the second experiment, the unusually high level, 250 μg/ml, gave inconsistent results and the incorporation of glucose into lipids was depressed by adrenalin.

Intravenous noradrenalin in 12–16 weeks old chickens, while producing hyperglycaemia, gave no increase in plasma NEFA concentration (Heald *et al.*, 1965). Intraperitoneal noradrenalin produced a gradual rise in plasma NEFA concentration up to 4 weeks of age but at 8 weeks of age the response was much reduced. The observed increased hyperglycaemic response to noradrenalin and the decreased adrenalin response with age (Freeman, 1969) led to the suggestion that age might play some part in these responses and there might be an interaction between the processes (Langslow and Hales, 1971).

1.2.4.4 Thyroid hormones

Since Rich *et al.* (1959) found that the administration of triiodothyronine (T_3) to humans resulted in an elevation in the plasma NEFA concentration, the regulation of adipose tissue metabolism by thyroid hormones has been clearly established (for review see Fain, 1980) though there are conflicting data with regard to the mechanisms involved.

Epinephrine-induced lipolysis was markedly depressed in fat pads from hypothyroid rats and the lipolytic action of epinephrine was greatly exaggerated in fat pads from hyperthyroid rats (Debons and Schwartz, 1961). The weight of adipose tissue from the hypothyroid rats was 57% greater and that of hyperthyroid was 46% less than that of controls. There was no change in the total number of fat cells indicating that it is changes in the size rather than the number of fat cells which were responsible for the alteration (Malbon *et al.*, 1978). The sensitivity of fat cells to all lipolytic agents is influenced and the ability of lipolytic agents to elevate cAMP is regulated by thyroid hormones (Fain, 1980).

In birds, the thyroid is necessary for normal growth and development, and growth is markedly retarded following thyroidectomy. Depressed thyroid activity as a consequence of goitrogen administration is reflected in reduced metabolic rate, increased fat deposition and growth depression (Ringer, 1976). The retarded growth brought about by goitrogen can be restored to normal by thyroid hormone injection (Singh *et al.*, 1968). In intact animals, low levels of thyroid hormone administration give little to moderate growth stimulation. Singh *et al.* (1968), using doses ranging from 1–6 μg/100 g body weight/day, indicated that thyroxine in small doses improved growth of chickens; when administered beyond

physiological doses (6 μg/100 g), however, it depressed growth rate.

Hypothyroidism in chickens results in increased fat deposition (Ringer, 1976) and thyroid hormones have been shown to be able to diminish fat deposition. May (1980) observed that feeding 3,5,3,-triiodothyronine at 0.25 ppm of the diet over a 54-day period reduced abdominal and carcass fat significantly in both male and female birds, water content was increased and carcass protein was not affected.

1.2.4.5 Adenosine

Fain (1973) found that the addition of purified adenosine deaminase from calf intestine to isolated rat adipocytes increased basal lipolysis and cAMP accumulation. The lipolytic sensitivity to catecholamines, but not the maximum response, was increased by adenosine deaminase. Subsequent findings led to the hypothesis that the endogenous rate of cAMP accumulation and lipolysis appears to be restrained by the presence of adenosine and this inhibitory constraint is released by the removal of adenosine (Fain, 1980). From these findings, Fain (1980) concluded that adenosine is a potent endogenous regulator of lipolysis and cAMP accumulation by rat adipocytes.

It would appear however that this mechanism is much less important in the chicken. In chicken fat cells there is no stimulation of cAMP accumulation by adenosine deaminase (Boyd *et al.*, 1975). Fain and Shepherd (1979) found that chicken adipocytes have an endogenous high level of adenosine deaminase and they explained that adenosine is unlikely a feedback regulator of adenylate cyclase released during incubation of fat cells with lipolytic hormones. Chicken fat cells appear to release less adenosine and deaminate more adenosine to inosine than do rat fat cells. The observation that chicken fat cells have little adenosine present in the incubation medium probably accounts for the lack of a response to adenosine deaminase and theophylline. The physiological effects of adenosine are mediated at an extracellular site that inhibits the ability of hormone-receptor complexes to activate both adenylate cyclase and lipolysis and these effects are blocked by methylxanthines (Fain and Shepherd, 1979).

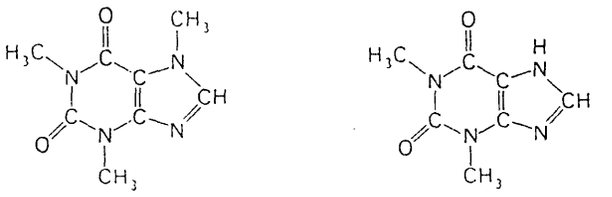

Figure 1.2: The structures of theophylline and caffeine

1.2.4.6 Methylxanthines

Theophylline and caffeine are methylxanthines and the main alkaloids in tea and coffee respectively. They are found to be lipolytic and potentiate the effects of catecholamines on cAMP in rat (Vaughan, 1961; Butcher *et al.*, 1965; Butcher *et al.*, 1968) and chicken (Langslow and Hales, 1969; Langslow, 1972) adipose tissue. Their structures are shown in Figure 1.2.

Within the cell of adipose tissue, cAMP is rapidly hydrolysed by phosphodiesterase which is highly sensitive to many pharmacological agents and the mostly widely used inhibitors are methylxanthines. Butcher and Sutherland (1962) observed that cAMP phosphodiesterase is blocked competitively by methylxanthines with theophylline being the most powerful.

Table 1.1 showed the effects of theophylline and epinephrine on cAMP accumulation in chicken fat cells (Langslow and Hales, 1969). *In vivo*, theophylline increased the plasma NEFA concentration in 1-day-old chickens but not in 19-day-old foetuses (Freeman and Manning, 1971). This was also reported by Langslow (1972) who found neither theophylline nor caffeine produced any significant lipolytic action in foetal adipocytes but both were potent stimulators of lipolysis in neonatal adiocytes.

Later findings, however, suggest that the lipolytic action of theophylline involves more

Table 1.1: Effects of theophylline on adrenalin stimulated lipolysis and dibutyryl 3',5'-cAMP and theophylline on lipolysis in chicken adipose cells (Langslow and Hales, 1969)

(a) Effects of theophylline

	Glycerol release[a]	NEFA produced during 1 hour incubation[a]
Controls	0.061±0.005	—
10 μg adrenalin/ml	0.264±0.010**	—
10 μg adrenalin/ml + 10 mM-theophylline	3.26±0.07**	—
10 mM-theophylline	2.57±0.08**	—

(b) Effects of dibutyryl 3',5'-cAMP and theophylline

Dibutyryl cAMP concentration mM	Theophylline concentration mM	Adipose tissue pieces glycerol release[a]	Isolated fat cells glycerol release[a]
0	0	0.41±0.05	0.061±0.005
100 μm	0	—	0.076±0.002
1	0	0.85±0.16**	0.454±0.030 ***
10	0	4.20±0.61***	2.88±0.17***
0	1	—	1.43±0.14***
0	10	1.44±0.31**	2.57±0.08***
1	10	1.48±0.37**	3.48±0.10***
10	10	4.71±0.91***	—

a. μmoles/100mg dry wt./hour

than an inhibition of cAMP phosphodiesterase. Experiments indicated that an inhibitor accumulates if a sufficient concentration of fat cells are incubated per ml and this inhibitor's effects are antagonized by methylxanthines (Fain, 1980). This inhibitor is probably adenosine (Schwabe *et al.*, 1973) and part of the effect of methylxanthines is due to antagonism of endogenous adenosine (Fain, 1980).

In isolated chicken adipocytes, theophylline did not appear to affect cAMP concentration in either the presence or absence of glucagon (Malgieri *et al.*, 1975). The ability of glucagon alone to maximally stimulate cAMP accumulation and the lack of a response to methylxanthines in isolated chicken adipocytes (Langslow, 1972) may be due to a low level of endogenous adenosine. An adenosine analogue resistant to deamination, N^6-phenylisopropyladenosine, was shown to lessen the rise in cAMP due to glucagon and this effect was reversed by methylxanthines. These observations were taken to indicate that methylxanthines have the expected effects on cAMP accumulation in chicken adipocytes if endogenous adenosine is present and the effect of methylxanthines on lipolysis and cAMP accumulation appear to be due to blockage of adenosine inhibition of adenylate cyclase activation rather than any effect on phosphodiesterase (Fain, 1980).

1.2.4.7 Non-esterified fatty acids (NEFA)

The fatty acids released to the medium during the activation of lipolysis by hormones appear to be the major feedback regulator of adenylate cyclase in rats. The most important factor was found by Fain and Shepherd (1975) to be the ratio of non-esterified fatty acid to albumin in the medium. Once the primary binding sites on medium albumin are saturated with NEFA, there is an inhibition of both adenylate cyclase and triglyceride lipase. The inhibition of cAMP accumulation by NEFA : albumin ratios above 2 was rapid in onset and with near maximal effects being seen after 30 seconds (Fain and Shepherd, 1975). Rodbell (1965) had previously demonstrated that lipolysis by isolated rat adipocytes in the presence of hormones virtually ceases when the ratio exceeds 3.

The behaviour of chicken adipocytes differs from that of the rat in that neither lipolysis nor cAMP accumulation is markedly inhibited by increased NEFA to albumin ratios as high

as 12 (Malgieri *et al.* 1975). Fain and Shepherd (1979) concluded from these observations that the prolonged elevation of cAMP seen when chicken fat cells are incubated with lipolytic agents (e.g. glucagon) results from an absence of feedback regulation of chicken fat cell adenylate cyclase by the NEFA to albumin ratio.

1.3 Factors Influencing Fat Deposition and Methods to Reduce Fat Content of Chickens

1.3.1 Reducing fatness by genetic means and consequences of selection

Irrespective of the contribution of genetic selection to the creation of the presently recognized problem in broilers, there is obviously scope for reducing fatness by genetic means and the sizeable correlated responses in body fat, in food consumption and in feed efficiency show that body composition can be manipulated either by direct or indirect means (Pym *et al.*, 1979). Estimates of heritability for abdominal fat are moderate to high which suggests that reducing body fat by selection with little or no effect on growth rate is feasible (Becker *et al.*, 1984; Cahaner, 1986).

1.3.1.1 Direct selection for decreased fat content

Though some breeders had become aware of excessive fat in their broilers prior to the 1980's, approaches based on selection were hampered by the lack of a simple, convenient, nondestructive, economic and effective method of measuring fatness in the live birds. The advantages and limitations of the few indirect measurement techniques available, such as tritiated water, ultrasonic and caliper fat measurements, were discussed by Pym (1979). So far two techniques, the use of abdominal calipers and the measurement of plasma very low density lipoprotein (VLDL), have shown good prediction of fatness in the live birds.

The size of abdominal fat pad generally determines whether the carcass is regarded as acceptable or not. As shown in Figure 1.3, there appears to be reasonable agreement between abdominal and total body fat (Hood, 1982; Whitehead and Griffin, 1984). Pym

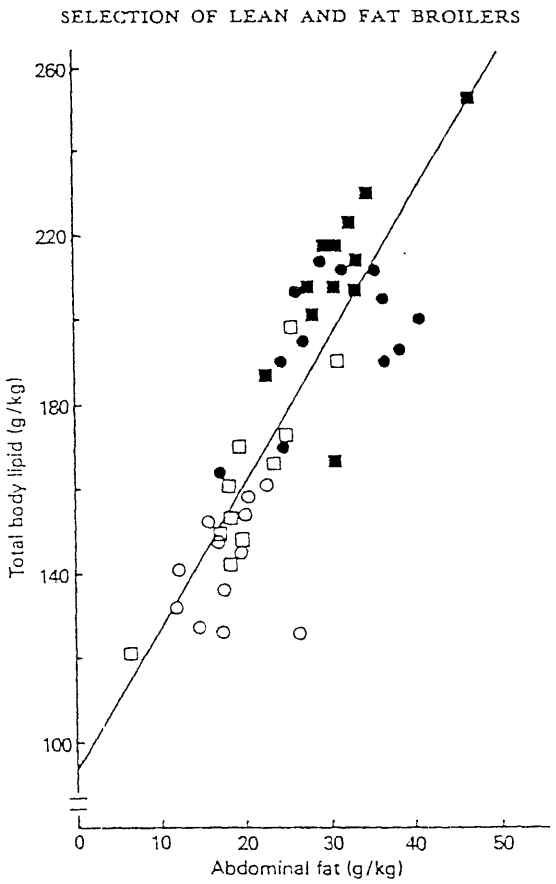

Figure 1.3: Relationship between abdominal fat and total body lipid in broilers at the third generation of selection (Whitehead and Griffin, 1984)

(1979) summarized some of the published results and showed that the correlation between abdominal and total fat was within the range of 0.40 and 0.88. It has been suggested that selection for abdominal fat should reduce fat in other locations without changing the fat-free weight (Pym, 1979; Becker *et al.*, 1979). The abdominal caliper has been developed, which measures the relative thickness of the fat pad between the inside of the cloaca and the outside of the body wall. Correlations as high as 0.8 between this measurement and abdominal fat have been reported (Pym and Thompson, 1980).

Another very useful technique is the measurement of plasma VLDL. The correlation between plasma VLDL and total carcass fat may be as high as 0.70 when a low fat diet is used and suitable precautions are taken to exclude the effects of starvation on plasma VLDL. A simplified assay for VLDL has been described for practical application of this technique (Whitehead and Griffin, 1982). Grunder and Chamber (1988) reported that indirect selection against abdominal fat using VLDL measurement was at least as effective as direct selection.

Some experiments have shown the effectiveness of selection against body or abdominal fat levels. LeClercq (1984) found that abdominal fatness in his high-fat selection line was four times higher than that in his low-fat selection line after 7 generations of sibling selection on the basis of the direct measurement of abdominal fat content. A two-fold difference between fat and lean lines after only two generations of selection for abdominal fatness was found when individuals were slaughtered for assessment of degree of abdominal fatness (Cahaner *et al.*, 1986). Figure 1.4 shows results from the study of Whitehead and Griffin (1984) and it can be seen clearly that after only three generations of selection on the basis of high or low plasma VLDL for low plasma VLDL, a substantial decrease in abdominal fat by 49% and total body lipid by 34% had resulted. There were no differences between the lines in body weight at 7 weeks. This, according to the authors, may simply be due to the low correlation between body weight and fatness. Body protein content, efficiency of feed utilization (FCR) and dietary protein in lean line were also improved. This correlated response of FCR to the selection for low body fat is consistent with observations by Whitehead *et al.* (1984), who showed that plasma VLDL concentration was significantly correlated with

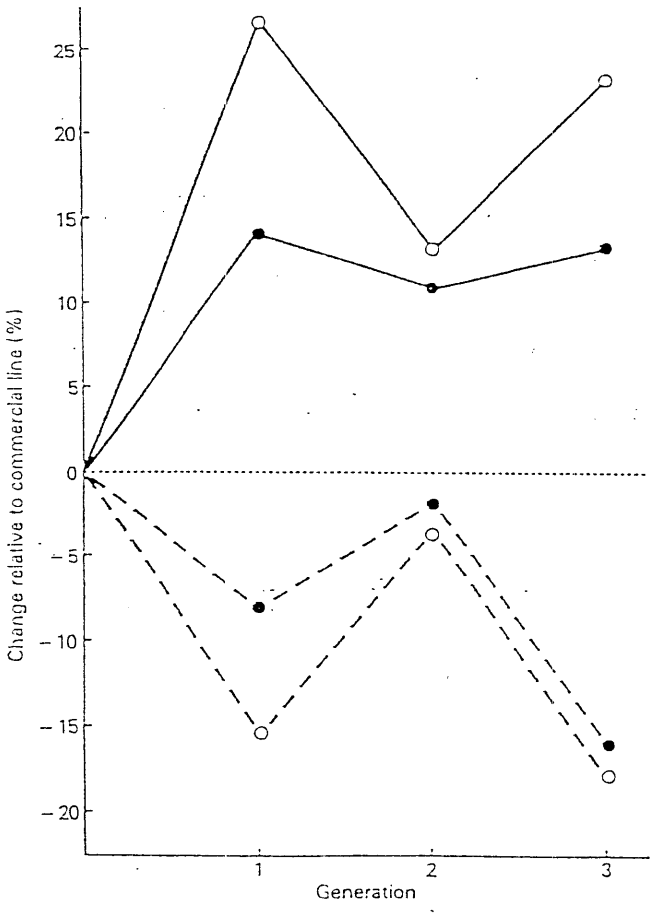

Figure 1.4: Change in the proportion of abdominal fat (o) and total body lipid (•) in fat (—) and lean (- -) lines relative to the commercial line (Whitehead and Griffin, 1984)

FCR as well as with body fat content, and agrees also with the results of Touchburn *et al.* (1981). Their further selection over the fourth generation resulted in continued divergence in the selection trait and the correlated response of total body lipid and protein contents and the efficiency of conversion of food and dietary protein.

Using abdominal calipers, Pym (1985b) made a comparison of a line selected for increased lean tissue growth rate with a similarly constituted line selected for growth rate alone. After five generations of selection, the lean tissue growth rate line resulted in similar responses in growth rate to the line selected for growth rate alone, but the latter line had 33% more fat than the former line. Despite the difference in fatness, there was no difference in FCR from 4-8 weeks of age in these two lines which was inconsistent with the result of Whitehead and Griffin (1984) mentioned previously. This was unexpected since the decreased fat deposition with its high energetic cost, and greater lean tissue deposition should lead to an improvement in FCR.

In none of the above four studies was there any difference in growth rate between high- and low-fat birds but significant divergent responses in fatness were obtained. Pym's study (1985b) has shown that, by combining selection for the two traits, i.e., increased growth rate and reduced abdominal fat, it is possible to obtain substantial improvement in lean tissue growth rate compared to selection for growth rate alone.

1.3.1.2 Indirect selection for decreased fat content

Since fat is deposited with a much greater energetic efficiency than protein (75% compared with 44% in rats, Pullar and Webster, 1977; 89% compared with 65% in chickens, Pym and Farrell, 1977), it is energetically more efficient to deposit fat. However, because of the higher energy density of fat compared to protein (39 compared with 23 kJ/g) on a dry weight basis, the two are deposited with roughly identical energy cost (53 kJ ME/g in rats, Pullar and Webster, 1977). And since protein is associated with about 70% water in muscle tissue and fat with only about 10% water in adipose tissue, theoretically it should cost three times as much energetically to deposit the same weight of adipose tissue as muscle (Pym, 1985a). Given that adipose tissue in birds is energetically dense, it would seem axiomatic

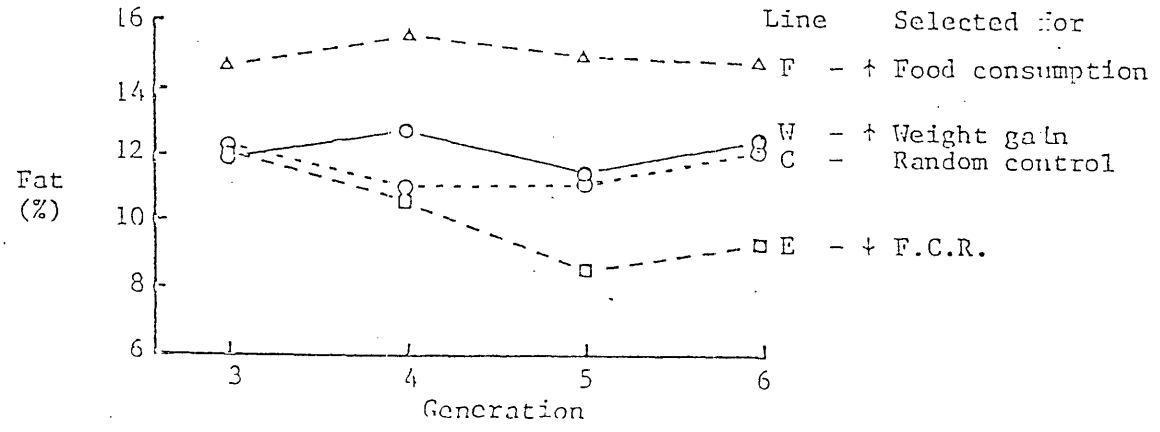

Figure 1.5: Fat expressed as a percentage of whole body weight in female chickens from four lines (Pym, 1979)

that selection for improved feed efficiency would result in a reduction in body fat and an elevation in body moisture (Pym, 1985a). Indeed, as early as 1935 Brody predicted that inefficient strains would store less protein and more fat than strains with higher efficiency. Though some experiments with rats and mice showed different results and the correlated response in fatness seemed to plateau after a few generations of selection (Pym and Solvyns, 1979), most of the related studies have shown that selection for improved FCR results in a decrease in fatness (Thomas *et al.*, 1958; Washburn *et al.*, 1975; Pym and Solvyns, 1979; Chambers *et al.*, 1983). Figure 1.5 shows results from a study of Pym (1979) on related changes in body composition in a hatch of birds from the four lines selected for increased food consumption, increased body weight gain, decreased FCR and random control in the 9th generation. In both sexes, selection for decreased FCR resulted in a reduction in the proportion of body fat.

There is ample scope for genetic improvement in body composition, particularly in lean tissue growth in commercial broiler chickens. Selection programmes which optimize economic response with respect to growth rate and feed efficiency should, according to the results of the previous studies, lead to a reduction in total body fat.

1.3.1.3 Consequences of selection: energy and nitrogen metabolism of genetically fat and lean chickens

Due mainly to the fat problem in the chicken, commercial breeding research programs now include leanness or FCR as selection parameters. Consequently, broiler carcass will become progressively leaner and there will be some modification of the metabolism and nutritional requirements of these birds. This area has been reviewed recently by LeClercq (1985), MacLeod and Geraert (1987) and LeClercq (1988).

A. Energy metabolism

Using their own selected fat (F) and lean (L) lines, LeClercq and Saadoun (1982a) measured metabolizable energy (ME) contents of diets in two experiments and found no difference between the two lines (12.4 *vs* 12.3 MJ/kg in the first experiment and 13.1 *vs* 13.2 MJ/kg in the second experiment). This was in agreement with the result of Sorensen *et al.* (1983) with a fat line and a control line but was different from that of Pym *et al.* (1984) who found differences in metabolisability of dietary energy after ten generations of selection for increased weight (line W), increased feed consumption (line F), increased conversion of food to gain (line E) or random (controls, line C) with line E being the leanest and line F being the fattest (73% for W, 63% for F, 76% for E and 73% for C).

LeClercq and Saadoun (1982b) reported that the maintenance requirement of line F was always superior to that of L chickens (450 *vs* 373 in experiment 1 and 456 *vs* 440 kJ ME/kg in experiment 2), but this difference was not significant. Efficiency of fat and lean deposition was about 2.5 kJ for 1 kJ of lean material and 1.1 kJ for 1 kJ of fat. However, by selecting indirectly for leanness and using FCR as the selection parameter, Pym *et al.* (1984) observed a significant difference in maintenance energy at the 10th generation between the lines selected: 671 (W), 866 (F), 701 (E) and 742 (C) MJ/W/d. They also found an improvement in efficiency of food utilisation in line E. They attributed this to an improvement in metabolisability of dietary energy, a decrease in maintenance energy requirement, an increase in net availability of ME (0.68, 0.76, 0.85 and 0.73 respectively) and a reduction in fat content of the tissue deposited in this line. Similar results were also

reported in the study of genetically lean and obese mice (Miller *et al.*, 1979) in which the lean mice were more efficient than obese mice in maintaining energy balance when food intake was decreased below, or increased above maintenance.

Another aspect of energy metabolism concerns the relationship between growth rate or fat content and energy concentration of the diet. Touchburn *et al.* (1981) reported a significant effect of ME concentration on growth rate, feed conversion and abdominal fat proportion when three diets with different ME levels (11.3, 13.0 and 14.6 MJ/kg) were compared. No interaction was found between lines and ME content for growth rate and feed conversion, but genetically fat broilers exhibited a more rapid rate of fat deposition than lean birds when the dietary energy intake increased.

B. Nitrogen metabolism

It has been shown that gross efficiency of protein retention was increased by selection for leanness in all the French (LeClercq, 1983), British (Whitehead and Griffith, 1986) and Australian (Pym *et al.* 1984) experiments.

Pym *et al.* (1984) found the line selected for increased food consumption ate 40% more food, produced 30% more heat and retained 70% more energy and 30% more nitrogen than the line selected for improved feed conversion efficiency. Sorensen *et al.* (1980) showed that selecting a broiler line on growth rate with low-protein diets induced an increasing tendency for fattening as compared to the control line given a diet with a normal protein level. Touchburn *et al.* (1981) reported that growth rate of fat birds was not modified by a 16% protein diet as compared with 20% and 24% protein diets, while that of lean birds was significantly reduced by the lower protein diet. These findings were confirmed several generations later with the same strains of birds (LeClercq, 1983) in which four protein levels were compared (15.2, 17.2, 19.1 and 21.1%). Fat birds displayed identical growth rate irrespective of the protein concentration of the diet while the growth rate of lean birds was diminished when protein levels decreased below 19.1%. Using five diets of different protein levels, Whitehead (1988) reported similar results and showed that lean line birds were significantly heavier than fat line birds when fed the higher levels of protein but exhibited a greater weight depression on the diets of lower protein levels, suggesting that

the lean line is less able to tolerate low protein. It may be concluded that fat chickens have lower protein requirements when related to a dietary protein concentration.

LeClercq (1985) summarized the results of related experiments undertaken on this subject with different strains of lean and fat birds and concluded that lean birds always displayed a higher protein gain to protein intake ratio. Pym *et al.* (1984) pointed out that there was a high nitrogen intake at a given nitrogen balance in their line F indicating a high nitrogen requirement at maintenance. Any beneficial effect upon efficiency of feed utilisation resulting from an increase in feed consumption in line F would have been offset by the low metabolisability of dietary energy, the high maintenance energy (as mentioned above) and nitrogen requirements and the high energy content of the tissue deposited in this line.

LeClercq (1985) cautioned that two kinds of artefact could be involved. One is the effect of a different genetic potential for protein deposition. Since the lean birds in the experiments mentioned above showed a higher potential for protein deposition which could *per se* explain the observed differences between the lines. The second one is due to the use of only one experimental diet when comparing lean and fat birds. Since absolute protein and energy requirements are probably different, it is impossible to compare protein utilisation by providing only one diet and thus experiments undertaken with several diets containing different protein levels are likely to be more conclusive.

The apparent improvement in protein utilisation by genetic selection for leanness suggests that there are some metabolic changes either in lean lines or in fat lines which partition differing quantities of amino acids away from protein synthesis and either to heat production or to fatty acid synthesis. It has been observed in some experiments that plasma uric acid frequently reaches high levels in fat chickens predominantly before 5 weeks of age and total plasma free amino acids are lower in fat than in lean birds. Either fed or starved fat line birds can incorporate more ^{14}C from leucine into body fat than lean line birds (LeClercq, 1985).

From two experiments with lean and fat chickens, LeClercq and Saadoun (1982b) concluded that the main difference between the two lines is a systematic tendency for the fat chickens to preferentially use feed above the maintenance for lipid synthesis and this was

observed even when the birds were restricted and pair-fed. The higher percentage of lipid in the liveweight gain explained the slower growth rate and the higher FCR of fat birds when they were pair-fed.

The study of Geraert *et al.* (1987) achieved similar ME intake, heat production, net availability of ME for maintenance and gross efficiency of energy retention for both genetically fat and lean line chickens. In lean line birds, the partition of retained energy between fat and protein did differ with increased protein deposition and decreased fat retention. The difference between the lines was then attributed mainly to changes in protein metabolism, with more amino acid being catabolized for energy in fat birds and this was confirmed by the increase in uric acid excretion. Similar observations were also obtained in the study conducted by Pullar and Webster (1974) in the obese rat and in the experiments of Tomas *et al.* (1988) in which they used Pym's lines described above and found the significantly higher rate of N^T-methylhistidine excretion in line F than that in line E. A later study (MacLeod *et al.*, 1988) showed that the proportion of energy retained as crude protein and the efficiency of crude protein retention were significantly greater in the lean birds; a 15% higher rate of amino acid oxidation was close to a 17% higher total nitrogen excretion in the fat line. These results were compared with earlier calorimetric work (Geraert *et al.*, 1987) and these two sets of data agreed closely. These findings have led to the general conclusions that selection for leanness and fatness in broilers has produced lines which differ markedly in rate of fat and protein deposition at similar intakes of energy and nitrogen. The leanness attained is attributable not to increased energy expenditure but to a change in the partition of retained energy between fat and protein deposition. Fowls directly or indirectly selected for high abdominal fat content on criteria other than feed intake, exhibit a higher rate of protein catabolism than lean counterparts. The amino acid carbon chain is therefore being diverted from incorporation into protein to the pool of energy metabolism substrates. This seems doubly fortunate in commercial terms; the indirectly selected character with leanness, has a desirable correlated character, increased efficiency of protein retention (MacLeod and Geraert, 1987; MacLeod *et al.*, 1988).

C. Causes of the differences between fat and lean lines

The primary cause of the metabolic differences between genetically fat and lean lines still remains uncertain. It has been observed that neoglucogenetic pathways of obese mammals are often more active than those of normal subjects (Felig *et al.*, 1974). Touchburn *et al.* (1981) concluded that the fundamental mechanism responsible for the fattening of fat line birds appears to be a greater insulin release from the pancreas soon after hatching. This was confirmed in a later study conducted by Simon and LeClercq (1982), in which they showed an impairment of the glucose-insulin balance of fat birds during the period when adiposity is most divergent (6 and 8 weeks in the first study, and 5-8 weeks in the second study) and an enhanced insulin release in response to a glucose load or a meal which diverts nutrients towards lipogenesis and lipid deposition in the fat line. In a later review on this subject, however, LeClercq (1988) concluded that the differences between his fat and lean lines were due to differences in tissue sensitivity to hormones more than to hormone secretions themselves. The liver of fat line has been shown to be more sensitive to insulin, which leads to an enhanced inflow of glucose and acetyl Co-A derived from amino acids catabolism. Moreover, control of fat cell multiplication and differentiation could also be a key factor. A defect in thyroxine (T_4) to triiodothyronine (T_3) deiodination is also noticed, though it accounts for only a small proportion of differences. Plasma corticosterone concentration has been reported to be higher in fat birds (Akiba, 1988).

1.3.2 Reducing fatness by nutritional means

It has been demonstrated frequently and experimently that carcass composition can be manipulated by dietary means since Combs (1962) first recognized this in 1962.

1.3.2.1 Feed restriction

At zero energy balance, (i.e., maintenance), broiler chickens are in positive protein balance and negative fat balance (Pym and Farrell, 1977). A number of studies have been made on the effects of both early and late feed restriction in reducing body fat. Restriction during early growth might reduce fat cell hyperplasia and therefore, limit the potential for growth of fat (March and Hansen, 1977). Restriction at the end of the growing period, i.e., during

the final week or so, might be economically beneficial due to the restricted feed intake. At present, neither of these possibilities looks very promising for commercial application due to various limitations including a depression in final body weight.

Attempts to reduce abdominal fat by energy restriction at a young age have produced inconsistent results. Restriction of energy intake in the 0-3 week period had no significant effect on abdominal fat when measured at 8 weeks of age (Griffith *et al.*, 1977). Nitson *et al.* (1984) observed that a severe restriction (less than 75% *ad libitum*) was necessary to depress body fat between 2 and 4 weeks of age but there was a depression in growth. Birds fed at maintenance from 6 to 12 days of age have been reported to be leaner, more efficient in converting food to body weight and were no lighter than the *ad libitum* fed controls at slaughter age (Plavnik *et al.*, 1986). When the birds were maintained by energy restriction for only a few days during early stages of growth and then fed *ad libitum* for the remainder of the growing period, the feed efficiency was improved and fat deposition was decreased (Plavnik and Hurwitz, 1985). The results indicate that feed restriction reduced the number of fat cells developing early in life and this delayed maximum fat deposition. Using different restriction programs from 7 days of age, carcass fat and abdominal fat pad were reduced and this was associated with the decreased final body weight (Jones and Farrell, 1987). Their investigations on feed restriction and the cellularity of adipose tissue showed the effect of early restriction on reducing fat cell size. By using rice hulls to dilute the diet, a significant decrease in AFP was achieved. But again, this was accompanied by a depression in growth rate (Jones and Farrell, personal communication, 1988). The feasibility of these approaches depends upon compensatory growth, a phenomenon which still remains controversial in poultry (Plavnik and Hurwitz, 1985). It was suggested that as the duration of restriction increased, complete growth compensation became increasingly harder to achieve (Plavnik and Hurwitz, 1985; Jones and Farrell, 1987). Under some circumstances, restricted feeding has been shown to result in an increase rather than a decrease in carcass fat and the possible reasons and implications were discussed by Cherry (1987).

Feed restriction during the final week or so of growth has also been explored and the results have indicated the effect on reducing fat content but also body weight (Auckland

Table 1.2: Influence of dietary ME and crude protein on body weight and body composition of broilers (data from Jackson *et al.*, 1982a,b)

Diet content		Body	Total body lipid	Abdominal fat
ME (MJ/kg)	CP (g/kg)	(g)	(% of dry)	(% of live)
10.9	260	1645	37.5	1.46
11.7	260	1693	39.3	1.72
12.5	260	1721	42.4	2.08
13.4	260	1764	42.6	2.13
14.2	260	1790	45.6	2.39
15.0	260	1797	47.9	2.70
13.0	160	1625	50.0	3.12
13.0	200	1734	46.2	2.55
13.0	240	1766	42.4	1.92
13.0	280	1762	39.4	1.67
13.0	320	1762	39.2	1.73
13.0	360	1762	38.3	1.49

and Fulton, 1973; Arafa *et al.*, 1983).

1.3.2.2 Energy levels and protein to energy (P:E) ratio

The dietary variable which has the greatest effect upon fatness in chickens has been recognized for some time to be the ratio of dietary protein to energy (P:E). A large body of data has documented its effects on the body fat content of broilers since Fraps (1943) and Combs (1962) observed that the fatness of chickens was progressively increased as the P:E ratio of the diet was reduced below a critical level.

The ratio can be altered by either changing energy level at a constant protein level or changing protein level at a constant energy level. The results in Table 1.2 from the study conducted by a group in Canada show the typical rates of response of body fat components to changes in dietary P:E ratio (Jackson *et al.*, 1982a,b). From Table 1.2 it can be clearly seen that two ways of altering the P:E ratio were equally effective in influencing body fat content. The proportion of abdominal fat and total fat were changed by factors of 2.1 and 1.3 respectively when the P:E ratio varied from 12 to 28 g/kJ. The data, however,

also illustrated that growth rate was depressed whenever either the protein or the energy concentration of the diet became inadequate. It has been concluded that minimising body fat content at optimum growth rate would be best achieved by increasing dietary protein level rather than by decreasing energy concentration (Whitehead, 1986). Pesti and Fletcher (1984) suggested that an effective way of reducing fatness would be to feed normal starter diets followed by finisher diets with higher than normal P:E ratio. This could be achieved by either decreasing energy or increasing protein in the finisher diet, but both methods have commercial disadvantages in that the first one incurs a growth penalty and the second one involves more expensive diets. Therefore, such means for growing leaner birds seem uneconomic. In Figure 1.6 it can be seen that although the yield of carcass fat is markedly influenced by dietary protein and energy concentration, the protein yield is little influenced by diet composition (Summers, 1988). The reason that the intake of protein beyond required levels reduces fat deposition has been explained in terms of the need of the birds to expend more energy in order to eliminate excess nitrogen from the body and thus having less energy available for the synthesis of body fat (Bartov, 1979). Dietary protein has been shown to inhibit hepatic fatty acid synthesis when added to the diet at the expense of carbohydrates though it has been difficult to separate the specific metabolic effects of additional dietary protein from those of reduced carbohydrates intake (Rosebrough *et al.*, 1982).

The final assessment of the use of dietary P:E ratio to manipulate fatness would require detailed consideration of the economic factors involved.

1.3.2.3 Protein quality and amino acid (AA) supplementation

A study conducted by Griffiths, Leeson and Summers (1977) showed that the addition of a low quality protein (feather meal) was equally as effective in reducing abdominal fat pad size as a high quality protein (methionine supplemented soyabean meal) under conditions in which neither produced a growth response. Similar results have been achieved with non-protein nitrogen sources (Velu *et al.*, 1972). Nir (1984) observed that the inclusion of feather meal or sterilized chicken manure in the diet did not consistently reduce abdominal fat and he suggested that the effect of excessive dietary protein on fat deposition depends more on its

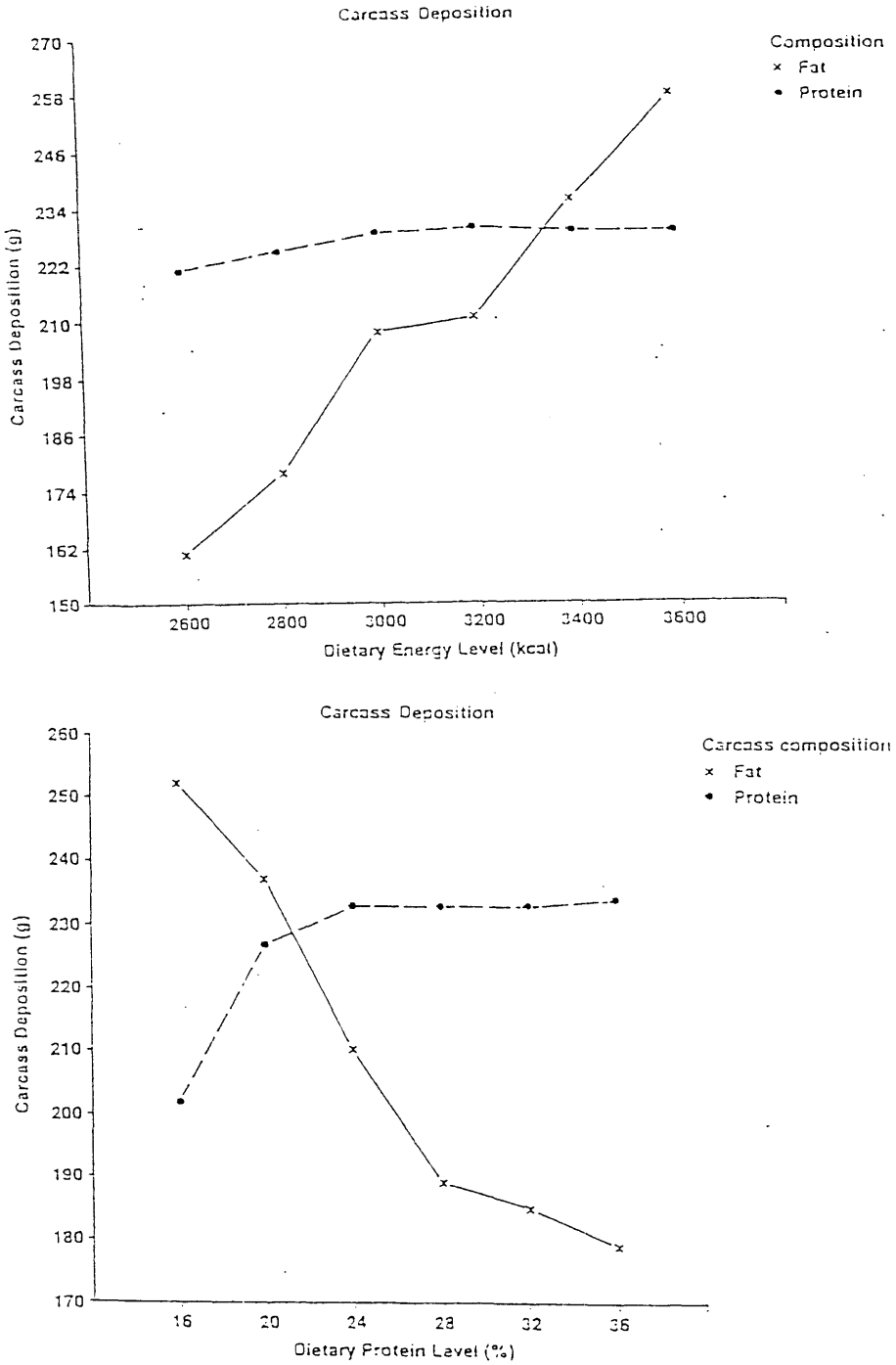

Figure 1.6: Effects of dietary protein and energy level on carcass deposition (Summers, 1988)

digestibility rather than its amino acids composition. Cherry (1987) further suggested that a combination of soyabean meal and a poor quality protein source of adequate digestibility might be a feasible approach to reducing carcass fat deposition.

Available information concerning the influence of individual amino acids on fat deposition is somewhat confusing and most of the studies have focussed on lysine and methionine. There is evidence that methionine and lysine concentrations exceeding levels needed to sustain optimal growth may decrease body fat (Nir, 1984). Reported effects of lysine are particularly confusing with its addition to the diet having been shown to reduce hepatic lipogenesis (Yeh and Leveille, 1969) and to enhance lipogenesis (Rosebrough *et al.*, 1982). It has been cautioned that amino acids, particularly methionine can depress growth at high levels of supplementation (Cherry, 1987). Recent work by Farrell *et al.* (1989) showed that the addition of 20% above normal level of either methionine, lysine or both had little effect on reducing fat carcass content, but when the addition was combined with feed restriction, the proportion of abdominal fat was decreased significantly compared with either the control or restricted birds without supplement. This may be related to the findings of Gous (1976) that the ability of birds to absorb lysine may be improved by feed restriction.

From a number of studies, Bertram (1986) concluded in a comprehensive review on the influence of DL-methionine on the fat content of broiler carcass that the carcass quality of poultry can be improved by adjusting the methionine content of the feed -- especially during the finisher phase -- to 110–120% level. Thus, the requirement for methionine according to optimum carcass quality — increase of protein and decrease of fat — is higher than according to weight gain *per se*.

In general, there appears to be no entirely satisfactory theory to explain the effects of amino acids supply on body composition. As suggested by Lipstein *et al.* (1975), variation in essential amino acids : energy ratios can probably be considered in the same way as P:E ratio.

1.3.2.4 Dietary fat and nutrient density

Bartov (1979) quoted numerous studies to show that adding fat to the diet at the expense of carbohydrates has no substantial effect on the body fat if P:E ratios are not changed although this does not necessarily mean that the dietary fat has no effect on carcass composition. Fat in diet is often associated with fatter birds and this is explained as that the inclusion of fat in the diet usually results in diets of higher nutrient density (Whitehead, 1986). As metabolizable energy increases at a constant P:E ratio, so too does growth rate and body fat content (Farrell, 1974a; Fisher and Wilson, 1974).

It has been cautioned that although the substitution of fat for carbohydrates reduces hepatic fatty acid synthesis and the activity of associated lipogenesis enzymes, there is work indicating that this effect is due to a reduction in carbohydrates and not to fat *per se* (Bartov, 1979; Fisher, 1985). Hillard *et al.* (1980) showed that fatty acid synthesis and the enzymes involved in synthesis were decreased by dietary fat, but if the dietary carbohydrates remained constant, fat had no effect. High concentrations of dietary fat may decrease fatty acid synthesis not through inhibition of the enzymes but through insufficient substrate for fatty acid synthesis (Enser, 1985). Reduced lipogenesis does not necessarily affect carcass fat because under such conditions a high proportion of body fatty acids is derived directly from dietary fat and this does affect the composition of carcass (Bartov, 1979)

Fisher (1985) summarized his own and others studies and concluded that 'the trend, in practice, towards higher nutrient density feeds containing more added fat has contributed to the observed increase in body fat levels. However, whether this is inevitable or a consequence of imperfect diet design, remains to be determined conclusively'.

Conflicting results have been reported on the effects of type of dietary fat. Cherry (1987) found no effect of degree of saturation on overall body composition, but the type of fat found in the carcass was associated with diet, i.e., feeding of unsaturated fat increased the amount of unsaturated fat in the carcass. Akiba (1988) on the other hand reported that feeding corn oil and chicken oil significantly decreased abdominal fat compared with feeding yellow grease, indicating that the degree of unsaturation of dietary fat had modified

the fatness of the broilers.

1.3.2.5 Salt and water

The inverse correlation between fat and water in the carcass has led to the suggestion that increased water consumption by broilers may inhibit fat deposition. Lightsey *et al.* (1983) found that higher than normal levels of salt in the diet reduced body fat and increased carcass water. But this is likely to be disadvantageous from the point of view of litter quality due to the elevated water intake and the possibility of toxicity from high levels of salt (Pym, 1987).

1.3.3 Environmental and management means

It has been suggested that environmental temperature influences the partitioning and the use of nutrients by birds because maintenance energy needs are lower at higher temperature and more of the feed consumed is available for fat deposition (Leenstra, 1984; Whitehead, 1986). At moderate temperatures results consistent with this hypothesis were observed by Kubena *et al.* (1972) and from these data Fisher (1985) calculated the overall rate of response to be 1.9 g fat/kg body weight per °C between 10 and 30°C.

Deaton *et al.* (1974) found that broilers reared in cages tended to be fatter than birds reared on deep litter and explained this in terms of the lower activity of the birds in cages. Evans *et al.* (1976) found, however, no difference in the fat content of breast and thigh meat between birds reared in cages and on litter.

Experiments reported on the influence of lighting systems on body composition have not given consistent findings. Van Es (1981) found that intermittent light gave less fat deposition but Leenstra (1984) found no effect.

Environmental and management factors generally have much less effect on body fat deposition compared with genetic and nutritional influence and the effect, for the most part, is related to the influence on feed or nutrient intake (Summers, 1988). Therefore, there is little interest in studying the influence of environmental and management factors on fat deposition to prevent excessive fat content in broilers.

1.3.4 Biotechnology

The approach to reducing fatness in livestock using the animal's serum antibodies to destroy its own fat cells has been developed at Britain's Hannah Research Institute (Conghlan, 1985). Sheep injected with fat cells from rats were shown to be leaner than untreated animals. So far no information has yet been published on poultry.

1.4 Repartitioning Agents

1.4.1 Beta-adrenergic agonists

1.4.1.1 Discovery of some beta-agonists as repartitioning agents

The effects of some β-adrenergic agonists on reducing fat content while increasing protein content were discovered around 1983 by two groups when they worked in different areas and with different species.

One group at the American Cyanamid Company initiated their project with the emphasis on screening large numbers of random compounds for their ability to increase lean muscle deposition and to limit the deposition of adipose tissue in growing animals. The basis of this research was the idea that systems controlling the shifts in nutrient partitioning during normal changes in physiological states, such as the onset of lactation and the period of rapid growth, could be manipulated by using exogenous chemical agents or hormones (Dalrymple, 1985). A rodent model system was used and a series of effective compounds were identified. The term 'repartitioning agents' was coined to describe these compounds because of their effect on increasing lean mass deposition while reducing body fat. These materials are analogues of catecholamines adrenalin and nor-adrenalin and are referred to as β-agonists because of their interaction with the β-receptors (Buttery and Dawson, 1987). The initial active compound discovered in their studies was clenbuterol and its effect was subsequently tested in the major meat producing species. Cimaterol was also evaluated at the same time.

The structure of clenbuterol, cimaterol, and epinephrine is shown in Figure 1.7.

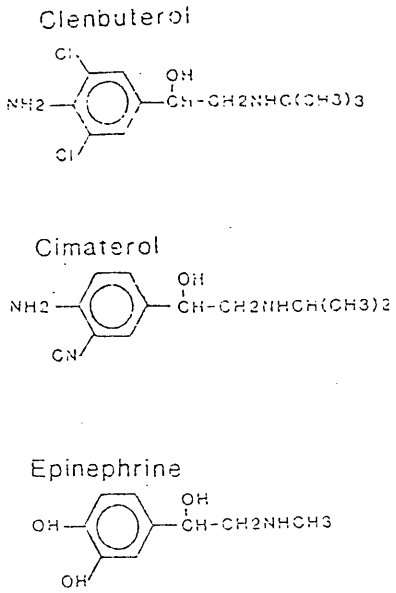

Figure 1.7: The structures of clenbuterol, cimaterol and epinephrine

While this American group worked on commercially important domestic livestock, another group at St.George's Hospital Medical School in the UK discovered an unexpected anabolic effect of some adrenergic agonists in their research on the sympathetic control of rat brown adipose tissue (BAT). Their studies into the effects of β-adrenergic agonists on metabolic rate and BAT activity indicated both β_1- and β_2-adrenergic agonists can stimulate oxygen consumption, raise BAT temperature and the activity of the mitochondrial proton-conductance pathway in this tissue. A β_2-adrenergic agonist, clenbuterol, was found to be as effective as the β_1-adrenergic agonist, propranolol. Having studied the acute effects on thermogenesis, the chronic effects of clenbuterol on heat production and energy balance were then investigated and it was in these studies that some unexpected changes in body weight and body composition were found (Emery *et al.*, 1984). These studies are described in more detail below (section 1.4.1.2.D).

1.4.1.2 Effects of β-agonists on carcass characteristics and performance

Treatment with either clenbuterol or cimaterol has been shown to alter the carcass composition of sheep, cattle, pigs and poultry by increasing body protein and reducing body fat. These compounds did not have consistent effects on liveweight gain, feed consumption or feed conversion efficiency though in some studies there was a small increase in gain, or a small decease in feed consumption or both, yielding improvement in efficiency of weight gain. In some studies where there was no change in gain-to-feed ratio, when efficiency was calculated on the basis of meat production, i.e., lean body mass, there was a marked improvement in efficiency of production.

A. In ruminants

So far, the most dramatic effects have been seen in ruminants. The few studies with steers showed the remarkable repartitioning effect of these two β-adrenergic agonists, clenbuterol and cimaterol, but with little effect on liveweight gain and feed conversion (Ricks *et al.*, 1984a; Quirke *et al.*, 1985; Allen *et al.*, 1985a; Williams *et al.*, 1987). The main results from the study of Williams *et al.* (1987) are the increase in carcass yield achieved as a result of both an increase in carcass weight and also a reduction in the weight of non-carcass components (notably liver, heart, gut, hide perinephric and channel fat). Analysis of the nitrogen and fat contents of the carcass and non-carcass components indicated that clenbuterol specifically promoted muscle gain and hence repartitioned nitrogen within the body. The improvement in total nitrogen gain was less than the increased retention in carcass, while the effect on fat was similar in both components.

The dramatic repartitioning effects of clenbuterol and cimaterol in sheep are consistent and well documented (Baker *et al.*, 1984; Dalrymple *et al.*, 1985; Thornton *et al.*, 1984; Thornton *et al.*, 1985; Quirke *et al.*, 1985; Beermann *et al.*, 1986; Hamby *et al.*, 1985). As with cattle, there is little or no effect on growth rate and feed conversion in sheep. Thornton's group at CSIRO found a 30% reduction in fat which was replaced with an equivalent amount of lean tissue and the response to clenbuterol was similar in ewes, wethers and ram lambs (Thornton *et al.*, 1984; Thornton *et al.*, 1985).

B. In swine

The results from the trials with finishing swine showed the effects of the two β_2-adrenergic agonists are not as large as those seen in ruminants (Dalrymple *et al.*, 1984a; Ricks *et al.*, 1984b; Jones *et al.*, 1985; Prince *et al.*, 1985; Quirke *et al.*, 1985; Fitzsimons *et al.*, 1986; Moser *et al.*, 1986). Trials with young pigs treated with cimaterol failed to show effects on any of the growth or carcass variables (Mersmann *et al.*, 1987). The observation that muscle marbling tended to be increased was not seen in cattle and no explanation for this apparent difference between species has been given.

C. In poultry

Four different studies with broiler chickens, as distinguished by a space, are summarized in Table 1.3. In each study, values with the same superscript within a column are not significantly different ($P>0.05$). Improvements in performance and carcass parameters, especially at 1 ppm clenbuterol (3.3% in gain, 3.0% in feed efficiency and 0.8% in carcass composition), were demonstrated in over 20 broiler floor pen trials (Dalrymple *et al.*, 1984b). The magnitude of the change is quite small compared with those observed in ruminants. Although proximate analysis of the ground whole carcass indicated alteration in carcass composition in favour of the repartitioning agents, neither β_2-adrenergic agonist showed much effect on the abdominal fat pad (Dalrymple *et al.*, 1983; Dalrymple *et al.*, 1984b; Dalrymple and Ingle, 1987). But in the six trials carried out in West Germany with cimaterol in broiler chickens, a significant 10% on average reduction abdominal fat was found (Scholtyssek, 1987).

A study with mature coturnix quails resulted in significantly greater weight gains, eviscerated carcass yields and lower moisture (Merkley, 1988). AFP was not affected and abdominal adipocyte diameter was significantly larger in treated birds. Feeding cimaterol to Pekin ducks led to a lower weight gain, but an improved FCR, breast and leg muscle weight, carcass yield and lower skin fat (Dean and Dalrymple, 1988).

Table 1.3: Effects of clenbuterol (CL) or cimaterol (CM) on the growth performance and carcass characteristics of poultry

CL or CM	Level (ppm)	Weight gain (g)	Carcass yield at 50-51 days (%)	FCR	AFP (%)	
					Male	Female
CL*	0	1080^a		2.21^a		
	0.25	1123^a		2.14^b		
	0.5	1124^a		2.13^b		
	1.0	1124^a		2.13^b		
	2.0	1113^a		2.13^b		
	4.0	1103^b		2.15^b		
	0	1198^b	69.8^b	2.20^a	3.44^a	4.24^a
	1	1259^a	70.4^a	2.09^b	3.36^a	3.88^b
	0	1156^b	69.9^b	2.30^b	3.76^a	
	1	1194^a	70.7^a	2.23^a	3.61^a	
CM**	0	1245^b	70.3^b	2.24^a	2.82^a	
	0.125	1281^b	70.8^b	2.19^b	2.77^a	
	0.250	1301^a	70.9^a	2.18^b	2.82^a	
	0.5	1295^a	71.3^a	2.16^b	2.75^a	
	1.0	1268^b	71.0^a	2.21^a	2.80^a	
CL	1.0	1284^a	71.0^a	2.18^b	2.83^a	

		Body composition					
		Fat (%)		Protein (%)		Water (%)	
		Male	Female	Male	Female	Male	Female
CL*	0	19.7^a	21.1^a	16.7^b	16.0^b	61.4^a	59.4^b
	0.25	18.0^a	19.2^b	17.1^b	16.9^a	62.0^a	61.1^a
	0.5	17.7^a	19.0^a	17.6^a	16.7^a	61.8^a	60.9^a
	1.0	16.6^b	19.3^b	17.2^b	16.9^a	62.8^a	61.7^a
	2.0	17.0^b	18.7^b	17.3^b	16.9^a	63.0^a	61.4^a
	4.0	17.5^b	19.2^b	17.0^b	17.0^a	62.7^a	60.0^a
	0	16.4^a	17.7^a	18.8^a	18.6^b	62.4^b	61.0^a
	1	15.1^b	16.4^b	18.9^a	19.0^a	63.4^a	61.9^a
	0	16.8^a		18.8^a		61.8^b	
	1	15.9^a		19.2^a		62.9^a	
CM**	0	17.6^a		19.2^a		61.0^b	
	0.125	16.2^b		20.0^a		61.8^b	
	0.250	16.4^b		19.6^a		61.9^b	
	0.5	16.0^a		19.5^a		62.5^a	
	1.0	16.2^b		19.7^a		62.4^a	
CL	1.0	16.3^b		19.7^a		62.2^a	

** Dalrymple et al., (1984b); ** Dalrymple (1985).*

D. In rodents

With the expectation that chronic stimulation of BAT activity and thermogenesis would decrease energy efficiency and fat deposition, Rothwell *et al.* (1983) in the UK surprisingly found that the rats treated with 0.5 mg/ml clenbuterol subcutaneously twice daily for 18 days gained significantly more weight (146 g) than rats treated with saline (124 g) or propranolol (125 g). Subsequent carcass analysis showed that the greater weight gain of the clenbuterol-treated rats was due to an increase in lean body mass. Further studies using higher doses of clenbuterol confirmed the effect by Emery *et al.* (1984) and showed a 27% increase in weight gain, which was due to an increase in fat-free mass (i e. protein and water) with no increase in carcass fat content though the percentage of fat dropped markedly (from 18% to 13%). A similar but less profound effect in the study with another β_2-adrenergic agonist, fenoterol, was reported (Emery *et al.*, 1984). Therefore these studies led them to the conclusion that the observed effects on the growth of lean body mass could be a common feature of β_2-adrenergic agonists (Stock and Rothwell, 1986).

In a study with genetically lean and obese Zucker rats, no effects of clenbuterol on energy intake or expenditure were observed (Rothwell and Stock, 1987a). Clenbuterol exhibited potent anabolic effects on lean body mass with the increase in protein to fat ratio by 50 and 73% respectively, but it also increased thermogenesis and reduced body fat content (19%) in the obese mutants. Similar findings were obtained in a comparison between a normal diet (22% protein) and a low-protein diet (8% protein) and the results showed that clenbuterol may help to conserve body protein at the expense of fat in protein-deficient animals, resulting in a smaller but leaner body mass (Rothwell and Stock, 1987b).

1.4.1.3 Effects of clenbuterol on energy and nitrogen metabolism

A. In laboratory rats

The first study of these β_2-adrenergic agonists on energy balance was conducted by Rothwell *et al.* (1983) with clenbuterol on rats. The intake of the clenbuterol-treated rats was restricted to that of control animals. A 14 % increase in heat production and a highly significant 33% reduction in energy gain were seen in the treated rats. Using a higher dose

of clenbuterol twice daily, Emery *et al.* (1984) found an increase in both energy intake (23%) and energy expenditure (26%) with a small but non-significant decrease in energy gain. Based on these results, Stock and Rothwell (1986) pointed out that a 17% reduction in weight gain in clenbuterol-treated rats was due to clenbuterol's effects on increasing heat production (14%) and, despite the lower rates of weight and energy gain, fat-free mass was unaffected and the higher heat production was entirely at the expense of fat gain which was reduced by 60%. Final protein to fat ratio was 1.88 for control and 3.29 for clenbuterol treated rats. Thus, even though clenbuterol failed to stimulate growth of lean body mass in these feed-restricted rats, it helped to sustain the normal rate of protein deposition.

B. In farm animals

The first published study on energy and nitrogen balance with farm animals was conducted by Williams *et al.* (1987) on calves in which they obtained similar results to those seen in rats, i.e., the reduction in the total energy content of live weight gain (from 1077 to 897 MJ) in clenbuterol-treated calves and an increase in mean daily heat production (23.1 to 25.9 MJ/d). The effects of clenbuterol were reported to occur mainly in skeletal muscle: N^T-methylhistidine excretion was significantly decreased and the estimated fractional breakdown rate of muscle protein in clenbuterol-treated calves was only 0.66 of that of the controls.

The effects of clenbuterol on energy and nitrogen balance were also assessed in lambs (MacRae *et al.*, 1986; MacRae *et al.*, 1988). The first trial showed an elevated mean heat production of 0.3–0.8 MJ/d and mean nitrogen retention of 1.5–4 g/d. This was associated with reduced leucine oxidation. These results indicated that clenbuterol increased protein gain by 15–20 g/d and reduced fat gain by 25–30 g/d. The second study on lambs showed a marked increase in nitrogen retention by 2–3 g/d ($P<0.01$) throughout the 20-day treatment period, an increase ($P<0.001$) in energy expenditure and a reduction in leucine oxidation ($P<0.001$). The effect of the treatment was then calculated to result in a daily retention of 19 g more protein and 30 g less fat.

1.4.1.4 Effects of a withdrawal period

A withdrawal period is often required for registration of feed additives given to animals which will be slaughtered for human consumption. Such a period is specified to minimise contamination with tissue residues and to ensure that no carry over occurs of residual metabolic activity of the compound into humans. There is currently no published information on the presence of tissue residues remaining after a period of treatment with clenbuterol or cimaterol. However, their metabolic effects have been shown to be of short duration. MacRae *et al.* (1988) observed that withdrawal of clenbuterol resulted in rapid alterations in nitrogen and energy metabolism towards those expected of control animals of that weight. An earlier study also indicated that the heat production in clenbuterol-treated sheep was immediately returned to values not significantly different to those of pretreatment on clenbuterol withdrawal (Hovell *et al.*, 1987). These reports appear to suggest that these two β_2-agonists are very rapidly metabolized and that shortly after withdrawal of the compound from the diet there is little remaining metabolic activity.

A 5-day withdrawal period prior to slaughter of poultry did not negate effects on protein and fat in the final carcass compared with treatment up to slaughter (Dalrymple *et al.*, 1984b). The results from a study with pigs (Jones *et al.*, 1985) showed a compensatory increase in feed intake over a 7-day withdrawal period and during this time there was no compensatory loss of nitrogen or reduction in nitrogen, confirming, as Hovell *et al.* (1987) had reported, that after treatment nitrogen balance values reverted to normal. Fat deposition was initially increased, though, at slaughter, pigs subjected to a withdrawal period still had less fat than the untreated control. A recent report, however, showed that extended withdrawal (90 days) from clenbuterol may stimulate secondary hyperplasia in the 9-10-11th subcutaneous adipose depot in young steers (Schiavetta *et al.*, 1988). But 90-day is really impractical for a withdrawal period.

1.4.1.5 Side effects

Heart rate has been observed to increase immediately in both clenbuterol-treated calves and sheep (Herbert *et al.*, 1985; Brockway *et al.*, 1987) and the rapid return to normal has been taken to indicate that a tachyphylaxia, i.e., desensitisation, occurs in β-agonist treated animals. Accompanied with the increase in heart rate, a drop in blood pressure has been noticed (Brockway *et al.*, 1987) and deep rectal temperature was elevated by approximately 1°C (Herbert *et al.*, 1985). Continued administration had no further effect on heart rate or blood pressure but in sheep dose levels above 1.5 mg/day depressed appetite for up to 5 days; at all dose levels clenbuterol brought about a long term increase in metabolic rate (Brockway *et al.*, 1987).

The reported effects of clenbuterol and cimaterol on meat quality are somewhat inconsistent. Muscle glycogen is depressed in clenbuterol-treated rats (Williams 1987) and cimaterol-treated lambs (Allen *et al.*, 1985b). Depletion of glycogen reserves prior to slaughter limits the normal drop in muscle pH which in cattle causes 'dark cutting', i.e., undesirable darker meat. Higher post-mortem pH in the longissimus dorsi and a three-fold higher incidence of dark meat from cimaterol-treated sheep has been reported (Allen *et al.*, 1985a). The observed increase in the proportional area of glycolytic fibres would also increase the capacity of muscle to metabolize glycogen, which in turn will exacerbate any effects on pre-slaughter depletion of muscle glycogen (Williams, 1987). Based on these effects related to muscle glycogen levels at slaughter, a withdrawal period has been suggested before β-agonist-treated animals are slaughtered (Williams, 1987) so that normal post-mortem pH values could be achieved if the sheep are slaughtered immediately on arrival at the slaughter house (Allen *et al.*, 1985b). Thornton *et al.* (personal communication, 1987), however, observed no effect of clenbuterol on ultimate pH of muscle from sheep.

1.4.1.6 Modes of action of β-agonists

The effects of clenbuterol and cimaterol have been proposed as, at least in part, being due to the β_2-adrenergic activity arising out of their structural similarity to catecholamines (Ricks

than that from ruminants (Muir *et al.*, 1985). This is in agreement with the observed effect of these β-agonists on the fat content of broiler chickens (Dalrymple *et al.*, 1985). Lipolysis in adipocytes originating from poultry is hardly stimulated by β-agonist treatment (Muir *et al.*, 1985). The study on swine adipose tissue also demonstrated a direct lipolytic and antilipogenic effect of cimaterol (Peterla *et al.*, 1987).

The β-receptors in adipose tissue tend mainly to be of the β_1 type in rats (Lands *et al.*, 1967) although the agents used in nutrient repartitioning appears to be selective for the β_2-receptor. However, there is evidence to indicate the dual nature of the adipose β-receptor. In rats the β_1-adrenergic receptor has a roughly equivalent affinity for the two catecholamines while the β_2-receptor exhibits a greater affinity for adrenalin (Buttery and Dawson, 1987).

B. Muscle

Protein deposition represents the net balance between the rates of simultaneous protein synthesis and degradation. Studies on the effect of these β_2-adrenergic agonists on muscle protein metabolism have been conducted both on major livestock species and on laboratory rats. The protein anabolic properties of clenbuterol and cimaterol are mainly confined to skeletal muscle in rats (Reeds *et al.*, 1986) and in calves (Williams *et al.*, 1987). Reeds *et al.* (1986) observed significant increases in the weights of the gastrocnemius, soleus, plantaris and extensor digitorum longus muscles in rats fed clenbuterol. The increase in skeletal muscle protein was shown to be a result of fibre hypertrophy rather than hyperplasia (Beermann *et al.*, 1986; Maltin *et al.*, 1986a). This was confirmed in rats by an increase in both skeletal muscle protein and RNA without any increase in DNA (Reeds *et al.*, 1986) and by the increase in fibre cross-sectional area (Maltin *et al.*, 1986a). Evidence to show that the main effect of β_2-agonists is via a reduction in the rate of muscle protein degradation was seen in the significantly reduced urinary 3-methylhistidine : creatinine ratio (Buttery and Dawson, 1987) after 10 weeks of treatment. This has also been observed by Williams *et al.* (1987) with young veal calves given clenbuterol after 60 days of treatment. The Cathepsin B activity of muscle is reduced by cimaterol in sheep (Forsberg *et al.*, 1987) and broiler chickens (Morgan *et al.*, 1988a). In a study with broiler chickens, no difference was shown in

any of the RNA, DNA and protein ratios or activity due to dietary cimaterol treatment and differences in muscle weight obtained by cimaterol were therefore attributed to changes in fractional degradation rate (Morgan *et al.*, 1988b). Data with other β-adrenergic agonists also suggested a reduction in protein catabolism (Garber *et al.*, 1976; Li and Jefferson, 1977). MacRae *et al.* (1986) found a significant reduction in leucine oxidation in sheep, which might be indicative of a decrease in protein degradation. Clenbuterol has also been shown not only to inhibit but to reverse denervation-induced atrophy of rat soleus muscles (Maltin *et al.*, 1986b).

The main and so far seemingly the only study that showed a direct effect on protein synthesis was reported by Emery *et al.* (1984) who observed a 34% increase in the fractional rate of protein synthesis in rats given high doses of clenbuterol for 7 days.

Thus, it has generally been concluded that the main effect of β-adrenergic agonists on muscle protein metabolism is a decrease in fractional degradative rate.

C. Involvement of other hormones and growth factors

Emery *et al.* (1984) observed no changes in plasma insulin, growth hormone (GH), or triiodothyronine in clenbuterol-treated rats. But a highly significant decrease in insulin and an increase in GH levels were found by Thornton *et al.* (1985) in growing lambs. These authors attributed the repartitioning of dietary nutrients away from fat and towards protein deposition to these hormonal changes. A reduced insulin level was also reported by Beermann *et al.* (1985) in cimaterol-treated lambs. The insulin-stimulated fatty acids synthesis in swine fat cells was inhibited by specific beta-agonists epinephrine and clenbuterol (Liu *et al.* 1988). Perkins *et al.* (1983) have presented evidence that β-agonists can stimulate GH secretion by direct action upon pituitary while Thiel *et al.* (1987) showed that cimaterol can induce some increase in muscle mass in hypophysectomised rats. Beta-agonists showed little effects on plasma cortisol concentration in lambs (Beermann *et al.*, 1985; Galbraith *et al.*, 1988) nor on corticosterone in rats (Sharpe *et al.*, 1986).

Although these results are not always consistent between species, some involvement of alteration of endogenous hormone pattern seems likely at least in the ruminants.

1.4.2 Iodinated casein

Substances affecting the thyroid or having thyroid activity have been observed to influence growth rate and fat deposition in various farm animals (for review see Blaxter *et al.*, 1949). Irwin *et al.* (1943) reported that thyroactive iodocasein (protamone) slightly increased body growth rate, improved feather growth but increased mortality at the higher levels tested. In later experiments, iodinated casein generally tends to decrease growth and results in poorer feed efficiency, although the effects are variable and dose related (Turner *et al.*, 1944; Wheeler *et al.*, 1948; Boone *et al.*, 1950; Oloufa, 1955; Herbert and Brunson, 1957; Wilson *et al.*, 1983).

Iodinated protein prevented the development of subcutaneous fat in Barred Plymouth Rocks (Turner *et al.*, 1944). Herbert and Brunson (1957) found that iodinated casein significantly decreased carcass fat content. Feeding iodinated protein to starting pullets has also resulted in decreased abdominal fat, liver fat, and body weight (Roberson and Trujillo, 1975). Iodinated protein has been shown to improve growth, feathering, and market finish and to decrease carcass fat in ducklings (Scott *et al.*, 1959). But feeding iodinated casein to the growing male duck resulted in significantly poorer growth rate and FCR (Adams and Stadelman, 1978). Poorer FCR, greater shrink and dressing losses were shown by Wilson *et al.* (1983) and he also observed higher mortality, lower carcass grade and lower conformation scores caused by higher levels of iodinated casein.

It appears that there is an optimum level at which iodinated casein in the diet will decrease fat deposition while stimulating or at least not retarding growth. These effects have been achieved at the level of 100 mg/kg diet (Wilson *et al.*, 1983) and 0.22% (i.e. 2200 mg/kg diet, Herbert and Brunson, 1957). Turner *et al.* (1944) observed 100 mg/kg diet slightly depressed growth and reduced subcutaneous fat and four weeks was required to reserve the fat.

1.5 Summary, Approaches and Objectives of the Present Study

There is no doubt that the fat content of broilers has increased in recent years and there is great interest in the poultry industry to reduce excessive carcass fat. The origins of this increase in fatness, though still not fully determined, appear to be due mainly to the faster growth rate resulting from genetic selection together with several nutritional and environmental variables which tend to increase fat deposition. It is clear that genetic selection, nutritional manipulation and repartitioning agents all offer ways to solve the problem. The main point is how to do this efficiently. A practical solution must be delayed due to a lack of a viable method to evaluate broiler flocks on a commercial scale.

Genetic selection remains the most promising and efficient way in the longer-term. Both direct selection for leanness and indirect selection for efficiency have led to the desirable results, i.e., decreased fatness *and* improved feed and particularly nitrogen utilization. Based on the results of selection experiments and estimates of genetic correlations, it can be expected that the correlated effects of selection against fatness will be favorable.

Of all the factors affecting fat deposition in broilers, the nutritional aspect has been studied most intensively. Of the methods tried so far, control of protein to energy ratios of the diet and feed restriction are effective but economic factors must be taken into consideration. Again, the price structure of the meat industry should reflect the interest in reducing fatness. The use of inexpensive, poor quality protein sources to increase protein intake appears promising. The commercial use of feed restriction seems to depend upon the critical stages at which fat deposition can be permanently altered, the degree of restriction necessary to achieve optimal results and its commercial practicality.

Beta-adrenergic agonists have remarkable effects on decreasing fat content while increasing lean mass proportion in the larger species of farm animals but have proved much less effective in poultry. Further studies on their possible short-term and long-term toxicological effects and meat residue are required. Whether these repartitioning agents can be registered and applied in the meat industry depends largely on public acceptability, i.e., public health

concern. There has not been much work done with iodinated casein to manipulate body composition and the few published results so far are conflicting. It is worthwhile, however, to test its effects on modern broiler chickens using optimal levels.

In the present study, growth experiments were conducted to test the effects of four substances, cimaterol, theophylline, caffeine and iodinated casein, on the performance and fat content in experimental fat and lean line birds and commercial broiler chickens. Calorimetric work was done to test the possible changes in heat production and energy and nitrogen metabolism in fat and lean line birds. To explore the influence of cimaterol, theophylline and iodinated casein on physiological and biochemical functions in commercial broilers treated with these agents, heat production and energy and nitrogen balance were also measured in closed-circuit respiration chambers.