Factors Affecting Poor Breast Feathering in Modern Turkeys

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For Dad

Declaration

I declare that the work described in this thesis is my own composition and no part of it has been presented in any other thesis. The work presented was conducted by myself, with the exception of the confocal microscopy work described in Chapter 6 which was performed by Mr G. Robertson. All help and assistance received has been acknowledged.

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Abstract

Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) cause depressed welfare and are an important source of economic loss in market turkeys. Such lesions are common in modern turkeys and feathers may offer some protection to the breast from environmental challenges. Feather growth is poor in modern compared with traditional turkeys and feathers may be absent over the breast region. This study quantified and compared feather growth in a modern commercial turkey with that in an unrelated traditional turkey and investigated the lack of breast feathers in the large modern bird.

A comparative study found that feather growth had not increased with selection for body weight in the modern turkey and that the growth of breast feathers from the cranial region of the breast tract appeared to be impaired. Modern birds spent more time resting than traditional turkeys.

Three possible causes of poor breast feathering were examined. First, the reduction in feather growth was an adaptive response to increased heat production resulting from fast growth rates. Second, there was competition between muscle and feathers for essential nutrients such as amino acids. Third, selection for increased breast muscle mass has not resulted in an increase in feather number and was associated with stretching of the skin and poor breast feathering.

Modern turkeys reared at high (26°C) and low (15°C) ambient temperatures showed no differences in feather growth. These turkeys were also fed *ad libitum* or restricted quantities of feed. Turkeys on restricted feeding showed a general decrease in feather growth apart from the cranial breast feathers that were increased in length.

Nutrition experiments suggested that, in the modern turkey, protein was preferentially partitioned to feather growth and that the amino acids arginine and methionine were used for feather growth in preference to muscle growth. When crude protein concentrations in the diet of the modern turkey were deficient, feather growth was maintained at the expense of body and particularly breast muscle growth. The impaired development of cranial breast feathers was associated with rapid growth of the breast muscle in the modern turkey and was not related to a deficiency of specific amino acids.

No increase in feather follicle number and a reduction of the collagen content of breast skin in the modern turkey support the hypothesis that development of the integument has not increased in proportion to body weight.

It was concluded that the impaired growth of cranial breast feathers was caused by the rapid growth and sedentary behaviour of the modern turkey resulting in prolonged pressure on the feather tracts of the breast.

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CHAPTER 1: Introduction and Literature Review

Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) cause depressed welfare and are an important source of economic loss in market turkeys (*Meleagris gallopavo*) through downgrading and condemnation of carcasses. Much of the work investigating causes of these conditions has been directed towards the environment such as wet litter, litter type or feeder designs which may cause friction to the breast. One aspect that has been noted by many workers but has received little attention is feather cover on the breast. Breast blister and button development, caused by environmental factors, is likely to be influenced by the amount of feather cover protecting the breast. It is possible that by improving feather cover on the breast the incidence of breast blisters and breast buttons can be reduced. This thesis examines feather growth in a modern commercial turkey and an unrelated traditional turkey and investigates possible causes of the lack of breast feathers in the large modern bird.

In this introduction, descriptions of breast blisters and breast buttons and factors affecting their development are summarised first. Feathers and their structure and function will then be described.

1.1 Breast Blisters and Breast Buttons

The sternal bursa, located in the subcutis on the ventral and anterior aspects of the keel bone, is a normal structure of turkeys and chickens that first becomes apparent microscopically when the birds are about four weeks old (Miner and Smart, 1975; Miner *et al.*, 1975). The sternal bursa is a true synovial sac with a typical synovial membrane of loose to dense connective tissue lined with flat to cuboidal mesothelial cells, which may be stratified. When the synovial membrane of the bursa becomes thickened and the cavity contains serum-like fluid it is called a "breast blister" (Miner and Smart, 1975). Breast blisters can be of various size and wall thickness, containing fluid in amounts from a fraction of a millilitre up to 70ml (Miner *et al.*, 1975). Thickening of the synovial membrane is the physiological response to pressure (Miner *et al.*, 1975).

Breast lesions in the turkey are of two types. Breast blisters are true, fluid filled blisters. Breast buttons (Figure 1.1) are calloused lesions characterised by a thickened area of skin (Parry, 1968). Breast buttons are skin ulcers that occur on unfeathered skin over the anterior keel of the turkey (Newberry, 1992). Ulcer margins are raised and the centre is found to be filled with a firmly adherent, hard scab (Gonder and Barnes, 1987).

Both breast blisters and breast buttons are unsightly lesions that are trimmed during processing, slowing line speeds, and resulting in a downgraded carcass (McEwen and Barbut, 1992). Downgrading is most costly when whole carcass turkeys are required. Turkeys must have an intact carcass, free of blemishes and unsightly lesions to

qualify as a premium product. Consequently these lesions are an important cause of economic loss in the turkey industry (Tilley *et al.*, 1996). Parry (1968) reported that a survey of turkey processors in the Kansas, Oklahoma, Missouri and Nebraska areas found that of 1 045 000 heavy stags, 8.5% had breast blisters serious enough to cause downgrading representing a total loss of approximately $102 000 to the producers. Cherry (1967) reported an estimate that downgrading due to breast blisters cost the American broiler and turkey industries 12 million dollars per annum. The results of a 1967 survey into poultry grading suggested that in the United Kingdom 5% were downgraded due to breast blisters (Cherry, 1967). Since then breast blisters have been reported to occur in between 11 and 62% of turkeys at slaughter (Miner and Smart, 1975) with breast buttons accounting for the downgrading of 20 to 30% of the heavy male turkeys processed in British Columbia (Newberry, 1992).

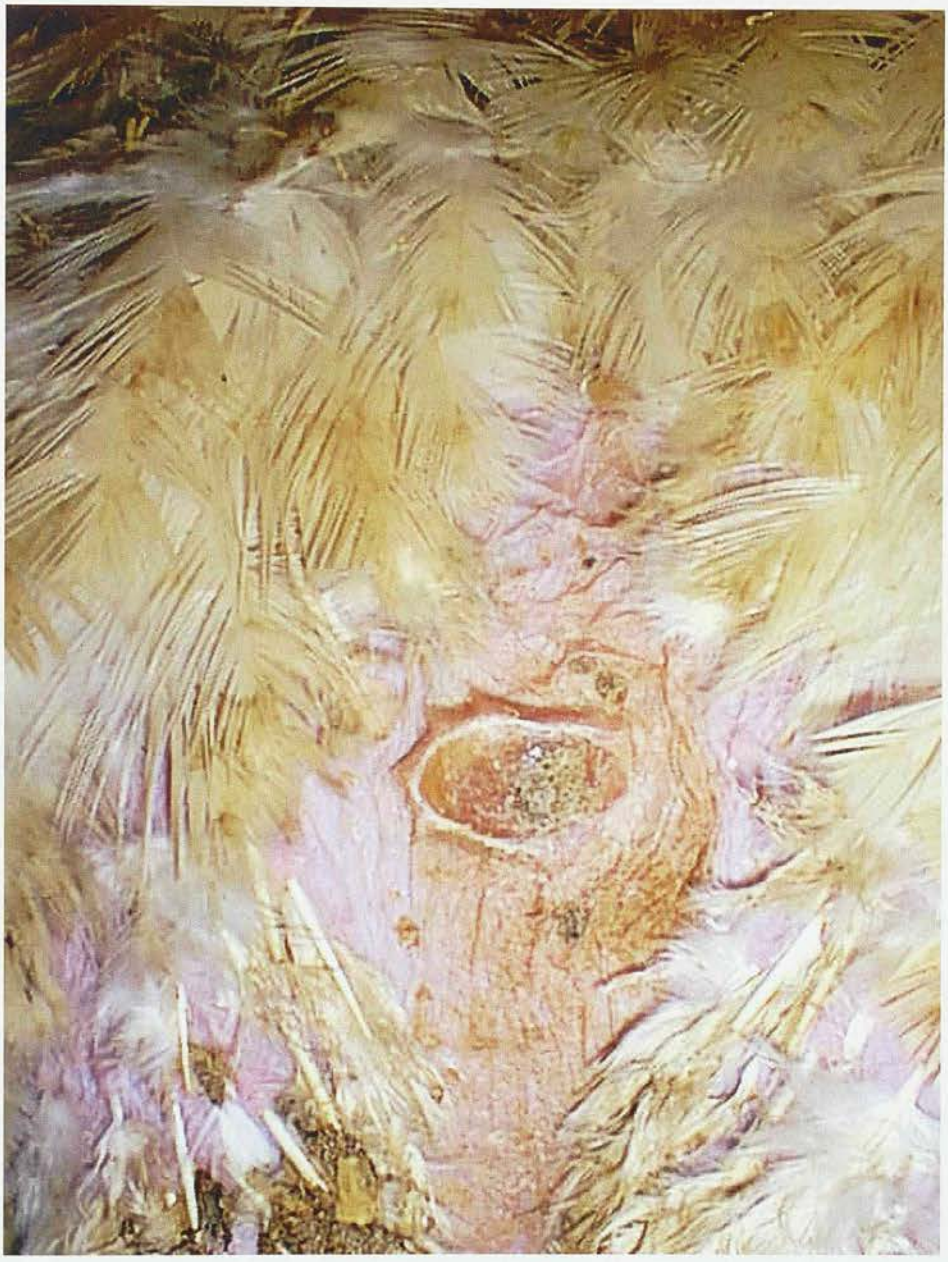


Figure 1.1 Breast button on the sternal apterium of a modern commercial turkey.

1.2 Factors Affecting the Development of Breast Lesions

The factors that give rise to the occurrence of breast lesions in turkey flocks are complex and not fully understood. However, certain factors are thought to have some influence on the frequency and severity of lesion formation (Parry, 1968). Studies investigating breast lesions usually refer to either blisters or buttons and this is reflected when the work of others has been referred to.

1.2.1 Infection

Miner and Smart (1975) investigated infection as a possible cause of blister formation. Bursas were taken weekly from 3 to 22 weeks of age, and no specific bacterial or viral infection was found. Bacterial cultures were also made of condemned bursas taken at processing and 91% were found to be sterile (Miner and Smart, 1975). The bacteria associated with blisters that were infected include staphylococci, streptococci and coliforms. These are likely to result from a secondary infection of the blister rather than being a causal factor. This finding was supported when antibiotics were found to have no effect on the incidence of blisters (Miner and Smart, 1975).

1.2.2 Management

Breast lesions are not due to a single cause, yet continual irritation through pressure or friction is probably the primary exciting factor (Miner and Smart, 1975). Adams *et al* (1967) also indicated that continuous irritation was required for breast blister

development. Good management can help reduce the incidence of breast lesions by minimizing the causes of irritation found in the poultry house environment.

The location of breast buttons in unfeathered breast skin suggests a contact dermatitis (Gonder and Barnes, 1987) as this skin is in most frequent contact with litter and wet litter has been associated with both contact dermatitis in chickens (Greene *et al.*, 1985) and pododermatitis in turkeys (Martland, 1984). Breast blisters or skin irritations have been observed when litter in the pen is wet. Martland (1985) found that the increase in pododermatitis caused by spraying the litter with water resulted in broiler chickens sitting on the litter for prolonged periods of time. The birds on the wet litter had a high incidence of breast skin lesions and breast blisters with the breast area caked with litter and faeces. May and Noles (1965) found that wetting the litter, to approximately double the moisture content, caused significant increases in incidence of breast blisters. A significant correlation between high litter moisture and breast buttons was reported by Gonder (1989). In contrast Tilley *et al* (1996) measured naturally occurring litter moisture and did not find it to be a significant factor in breast button development. Breast lesions may be caused by contact with ammonia or other noxious materials including faecal enzymes in the litter, as is the case with nappy dermatitis in humans (Berg *et al.*, 1986; Buckingham and Berg, 1986). High stocking densities are likely to affect litter condition and have been found to increase the incidence of breast blisters in broiler chickens (Cravener *et al.*, 1992).

Tilley *et al* (1996) showed that litter type significantly influenced the incidence of breast buttons in male turkeys, with the highest incidence found on coarse litter. Contrary to this finding, Miner and Smart (1975) found litter type to have little effect on breast blister development whereas incidence of blisters was found to decrease with increasing litter depth. The same study showed that birds raised in confinement on wire floors had the greatest number and most severe blisters.

Treatment of the litter with perlite and lime to improve its quality during work by Tilley *et al* (1996) did not significantly influence the incidence of breast buttons in male turkeys. Perlite was added to draw moisture away from contact with the breast skin of birds while they sat and lime was used to reduce pathogen load in the litter.

The tendency for poorer breast feathering and higher callus incidence at the end of the brooding period among poults brooded on wire or wood slat floors indicates that breast skin is likely irritated during the brooding period. However, apparent failure of early irritation to lead to blister formation suggests that additional factors must enhance severe blister development (Adams *et al.*, 1967). Tilley *et al* (1996) brooded male poults on crenelated paper to prevent early injury that might lead to the development of breast buttons but found no significant influence on the incidence of breast buttons.

Certain types of feeder, where the breast comes into contact with the feeder, increases the incidence of breast blisters (Miner and Smart, 1975).

Newberry (1993) investigated the role of temperature in the development of breast buttons and found that breast button incidence and size at 12 weeks increased in turkeys kept at cooler temperatures. This is contrary to the findings of Neufeld (1989), who observed a reduction in breast button incidence in flocks reared at progressively lower temperatures. A recent study found that increasing temperature increased the incidence of breast blisters and breast buttons but that increasing ventilation at the higher temperatures resulted in a similar incidence to that found at lower temperatures (Nixey, 1996). Temperature can also have indirect effects by influencing environmental conditions. Noll *et al* (1991) found that increasing temperature reduces litter moisture but also increases ammonia levels.

1.2.3 Age

The age at which a breast blister is most likely to be recognized in chickens, varies from seven to twelve weeks but could occur up to maturity (Miner and Smart, 1975). In turkeys, reports indicate that blisters are recognizable from 11 weeks of age to marketing (Adams *et al.*, 1967; Miner and Smart, 1975). May and Noles (1965) showed that breast blisters increase with increasing age of bird. Work by Newberry (1993) suggests that breast buttons first appear between four and eight weeks and the incidence increases between eight and 16 weeks although buttons can heal and disappear within a four week period. Recent work by Tilley *et al* (1996) found the incidence of breast buttons low prior to 10 weeks of age but thereafter increased to 18 weeks of age.

1.2.4 Genetic

A genetic basis for the tendency to develop blisters is evidenced by marked differences between sexes and strains of chickens and turkeys that are probably related to differences in body conformation, rate of growth and weight in relationship to age. These attributes of poultry are all heritable (Miner and Smart, 1975).

It has been documented that male chickens have up to 35 times as many blisters as females (May and Noles, 1965; Miner and Smart, 1975). In turkeys a sex difference has been observed on the processing line (Miner and Smart, 1975). Gonder and Barnes (1987) suggested that the increased incidence of breast buttons in males compared with females may be related to the increased water consumption by males compared with females. This could result in wetter litter in male houses, promoting adherence of irritating material to the breast skin. Differences in breast feathering patterns between the sexes may also exist, which could promote development of breast lesions if males are more poorly feathered than females (Gonder and Barnes, 1987).

The heavier the bird during the fast growing period, the greater the incidence of breast blisters (Miner and Smart, 1975). Faster growing chickens have been found to have significantly more breast blisters Miner and Smart (1975). Newberry (1993) found that breast buttons were associated with higher body weights at 8 and 12 weeks, although another report found no significant correlation between number of blisters and the live weight of turkeys (Cherry, 1967). Adams *et al* (1967) considered

that at the age when there is the highest occurrence of breast blisters, turkeys, and particularly the males, have reached a relative stage of growth where the differential between muscle development and skeletal development is such that they tend to become improperly balanced. Rasplicka and Fry (1963), from a study of the relationship between body conformation and breast blister formation, concluded that body conformation did affect the incidence of breast blisters and consequently led to strain variations. Turkeys with wide breasts and shallow bodies were more prone to develop breast blisters than those with wide breasts and deep bodies (Parry, 1968; Rasplicka and Fry, 1963). Tilley *et al* (1996) compared the three most popular commercial turkey strains and found that the strain of male turkey can significantly influence the incidence of breast buttons.

1.2.5 Skin Thickness

In work by Adams *et al* (1967) the thickness of the breast skin of turkeys was measured, and there was some indication that differences in thickness at an early age could be used to predict which turkeys would develop blisters. The thicker the skin the more likely blisters were to form. Fold of the breast skin thickness was measured by a caliper to the nearest 0.25mm (Adams *et al.*, 1967). With the exception of the 8 week examination, a highly significant correlation was observed between breast skin thickness and percentage of turkeys that subsequently developed breast blisters (Adams *et al.*, 1967). The turkeys that developed breast blisters had thicker breast skin over the keel bone than those that showed no evidence of blisters (Adams *et al.*,

1967). As the turkeys matured their breast skin increased in thickness (Adams *et al.*, 1967).

Reports suggest that the number and severity of blisters in chickens and turkeys are increased when pressure and friction on the sternal bursa cause the synovial membrane of the sternal bursa to enlarge (Miner and Smart, 1975).

1.2.6 Feather Cover

The observation that good feather coverage on the bird's breast may reduce blisters by protecting the breast area from various environmental conditions has also been reported (Adams *et al.*, 1967). Miner and Smart (1975) state that the amount and completeness of feather covering of the keel reduced the incidence of blisters in chickens. Kondra and Cavers (1947) agree that feathers have a protective action and suggested that the breast feathers reduce the effect of pressure on the bursa.

Gyles *et al* (1961) selected two divergent broiler breeder lines for a high and low incidence of breast blisters and found that the blister line was less well feathered and heavier. Their results indicated a very close association between the presence of blisters and poor feather coverage of the breast. Newberry (1993) found that heavier turkeys had more breast buttons, poorer feather cover over the keel and a wider area of unfeathered skin across the keel at 8, 12 and 16 weeks than lighter turkeys. Newberry (1992) earlier reported an association between breast button presence and width of unfeathered skin over the keel at 17 weeks. The positive association

between body weight and unfeathered skin width is probably due partly to feather loss and partly to the greater surface area of large turkeys and, therefore, greater distance between the first feather tract on either side of the midline (Newberry, 1993).

In an experiment by Miner and Smart (Miner and Smart, 1975) complete feather covering of the keel significantly influenced the number of blisters, as 60.5% of the turkeys with blisters had no feather cover, 37.4% had partial feather cover and 1.2% had complete cover. Breasts covered with sheepskin to protect against pressure and friction showed a significant difference between protected (12%) and unprotected (24%) turkeys in percentage of blisters (Miner and Smart, 1975).

Inadequate feather covering occurs naturally in birds with late feathering or poor feathering characteristics. Observations by Hocking (Hocking, 1995) suggest that feather growth in modern compared with traditional strains of turkey is poor and feathers may be absent over the breast region, exposing the skin to noxious material in the litter. Breast buttons may develop as a consequence of damage to the skin and feathers protecting the skin, due to abrasion when turkeys lie down on coarse litter material (Newberry, 1993).

Reduced feather cover has been produced artificially by clipping the feathers and had no effect on the incidence of breast blisters (Miner and Smart, 1975). Conversely Funk and Savage (1956) showed a higher incidence of breast blisters in birds that had breast feathers removed by plucking and suggested that feather coverage of the breast

was an important factor related to the incidence of breast blisters. Observations by Newberry (1993) show that buttons sometimes appeared to be located in line with the first line of feather follicles on either side of the keel and may have been located in feather follicles from which feathers were missing.

1.3 Prevention of Breast Lesions by Improving Feather Cover

Although much work has been carried out to examine the causes of breast blisters and buttons in both chickens and turkeys, little progress seems to have been made in reducing the incidence of these conditions. It is interesting that the most recent work that examined the incidence of breast buttons in turkeys focused on litter and strain (Tilley *et al.*, 1996) both of which have been looked at in previous work. It is likely that more than one factor causes breast blisters and breast buttons and the interaction between different factors requires further study. Breast blisters and buttons are a common problem in modern turkeys and, as noted above, feathers may offer some protection to the breast from possible causes of these conditions. As described in the previous section feather growth in modern compared with traditional strains is poor and feathers may be absent over the breast region (Hocking, 1995). Three hypotheses to explain these differences in feathering are now proposed: First, selection for increased breast muscle in the modern turkey may have resulted in poor feather cover over the breast if the skin has stretched to cover a larger area without the number of feathers and feather follicles increasing. Second, there may be competition between muscle and feather growth for essential nutrients such as amino acids or minerals. Third, in order to regulate body temperature resulting from fast growth rates in the

modern turkey a reduction in feather growth may be an adaptive response by the birds to their increased heat production.

By investigating the breast feathering of turkeys it may be possible to improve feathering in the modern bird and to reduce the incidence of breast blisters and breast buttons.

1.4 Feathers

The three principal functions of the feathers are flight, insulation and waterproofing. Feathers may also function in courtship, defence and aggressive territorial behaviours. It is interesting that little importance has been given to feather growth, with few studies concentrating on this area.

The rate and extent of feathering can have an effect on food conversion rate by influencing the birds energy requirements to maintain its body temperature. Feathers provide an excellent insulative cover, and so degree of feathering influences heat balance (Deschutter and Leeson, 1986). Richards (1977) reported that poorly feathered birds have a 60% higher metabolic rate compared with normally feathered birds. An increase in heat production is associated with a decrease in feed efficiency and Leeson and Morrison (1978) indicated that feed efficiency in laying hens was significantly correlated with feather cover.

It is also likely that feather cover will have a major influence on breast skin blemishes such as blisters and buttons by providing protection from mechanical damage. Before looking at feather growth and cover on the breast of turkeys it is useful to understand a little about feather cover and feather structure in general.

The majority of the work describing feathers has been extensively reviewed by Lucas and Stettenheim (1972) with little further work appearing in the literature. The description of turkey feathers has mainly been based on the bronze turkey and it is possible that differences may exist between this and the modern large white turkey.

1.4.1 Pterylae and apteria

Certain areas of the turkey such as the beak, eyes and feet are free of feathers with plumage also being absent in the tarsometatarsal region.

Contour feathers are confined to discrete tracts or pterylae (Figure 1.2) in the plumage covered regions with these being separated by extensive featherless spaces, apteria (Lucas and Stettenheim, 1972). These bare areas are usually covered by the contour feathers of adjacent pterylae. Apteria have no contour feathers, although semiplumes can sometimes be present.

The number of pterylae varies between different species of birds: nearly 70 have been identified in the domestic fowl (Lucas and Stettenheim, 1972). Pterylae are of physiological importance as centres of plumage growth and have therefore been

named individually (Voitkevich, 1966). By originating from tracts rather than being scattered randomly over the body, feathers can smoothly overlap each other and follow the natural contours of the bird.

Apteria allow feathers to provide an effective covering without overcrowding and probably evolved after the development of contour feathers (Spearman and Hardy, 1985). If feathers covered the entire surface, it would provide unnecessary extra weight, especially for flight.

The area of interest in relation to breast blisters is the sternal tract (Fig. 1.2). Lucas and Stettenheim (1972) give the following description of this area.

"Feathers at the anterior end of the sternal tract are widely spaced and transitional toward feathers of the adjacent pectoral and ventral cervical apteria. The remainder of the sternal tract is strong, as is its continuation caudally where it becomes the medial abdominal tract. The sternal tract is divided by the sternal apterium located on the ventral edge of the keel. This space, entirely devoid of down feathers, continues into the abdominal region where it is continuous with the medial abdominal apterium. The sternal and abdominal tracts have an approximately uniform width throughout their length."

It is on the sternal apterium that breast blisters and buttons are commonly found.

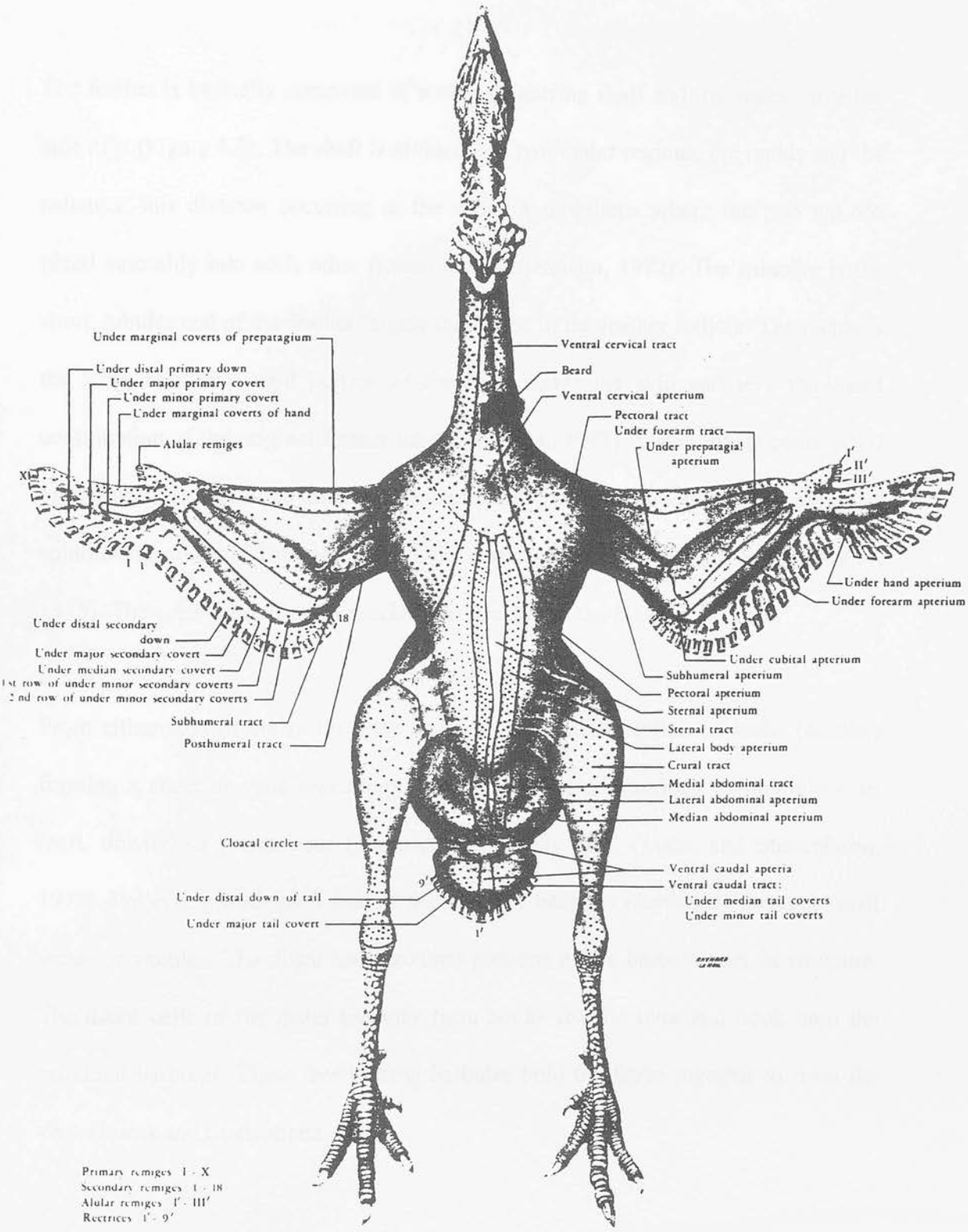


Figure 1.2 Ventral view of the pterylosis of an adult male Bronze Turkey.

From Lucas and Stettenheim (1972).

1.4.2 Feather Structure

The feather is basically composed of a central tapering shaft and the vanes on either side of it (Figure 1.3). The shaft is divided into two major regions, the rachis and the calamus, this division occurring at the superior umbilicus where the two regions blend smoothly into each other (Lucas and Stettenheim, 1972). The calamus is the short, tubular end of the feather largely implanted in the feather follicle. The rachis is the long, relatively rigid portion of the shaft above the skin and is a thickened continuation of the original feather tube (Spearman, 1971). The rachis is constructed of a central medulla of air-filled keratinised epithelial cells and a solid outer cortex of spindle-shaped cells. The cytoplasm of the cortical cells is filled with keratin (Auber, 1955). The cortex provides the mechanical strength of the feather.

From either side of the rachis arise a series of parallel branches or barbs (*barbae*) forming a sheet or vane (*vexillum*). The vane is either described as plumulaceous (soft, downy) or pennaceous (compact and closely knit) (Lucas and Stettenheim, 1972). Projecting from each side of the barb are barbules (*barbulae*) forming small vanes or vanules. The distal and proximal portions of the barbule vary in structure. The distal cells of the distal barbules form hooks that lie over and hook onto the proximal barbules. These interlocking barbules hold the barbs together to form the vane (Lucas and Stettenheim, 1972).

There are seven basic types of feather: contour feathers, remiges and retrices, downs, powder downs, semiplume, filoplume and bristle. These represent the main structural types of feathers (Lucas and Stettenheim 1972).

FEATHER STRUCTURE

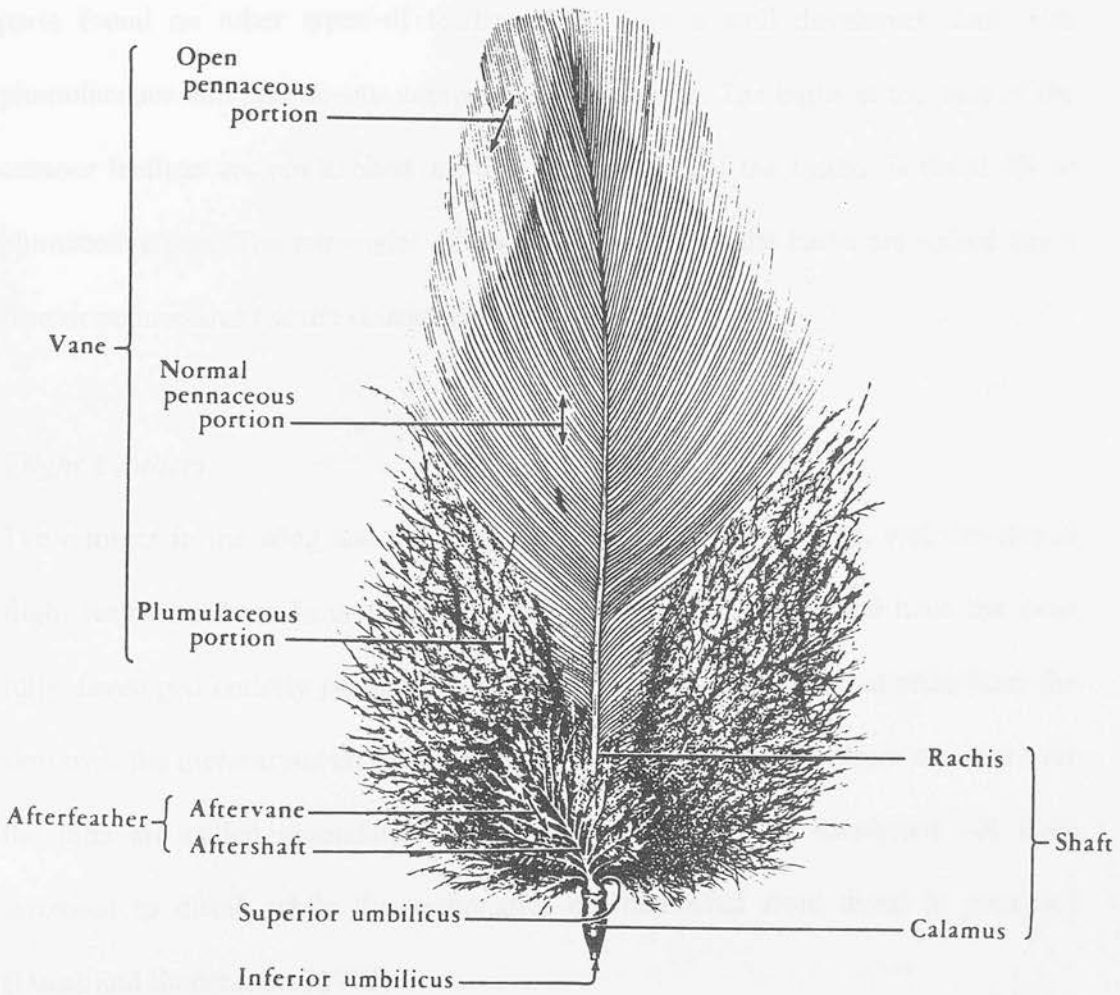


Figure 1.3 Main parts of a typical contour feather.

From Lucas and Stettenheim (1972).

Contour Feathers

The contour feathers (*pennae contourae*) are predominant on a bird's body and make up the surface plumage of adult birds. This feather type incorporates nearly all the parts found on other types of feather. They have a well developed shaft with plumulaceous and pennaceous components of the vane. The barbs at the base of the contour feathers are not hooked together and this part of the feather is the fluffy or plumaceous part. The remainder of the feather in which the barbs are united has a firm or pennaceous texture (Lucas and Stettenheim, 1972).

Flight Feathers

The remiges in the wing and the rectrices in the tail are large, stiff, well developed flight feathers. These feathers are generally asymmetric in form and have the most fully developed entirely pennaceous vane structure. The remiges that arise from the skin over the metacarpus are called primaries and those that arise from the skin over the ulna are called secondaries. Primaries (usually 10) are numbered I-X from proximal to distal, while the secondaries are numbered from distal to proximal (Lucas and Stettenheim, 1972).

Down Feathers

Down feathers (*plumae*) are small and fluffy and have a wholly plumaceous vane with the rachis being shorter than the longest barb or absent altogether. This type of feather occurs in the young chick and they also form a thermoregulatory undercoat in adults (Lucas and Stettenheim, 1972).

Powder Down

Powder downs (*pulviplumae*) are specialised down feathers that disintegrate producing powder (keratin) that is spread through the feathers during preening. They are found throughout the body among down and contour feathers. They are not present in all birds (Lucas and Stettenheim, 1972).

Semiplumes

Semiplumes (*semiplumae*) are plumaceous feathers that occur along the margins of the contour feather tracts as well as singly within the tracts and in the apteria. The semiplume can be distinguished from the down feathers as the rachis is longer than the longest barb (Lucas and Stettenheim, 1972).

Filoplumes

These feathers (*filoplumae*) have a long hair-like rachis with a bunch of flexible barbs at the tip. They are present in all feather tracts and generally accompany contour feathers. Filoplumes are the only feathers which do not have muscles attached to the follicles. They are thought to be concerned with the sensory input for controlling the position of larger feathers (Lucas and Stettenheim, 1972).

Bristles

Bristle feathers (*setae*) have a stiff, tapered rachis, and barbs that are generally restricted to the proximal end. Bristles occur on the head and around the eyes (Lucas and Stettenheim, 1972).

1.5 Skin

The skin of birds has a basic structure consisting of an outer epidermis and inner dermis. The epidermis is thin over most of the body and consists of three layers including the basal (germinative) layer, intermediate layer and outer cornified layer. The dermis is a layer of connective tissue composed of superficial and deep layers. The superficial layer contains loosely arranged layers of collagen but in the deep layer collagen fibrils are arranged more densely in interwoven bundles that run roughly at right angles to each other and mostly horizontally to the skin surface (Spearman and Hardy, 1985). Fat, feather follicles, smooth muscles that control movement of the feathers and nerves that supply the dermis and epidermis are also found in the deep layer (Pass, 1989). Arteries and veins supply large blood capillary plexuses just beneath the epidermis and in the deep dermis with some capillaries entering the dermal papillae of feather follicles (Lucas and Stettenheim, 1972). Sensory nerve endings (Herbst corpuscles) occur throughout the dermis, particularly associated with feather follicles (Spearman, 1971). The skin is glandless except for the uropygial (preen) gland, glands of the ear canal and glands in the vent region. The secretions from the uropygial gland, in combination with lipids from the keratinocytes, are involved in maintaining feather condition (Lucas and Stettenheim, 1972).

1.6 Follicle Development

Feathers are keratinised cellular derivatives of the epidermis and are formed in follicles that penetrate into the deep dermis (Spearman and Hardy, 1985). Virtually all feather follicles are formed during embryo development and all of the follicles formed at this stage will persist throughout life (Lucas and Stettenheim, 1972). Development of feathers in the embryo begins with formation of the feather germ, a condensation of dermal cells (dermal papilla) beneath a cluster of elongated epidermal cells (placode) (Lucas and Stettenheim, 1972). Cell proliferation results in the feather germ projecting upwards from the surface forming a dome shaped projection of the epidermis over the raised dermal papilla (Spearman, 1971). Downward growth of the cells at the base of the germ into the dermis produces an invagination of epidermis which constitutes the feather follicle (Spearman, 1971). Differentiation of the epidermis in the invagination results in an outer follicular part and inner cylindrical feather tube. At the base of the feather germ the epidermis thickens and forms a ring (epidermal collar) around the dermal papilla. The majority of subsequent growth originates from the epidermal collar. The basement membrane of the outer follicular epidermis is continuous with the inner surface of the feather tube. The cylinder of pulp, encircled by the feather epidermis, resembles the dermis and has a central (axial) artery (Pass, 1989).

1.7 Feather development

There are two basic components of the growing feather, the outer epidermis and the inner pulp. The epidermis differentiates into the barb ridges and rachis as it grows longitudinally. It also develops laterally from a basal layer to a keratinised layer which becomes the sheath (Pass, 1989). The feather while still in its sheath is called the pinfeather. The pulp also grows up with the epidermis and distally is covered by epidermis (pulp cap). Cells arising from the base differentiate as they progress to the peripheral regions of the growing feather (Deschutter and Leeson, 1986). Once the basic shape and structure of each barbule and barb has been formed the process of keratinisation begins (Lucas and Stettenheim, 1972). The feather sheath encloses the rachis and barbs as they develop but as the tip of the feather fully keratinises and dries after emergence from the follicle the sheath starts to split allowing the rachis and barbs to emerge and spread out. As this happens and the feather grows longitudinally the pulp regresses by degeneration and resorption of the connective tissues (Pass, 1989). The keratinised pulp cap remains, the new distal end of the pulp is re-epithelialised and the process is repeated. Some distal pulp caps disintegrate as they are exposed but others remain and are visible in the calamus. The calamus becomes completely keratinised when longitudinal growth is completed. It sits on top of the dermal papilla anchoring the feather in place and does not contain pulp (Spearman and Hardy, 1985).

1.8 Feather composition

Feathers are mainly composed of the insoluble and indigestible protein keratin (Graber *et al.*, 1971) and are made up of varying proportions of protein and water with small amounts of ash and lipid (Martin *et al.*, 1994). Feather moisture decreases with age and there is a proportional increase in protein concentration with age.

Fisher *et al.* (1981) reported that feather growth and feather composition can be influenced by dietary specifications, therefore reported feather amino acid values will be specific to the diet composition used. The composition of both total protein and individual amino acids was found to vary with age but no sex difference was observed (Fisher *et al.*, 1981). The methionine content of feathers decreased with age while that of threonine, isoleucine and valine increased (Fisher *et al.*, 1981). Stilborn *et al.* (1997) also showed that the protein content of feathers was influenced by age and not breed or sex. Total non-essential amino acids as a percentage of total essential amino acids were found to increase with age indicating that feather compositional changes had occurred (Stilborn *et al.*, 1997). During the first 6 weeks of life the change from down to full feather cover caused the greatest change in composition. Stilborn *et al.* (1997) confirmed the previous reports that feather methionine content decreased and valine increased with age but also showed that phenylalanine and alanine content increased initially before declining. Differences have also been found in the amino acid composition of the morphological parts of the feather (Harrap and Woods, 1964). Emmans (1989) used previously published

reports to produce mean values for the comparison of the essential amino acid contents of body and feather proteins. The mean values are reproduced in Table 1.1.

1.8.1 Keratin

Feather cells contain a mixture of proteins that are bonded together at the time of cell death to form the durable keratin complex, which is resistant to hydrolytic enzymes and bacterial decay (Brush, 1978). Keratins comprise approximately 85-90% of the total protein in feathers (Harrap and Woods, 1964). Feathers contain the pleated form of keratin. The pleated sheets of feather keratin are twisted to form β filaments. In feather keratin a cystine-rich zone occurs in the peripheral region of each filament and is sometimes regarded as a cystine-rich matrix, but it is joined to the pleated sheets, probably as side chains (Fraser and MacRae, 1980).

Table 1.1 The essential amino acid contents of body and feather proteins (g/kg). Values from Emmans (1989).

Amino acid	Body protein	Feather protein
Arginine	68	65
Cystine	11	70
Histidine	26	8
Leucine	71	70
Isoleucine	40	40
Lysine	75	18
Methionine	25	6
Phenylalanine	40	45
Threonine	42	44
Tryptophan	10	7
Tyrosine	31	50
Valine	44	60

The mixture of proteins in feather keratin can vary from site to site in the same species. A composite structure is provided by the mixture of filamentous proteins and randomly orientated side chains, which in engineering terms is known to provide great strength with elasticity (Fraser *et al.*, 1972).

Despite the common features in feather development, feathers show great morphological diversity, even on the same bird (Chuong, 1993). In order to produce the complex feather structure, the pattern of the feather must be genetically imprinted on the follicular epidermis so that only certain cells are keratinised (Spearman and Hardy, 1985).

1.8.2 Feather Strength

Feathers differ considerably in their mechanical properties from hairs, and the α fibrils in hairs and β fibrils in feathers are mainly responsible for these differences. Astbury and Marwick (1932) found that hairs and feathers differed in tensile properties, the latter being much less extensible before rupture of the keratin than hairs.

Purslow and Vincent (1978) found that the shape and size of the cortex of the shaft appeared to be the most significant feature in relation to bending behaviour. They also showed that differences in the shape of the cortex in the outermost primary and those proximal to it account for different mechanical properties. The shape and size of the cortex was shown to have some relation to the body weight of the bird (Purslow and Vincent, 1978).

The rachis of primary feathers is a cantilever beam structure whose bending behaviour is controlled by the flexural stiffness along its length (Bonser and Purslow, 1995). Bonser and Purslow (1995) investigated the stiffness of feathers using tension testing and concluded that the flexural stiffness of the whole rachis is principally controlled by its cross-sectional morphology rather than by the material properties of the keratin.

Melanic feather keratin has been shown to be more effective at resisting abrasive wear than non melanic keratin (Bonser, 1996).

1.9 Plumage Renewal and Moult

Moulting is the process whereby the growth of a new feather causes the shedding of an old feather. Feather follicles go through cycles of growth (anagen), followed by a rest period (telogen) (Spearman and Hardy, 1985). When feather growth is complete the mature feather remains held in the follicle. As feather growth is renewed the new feather grows up beneath the old feather loosening it from the follicle and finally pushing it out. Therefore once a new feather has been stimulated to grow in the follicle the moulting process is purely mechanical and is dependent on the developing generation of feathers.

The single generation of feathers that occurs as a result of a moult is collectively known as plumage. At any one time a bird may have feathers derived from more than one moult (Lucas and Stettenheim, 1972). Feather moult occurs over a prolonged period with neighbouring follicles moulting at different times to prevent baldness occurring (Spearman and Hardy, 1985). Each pteryla is a separate physiological moulting unit, with renewed feather growth commencing in the centre and then spreading to other follicles around it (Voitkevich, 1966). The sequences of plumage replacement in different pterylae are coordinated by a genetic plan of feather moult (Palmer, 1972). Collectively the feathers present on the body at one time regardless of when they first appeared are called the feather coat.

The initial coat of feathers is derived during development of the embryo and closely resembles the soft down feathers of the adult bird (Spearman, 1971). The first moult

occurs shortly after hatching and replaces the natal down resulting in a second generation of feathers. This second plumage provides the chick with a more mature feather coat similar in structure to the adult contour feathers. The second moult in a juvenile leads to the third plumage, although many second and third generation feathers will be present at the same time. The third moult occurs with the growth of the fourth generation of feathers, this being the definitive adult plumage (Lucas and Stettenheim, 1972).

The moulting process in adult birds occurs on a cyclic basis. Domestic poultry however, reproduce year round mostly under artificial lighting conditions and may not undergo the seasonal moult that would be seen in free-ranging poultry. Flocks are usually depleted before a seasonal moult would take place since moulting and egg production cannot occur together (Spearman and Hardy, 1985).

The control of moulting is extremely complex and only partially understood. The moulting process probably involves a combination of hormonal, seasonal, nutritional and feather follicle factors (Lucas and Stettenheim, 1972).

The involvement of sex hormones in the control of feather growth and moult has been studied mainly in the domestic fowl and pigeon (Spearman, 1971; Voitkevich, 1966). Oestrogen retards feather growth, while testosterone has little effect on feather formation although gonadectomy allows prolonged moult and continuous renewal of plumage (Spearman, 1971). Progesterone will stimulate feather growth in follicles that are already replacing a feather but will not stimulate feather development.

Peczely (1992) investigated hormone receptors involved in feather growth and found high levels of androgen and oestrogen binding sites during the rapid growth of new feathers. A multi-steroidal regulation of feather follicle function was suggested. Herremans *et al* (1993) demonstrated the presence of progesterone and oestrogen receptors in the feathers and skin of adult hens. The immunocytochemical study found both receptor types to be present in the dermal papillae and the epidermal germinative layer cells of growing and mature feathers.

Thyroxine initiates growth in adult feather follicles with the exception of the remiges that, like plumage growth in the young chick, are independent of hormones (Voitkevich, 1966). Administration of thyroxine can induce a moult in fowl. Removal of the thyroid stops feather formation on the body but the moult of wing feathers will continue. Administration of high concentrations of thyroxine will increase the speed of the moult cycle (Pethes *et al.*, 1982).

1.10 Factors Affecting Feather Growth

Genetics, hormonal balance (Section 1.9) and nutrition are involved in normal feather development (Fuller and Wilcke, 1942). The physical characteristics and appearance of the feather are controlled by factors that affect the development of the feather at the edge of the epidermal collar. Any infectious agent or systematic abnormality that alters the nutrients or blood supply available to the developing feather will alter its appearance. Additionally damage to the epidermal collar will be manifested as an abnormal feather (Pass, 1989).

1.10.1 Nutrition

The nutritional adequacy of the diet appears to be the major factor influencing both the structure and growth of feathers (Deschutter and Leeson, 1986). A feather grows from the epidermal collar of the papilla producing growth bars on the feather perpendicular to the rachis (Grubb, 1989). Translucent growth bars across the vane of a feather are frequently referred to as stress marks. These abnormalities represent segmental dysplasia that occurred in the developing barbs and barbules and represent a brief period of dysfunction in the epidermal collar. A brief period of food deprivation would be expected to induce these lesions. Under more severe nutritional conditions there may be insufficient energy or specific nutrients available for adequate deposition of keratin in the central rachis and the entire feather may break off where the rachis is weak (Grubb, 1989). In a structural study of broiler breeder feathers, Sparks (1994) observed no differences in the structure of the rachis, barb or barbule between *ad libitum* or restricted fed groups, suggesting that the restriction was not severe enough to cause feather abnormalities. The general appearance of feathering in restricted turkeys was found to be much better than that of full fed turkeys (Etches *et al.*, 1993).

Ptilochronology is described as a technique that "uses the width of daily growth bars on a feather as an index of a bird's nutritional condition" (Grubb, 1989). This technique has been adapted by Brodin (1993) using radioactively labelled food. Ingested radioactive sulphur, ^{35}S, in amino acids is readily incorporated in growing feathers. After ingestion the radioactivity is deposited in the part of the feather that

develops during the next days and, using autoradiography, a picture can be developed showing radioactive segments of the feather as dark bands (Brodin, 1993). This technique has been used to study the nutritional status of food hoarding bird species (Brodin, 1993).

Amino acids

Dietary protein is required for the maintenance and growth of body and feather protein. There is variation in the proportion of total protein deposited as feather protein during growth and the amino acid composition of feather protein is different from body protein (Table 1.1) so nutrient requirements for feather and body growth will differ (Martin *et al.*, 1994). Diets with higher protein concentrations have been found to produce better growth and feathering than lower protein diets (Glazener and Jull, 1946).

Dietary amino acids play a critical role in feather development since 89-97% of the feather dry matter is protein (Fisher *et al.*, 1981). With each moult the protein in the old feathers is lost and new feather growth requires a supply of amino acids. Each successive juvenile feather coat is heavier than the previous one and will therefore require an increased input of amino acids. Deficiencies of the dietary essential amino acid concentrations reduce the growth rate of feathers and produce abnormal feather structures.

Improper growth of feathers can be seen in several ways. Among the most obvious are faulty structure of the feather itself and abnormal plumage patterns resulting in the poor appearance of the bird (Fuller and Wilcke, 1942).

Cystine and methionine, the sulphur containing amino acids, are the major amino acids involved in the synthesis of feather keratin (Wheeler and Latshaw, 1981). Cystine and methionine are required for general maintenance and growth in addition to their role in feather synthesis. The relative proportion of the sulphur containing amino acids is much greater in the integument than in muscle tissue, and marginal dietary deficiencies of these amino acids will often be initially manifested as abnormal feathering (Deschutter and Leeson, 1986). Leong *et al* (1959) observed poor feathering in the absence of supplements of methionine in chickens fed on low protein diets and found that the requirements for methionine and cystine increase with increased dietary protein concentrations. Cystine and methionine are frequently the first limiting dietary amino acids, therefore accurate determinations of the sulphur containing amino acid requirements during various stages of growth are necessary to ensure optimum body and feather growth.

Sheridan and McDonald (Sheridan and McDonald, 1963) proposed that amongst the substrates required for feather and body growth the amino acids arginine and cystine could be the limiting factors. Both of these amino acids are required in relatively large amounts during feather synthesis and could be involved in an apparent interaction between feather protein and body protein synthesis.

D'Mello and Emmans (1975) noted poor feather development in turkeys fed diets containing low arginine concentrations. Chicks fed arginine deficient diets had feathers that were more brittle and easily broken with a thin and ragged appearance and the barbs were less well developed (Anderson and Warnick, 1967). Earlier reports indicated similar findings characteristic of an arginine deficiency including a spoon like appearance of the chick's primary and secondary feathers caused by the retention of an abnormally long sheath that covered the proximal end of the feather shaft (Sanders *et al.*, 1950). Furthermore normal barbs maintained the integrity of the vane only at the tip of the feather, with the remainder of the barbs and barbules limp and degenerate. The addition of arginine to the diet was shown to correct abnormal feathering of the chicks.

Feathers showing signs of amino acid deficiency have also been noted in chicks fed rations deficient in valine, leucine, isoleucine, phenylalanine, tryptophan, lysine, glycine and tyrosine (Anderson and Warnick, 1967). The feather abnormalities described above were also characteristic of these deficiencies although the degree to which the abnormality progressed varied with the individual amino acid and the severity of the deficiency (Anderson and Warnick, 1967). D'Mello (1975) observed poor feather development in turkeys on a low isoleucine diet but not in turkeys fed low leucine or valine diets.

A deficiency of dietary lysine during growth also results in feathers with no melanin pigment (Grau *et al.*, 1989). The failure to develop normal feather pigmentation in turkeys was found to be prevented by adding lysine to the diet (Anderson and

Warnick, 1967). One way by which lysine deficiency causes depigmentation is by inhibiting activity of the enzyme tyrosinase in the feathers thereby inhibiting formation of the melanin pigment (Owings and Balloun, 1959).

Although amino acid deficiency can be indicated by abnormal feathering, antagonisms or imbalances of various amino acids can often result in similar abnormalities of plumage (Deschutter and Leeson, 1986).

Vitamins and Minerals

Vitamins and minerals play a significant role in feather development. Supplee (1966) observed discoloured and atrophied calami of the flight feathers followed by bleeding of the feather pulp in turkey poults fed rations deficient in vitamin E and selenium.

Zinc is involved in keratogenesis (Underwood, 1971) and has been shown to be a structural component of the feather (Rahman *et al.*, 1961). A zinc deficiency therefore has a detrimental effect on feathers. Rahman *et al* (1961) found that pullets fed a zinc deficient diet had poor feathering, brittle feathers and unlaced thorny barbules on the feathers. In zinc deficient quail the feathers do little more than emerge from the follicle and when they do grow longer, they are ragged and break easily (Fox and Harrison, 1964). It would appear that zinc deficiency affects feather strength and feather growth.

Dietary deficiencies of pantothenic acid, folic acid, vitamin B12, pyridoxine, biotin and niacin are further nutritional causes of poor feather structure in poultry (Elliot, 1996).

1.10.2 Environment

Rizzi (1994) showed that traits concerning plumage had low to moderate heritability estimates and were greatly influenced by the environment. Housing conditions appear to influence the completeness of plumage. Feathers can be damaged as they grow due to abrasion on the surfaces within the housing environment. A comparison of new and built up litter showed that broilers were better feathered on new litter suggesting that litter quality can influence feather growth (Harris *et al.*, 1980).

Temperature and humidity have also been found to influence the rate of feathering. Radi and Warren (1938) reported that feather growth was more rapid at low temperatures and at higher humidity.

1.10.3 Genetics

The literature relating to single gene variation in the turkey has been reviewed by Savage (1990). Four mutations which affect feathers have been studied and a further nine single gene loci have been documented that directly influence plumage colour or patterns in turkeys. A brief description of the four mutations affecting feathering is given below.

Hairy

An absence of normal webbing caused by the separation of barbs from each other is characteristic of the abnormal hairy feathers described by Smyth (1954). These feathers are found in all pterylae of affected birds and the effect of the disorder is associated with barbule structure and numbers. The condition was established as being an autosomal recessive trait and the mutant gene given the symbol *ha*.

Naked

This mutation restricted feathering to the caudal margins of the wings, and to contour feathering on the elbows, wing webs, shoulders and breast. Extra hind toes and deformity of the feet and legs were associated with the naked condition that was inherited as an autosomal recessive trait. The naked (*na*) gene in the turkey was not found to be homologous with sex-linked nakedness of chickens (Savage, 1990).

Knobby

The knobby (*kn*) condition resulted from an autosomal recessive gene with complete penetrance. This caused a semilethal down abnormality affecting plumule structure in newly hatched poults. The number of plumules with large accumulations of undiferentiated cellular material in the distal segments was related to mortality. Although the plumage of juvenile and adult knobby mutants was rough, there were no other apparent feather abnormalities (Savage, 1990).

Late feathering

The late feathering (*K*) condition was inherited as a dominant sex-linked trait. Late feathering results in a reduction or absence of primary remiges in poults at hatching. An absence of flight and tail feathers at maturity identified the late feathering birds. A similar mutant that does not result in the absence of any feathers is present in chickens and is used extensively for feather sexing of day-old chicks. At present the gene is not used commercially for sexing turkey poults (Savage, 1990).

The recessive sex linked gene controls early feathering in chickens with the dominant allele producing late feathering birds (Sheridan and McDonald, 1963). In work with broiler chickens, Sheridan and MacDonald (1963) showed that there appeared to be competition between the body of the bird and its feathers for common substrates during the first few weeks of life. It is during this period that there is an extremely rapid increase in body size which could explain why later feathering birds are heavier at five weeks of age, whereas at ten weeks the early feathering birds are heaviest (Sheridan and McDonald, 1963). Energy is required for both body and feather growth, therefore slow feathering will allow more energy to be available for body growth during the period of rapid body weight increase. With the slow feathering birds the decrease in the amount of dietary protein directed into feather protein makes available more protein for skeletal and lean tissue (Ajang *et al.*, 1993).

1.11 Outline of Thesis

The aim of this thesis is to describe feather growth in a modern commercial turkey compared with that in an unrelated traditional turkey and to investigate the lack of breast feathers in the large modern bird. The experiments reported here quantified feather growth in the two strains of turkey and investigated three possible causes of poor breast feathering in the modern turkey.

Procedures common to several experiments are described in Chapter 2.

The aim of Chapter 3 is to obtain a quantitative comparison of feather growth and body growth in a modern commercial turkey strain with that in an unrelated traditional line. Differences in the growth of breast feathers between the two strains are confirmed. As the modern turkey is growing rapidly to a heavy body weight it is possible that this bird will be less active than the traditional turkey and spend a greater proportion of time resting. To test this hypothesis resting behaviour is examined in the modern and traditional turkey. An increase in time spent resting may increase breast feather loss or damage or change breast skin temperature in the modern turkey.

The modern turkey has been selected for rapid growth and this may lead to increased heat being produced that must be lost in order to maintain constant body temperature (Emmans, 1989). Restricted feeding may reduce heat production (MacLeod and Hocking, 1993). Chapter 4 investigates the hypotheses that a reduction in feather

growth is an adaptive response to practical ambient temperatures and that feather growth can be improved by restricted feeding.

The hypothesis that there will be competition between body and feather growth for essential nutrients predicts that body growth will be maintained at the expense of feather growth when protein concentrations in the diet are deficient. To test this hypothesis Chapter 5 compared feather and muscle growth in modern and traditional turkeys fed on diets with decreasing protein concentration. Supplementing a low protein diet with amino acids that are thought to be important for feather growth assessed the role of specific amino acid deficiencies on feather growth in the modern turkey.

If the breast skin of the modern turkey has stretched with selection for increased breast muscle it is possible that the skin may be damaged in some way. Chapter 6 investigates some of the possible effects of breast muscle selection on the skin and feathers in the breast region. The total number of feather follicles on the breast was counted to test the hypothesis that there has been no increase in the number of feather follicles with the increase in the body size of the modern bird. Skin was examined histologically for evidence of structural damage and collagen content assessed as an indication of possible structural differences in the skin of the modern and traditional turkey. It was hypothesised that pressure on the breast may result in damage to the blood supply to the feather follicles and this was also investigated in Chapter 6.

Chapter 7 provides a general discussion of the investigations into feather growth and poor breast feathering in the modern turkey.

An initial investigation into the effect on feather growth of increasing dietary zinc concentrations is reported in Appendix 1. The moulting patterns of modern turkeys in the commercial environment are described in Appendix 2. The hypothesis that commercial selection for increased breast muscle in the modern turkey has resulted in active selection against breast feathering is examined in Appendix 3.

CHAPTER 2: General Materials and Methods

This chapter describes the procedures that are common to most of the experiments in this thesis. Modifications to these procedures and all other methodology are described in the appropriate chapters.

2.1 Animals and Husbandry

Turkeys (*Meleagris gallopavo*) of two unrelated strains were used in this study. Modern commercial Large White male line breeding turkeys (BUT Big 5) were obtained from British United Turkeys Ltd, Chester, UK and traditional Nebraska Spot turkeys were obtained from a breeding flock maintained at Roslin Institute, Roslin, UK. Turkeys were reared in pens 1.5 x 2.4m, littered to a depth of 8cm with untreated white wood shavings (Anderson Alexander, Bo'Ness, UK) and containing a suspended bell drinker to provide water *ad libitum*. The photoperiod was 23 hours light and one hour dark for the first 24 hours followed by 14 hours light and 10 hours dark thereafter. Brooders were adjusted to obtain a spot heat of 38°C and an ambient temperature of 21-25°C with relative humidity 65-70%. Brooders were removed at 6 weeks of age and an ambient temperature of 18-20°C maintained by controlled ventilation and heating. All birds received a course of vaccinations against Erysipelas (Pasturella- Erysipelas Vaccine, Hoechst, Milton Keynes, UK) at 8 and 18 weeks and a combined vaccine for Newcastle Disease, Gumboro and Infectious Bronchitis (Nobi Vac, Intervet, Cambridge, UK) at 4, 12, 18 and 21 weeks of age. Males were desnooded at hatch and all turkeys beak trimmed between 7 and 10 days of age. Individual birds were identified by numbered wingbands.

The turkeys were fed on a series of conventional turkey diets, containing 280 (starter, 0-4 weeks), 240 (grower 1, 5-8 weeks), 200 (grower 2, 9-12 weeks) and 180 (grower 3, 13-25 weeks) g crude protein kg^{-1}. The formulations for these diets are shown in table 2.1.

2.2 Feather and Body Measurements

Food was removed 24 hours prior to killing to minimise the variation in body weight associated with gut fill. Turkeys were killed by intravenous injection of sodium pentobarbitone (Euthatal, Rhone Merieux Ltd, Harlow, UK) and body weight was recorded.

Feather and tract measurements were made in millimetres (mm) using a ruler with mm divisions that was filed down at one end so that the ruler started at zero. Feather and tract measurements were made before plucking. The length of the right and left sternal tracts were measured from the point of the first feather or follicle at the cranial end of the sternal tract to a point level with the caudal end of the keel bone. The widths of both sternal tracts were measured at the mid point of the keel bone from the outside edge of the vane of the outside feather to the outside edge of the vane of the inside feather without the feathers being moved. The sternal apterium was measured, at the same position on the keel, between the inside edges of the feathers of the two tracts again without disturbing the feathers. The back tract length was measured from the mid point, between the wings to the uropygial gland at the base of

the tail. The means of the right and left sternal tract measurements were used for statistical analysis.

For all feather length measurements the longest feathers were selected from each area described below in order to minimise any influence of differences in the rate of moulting between sexes and strains. The ruler was placed at the base of the feather shaft at the level of the skin and the feather measured to the tip. The sternal tract was divided into two areas, cranial and caudal, at the point where width was measured and the lengths of one feather from the centre of the cranial and caudal region of each sternal tract were measured. The back tract was also divided into two areas, cranial and caudal, at the mid point of the tract. Two feathers from the cranial and caudal region of the back, two feathers from the left and right wings and two feathers from the tail were measured for each turkey. Feather lengths from the same area, for example the 4 wing feathers, were combined and the mean length used for statistical analysis. This resulted in mean feather lengths for the cranial breast, caudal breast, back, wing and tail.

Feather follicle density on the breast tract was measured by plucking an area of the left tract and counting the individual follicles within the fixed area ($4cm^2$) of a cardboard template. Care was taken not to stretch the skin. If skin samples were required for histology a small piece of skin was dissected from the centre of the right sternal tract. A sample of feathers from the wing, tail and back was obtained and sealed in a plastic bag.

The turkey carcass was scalded in a tank of hot water (58°C) for 15 seconds and immediately plucked in an automatic wet defeathering machine (Commodore, Wallbridge, Southampton, UK). Any feathers that had not been removed were plucked by hand. When the carcass was visibly dry, after hanging on shackles to drip, and there was no obvious surface moisture it was re-weighed and the weight of feathers estimated as the difference in weight before and after plucking. After plucking, the left breast muscle was dissected cleanly from the carcass, taking care to avoid blood loss, and weighed.

Carcasses required for analyses were frozen at –20°C.

2.3 Carcass Processing

Frozen feather free carcasses were allowed to partially defrost so that they were still firm but not solid. A band saw (AEW 350, Dalziel and Watson Food Machinery, Bellshill, UK) was used to cut large carcasses into manageable pieces before being chopped more finely in a bowl cutter (A. Stephanu. Sohne, Hameln, Weser, Germany). The chopped carcass was then put through a mincer (Hobart Manufacturing Company, Troy, Ohio, USA) three times until it was finely ground and well mixed. Equipment was cleaned between each carcass to avoid any mixing of the samples. Duplicate samples of approximately 100g of mince were weighed accurately into pre-weighed foil trays and subsequently frozen at –20°C.

2.4 Carcass Analysis

Carcass Dry Matter

Weighed frozen mince samples were placed in a freeze drier for one week until completely dry. Samples were removed from the freeze drier and left overnight to equilibrate before being weighed. Dry matter % and moisture % were calculated and a mean obtained for the duplicate samples.

Oil Extraction

Dry samples were crumbled and then re-dried in an oven at 100°C for one hour and allowed to cool in a dessicator. Approximately 3g of sample was weighed accurately into an oil extraction thimble (Whatman extraction thimbles, 26mm x 60mm, Merck, Poole, Dorset, UK) in duplicate and the thimble sealed with cotton wool. Oil extraction was carried out using a Tecator Soxtec System HT 1043 extraction unit (Tecator, Didcot, UK). Oil extraction cups (Tecator, Didcot, UK) were weighed and filled ¾ full with petroleum spirit (Merck, Poole, Dorset, UK) before attachment to the extraction unit. The thimbles were immersed in the petroleum spirit and the oil was extracted for 1.5 hours then rinsed for a further 1.5 hours. The cups containing the oil were removed and placed in an oven at 100°C overnight and then placed in a dessicator to cool before being re-weighed. Thimbles were removed and allowed to dry overnight before the oil free material was removed for nitrogen and ash determinations. Carcass fat percentage was calculated and a mean obtained for the duplicate samples.

Nitrogen Determination

Oil free material was ground to a fine powder, dried in an oven at 100°C for one hour and allowed to cool in a dessicator. Approximately 0.2 to 0.3g of samples were weighed accurately into tin foil cups (LECO Corporation, Stockport, Cheshire, UK) in duplicate. Samples were analysed for crude protein content using a protein/nitrogen analyser (LECO Corporation, Stockport, Cheshire, UK). Mean carcass crude protein % was calculated during the analysis.

Ash Determination

Ground, oil free material was dried in an oven at 100°C for one hour. Approximately 0.5 to 1g of samples were weighed accurately into pre-weighed glass beakers in duplicate. Beakers were placed in a muffle furnace and ashed overnight at 550°C. On removal from the furnace the beakers were placed in a dessicator and allowed to cool before being re-weighed. Carcass ash percentage was calculated and a mean obtained for the duplicate samples.

Feather Dry Matter

Feather samples were finely chopped using scissors, about 10g weighed accurately into pre-weighed foil containers in duplicate and the samples frozen at –20°C. Weighed frozen feather samples were placed in a freeze drier for 48 hours until completely dry. Samples were removed from the freeze drier and left overnight to equilibrate before being re-weighed. Dry matter percentage and moisture % were calculated and a mean obtained for the duplicate samples.

2.5 Injection Procedure

To give intravenous injections, birds were restrained and the brachial vein (wing vein) exposed by removing a few feathers. Sodium pentobarbitone was injected with the syringe needle (25G) pointing towards the body.

2.6 Experimental Design

Where both traditional and modern turkeys were included in an experiment the two strains were housed in separate pens within the blocks of the experimental design. Each pen was then taken as a unit of replication for statistical analysis. Strains were housed separately because their difference in size would have resulted in competition, particularly for feed, and this could have affected the growth of both body and feathers differently in the two strains.

Difficulties were experienced in obtaining sufficient numbers of traditional turkeys for some of the experiments resulting in different numbers of traditional and modern turkeys housed per pen. The number of modern turkeys was not reduced to correspond with the traditional turkeys because of the higher level of mortality observed in the modern strain. Stocking density was not expected to affect feather growth.

2.7 Statistical Analysis

Data obtained in this study were tested to ensure that the residuals were normally distributed and independent of the mean before parametric tests such as analysis of variance (ANOVA) were performed.

Table 2.1 Specification and ingredients of standard turkey diets

Diet	Starter 0-4 weeks	Grower 1 5-8 weeks	Grower 2 9-12 weeks	Grower 3 13-25 weeks
Metabolisable energy (MJ/kg)	12.0	12.0	12.0	12.0
Crude protein (g/kg)	280	240	200	180
Calcium (g/kg)	16	13	12	11
Phosphorus (g/kg)	10	10	9	8
Ingredients (g/kg)				
Maize	152.0	0.0	0.0	0.0
Wheat	304.0	456.9	455.0	390.8
Wheatfeed	0.0	200.0	200.0	200.0
Barley	0.0	0.0	91.5	210.1
Soya	385.0	228.1	172.0	117.2
Fish meal	100.0	67.2	30.0	30.0
Limestone	7.0	3.3	4.4	7.0
Dicalcium phosphate	10.0	10.8	12.1	8.6
Sodium chloride	2.0	3.0	3.0	3.0
Methionine	2.0	1.2	1.0	0.8
l-Lysine HCl	1.0	1.5	2.0	2.5
Vitamin / mineral mix	7.0	7.0	7.0	7.0
Pellet binder	10.0	10.0	10.0	10.0
Soya oil	20.0	10.0	10.0	10.0

Starter (0-4 weeks)
Supplied per kg diet: Cu 14 mg, I 1.4 mg, Fe 112 mg, Mn 140 mg, Zn 112 mg, Se 0.28 mg, Mo 0.7 mg; Co 0.7 mg, retinol 5.0 mg, cholecalciferol 175 µg, α-tocopherol 47 mg, menadione 4.2 mg, riboflavin 9.8 mg, thiamine 2.8 mg, nicotinic acid 70 mg, pantothenic acid 21 mg, biotin 280 µg, pyridoxine 7 mg, cyanocobalamin 21 µg, folic acid 1.4 mg.

Grower (5 weeks +)
Supplied per kg diet: Cu 12 mg, I 1.2 mg, Fe 96 mg, Mn 120 mg, Zn 96 mg, Se 0.24 mg, Mo 0.6 mg; Co 0.6 mg, retinol 4.3 mg, cholecalciferol 150 µg, α-tocopherol 40 mg, menadione 3.6 mg, riboflavin 8.4 mg, thiamine 2.4 mg, nicotinic acid 60 mg, pantothenic acid 18 mg, biotin 240 µg, pyridoxine 6 mg, cyanocobalamin 18 µg, folic acid 1.2 mg.

CHAPTER 3: Comparative Study

3.1 Introduction

Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) may cause depressed welfare and are an important source of economic loss in market turkeys through downgrading and condemnation of carcasses. Such lesions are common in modern turkeys and feathers may offer some protection to the breast from possible instigating causes of these conditions.

Observations by Hocking (1995) suggest that feather growth in modern compared with traditional strains is poor and feathers may be absent over the breast region exposing the skin to noxious material in the litter. Hocking (1995) found that from 8 weeks of age feathers in the anterior portion of the breast of modern male line turkeys were sparse and short leaving the skin exposed to the environment. However, the breast of the traditional turkey was covered in a thick coat of normal feathers. This study suggests that there are differences in breast feathering between the modern and traditional turkeys. It is possible that feathering in the modern turkey is poor in areas other than the breast and differences in feathering between the modern and traditional turkeys might also be found in these areas. Comparing the two strains of turkey might give an indication as to the cause of the differences in feathering and the traditional turkey, with good feather cover, could be used as a control. As well as investigating feather growth in the two strains of turkey there may be other differences which are

related to feathering. One example might be differences in behaviour between the modern and traditional turkeys. The time a turkey spends resting could be related to poor feathering on the breast due to increased friction causing loss or damage to the feathers. Alternatively increased skin temperature at the breast could result in a reduction in feather cover to allow for increased heat dissipation.

By comparing modern with traditional strains and investigating factors affecting the growth of breast feathers in turkeys it may be possible to devise management practices that improve feathering in the modern bird and reduce the incidence of breast blisters and breast buttons.

It was predicted that feather growth would be poor on the breast of the modern turkey and that there would be differences in feather growth between the modern and the traditional turkey. Experiment 1 (Section 3.2) compared feather and body growth in a modern and a traditional turkey during the period of rapid growth. Experiment 2 (Section 3.3) examined resting behaviour in the modern compared with the traditional turkey because increased resting may cause damage to the breast feathers of the modern bird. Poor breast feathering or increased resting may result in differences in the breast skin temperature of the modern compared with the traditional turkey. To test this hypothesis Experiment 3 (Section 3.4) compared breast skin temperature in the modern and traditional turkey.

3.2 Experiment 1: Feather and Body Growth Comparison

A comparative study of modern and traditional turkeys from hatch to 25 weeks of age was carried out. The aim of the study was to quantify and compare feather and body growth in a modern commercial turkey strain (BUT Big 5 Male Line) with that in an unrelated traditional line (Nebraska Spot). Carcass analysis was used to compare the growth of carcass and feather protein in the two strains.

3.2.1 Materials and Methods

Seventy-two (36 male and 36 female) day-old BUT Big 5 male-line turkeys and 48 (24 male and 24 female) day-old Nebraska Spot turkeys were allocated to 12 pens using a randomised block design. The twelve pens were arranged as 3 blocks with each block consisting of one pen of each strain and sex. Twelve day-old poults were placed in each of the BUT pens and 8 in each of the Spot pens. Turkeys were fed on standard turkey starter, grower and finisher diets (Chapter 2) throughout the trial and the food intake per pen was recorded weekly. The turkeys were also weighed as a pen each week. After 2, 4, 6, 8, 10, 15, 20 and 25 weeks, 3 birds of each strain and sex (one bird from each pen), selected at random at the start of the trial, were removed from the experiment and killed by injection of sodium pentobarbitone. Feather and body measurements were recorded by the methods described in Chapter 2. The carcasses from birds at 4, 6, 10 and 25 weeks were frozen and prepared for carcass analysis (Chapter 2).

The feather and body measurement data for BUT males, BUT females, Spot males and Spot females were plotted separately against age and growth curves (Gompertz functions) or exponential curves were fitted to the data. The Gompertz model has an equation of the form:

$$y = A + C \exp(-\exp(-B(x - M)))$$

where 'exp' means '*e* to the power of', A is a constant, C is the upper asymptote, B is the rate parameter (the rate of decay of growth rate), x is the age (weeks) and M is the point of inflection.

The exponential model has an equation of the form:

$$y = A + BR^x$$

where A is the upper asymptote, B is the scaling parameter, R is the rate parameter and x is the age (weeks).

Multiple regression analysis was used to fit a single curve for feather and body growth traits. Sequential fitting of the linear (C and A) and non linear parameters was conducted to test for differences in parameters of the model for the two strains and sexes. Analysis of variance was used to test for differences in carcass composition data in a linear model with effects for age, strain, sex and their interactions.

3.2.2 Results

Feather and Body Traits

Figures 3.1, 3.2 and 3.3 show the change in body weight, feather weight and breast muscle weight respectively for male and female modern BUT Big 5 male line and traditional Nebraska Spot turkeys from 2 to 25 weeks of age. A Gompertz growth curve was found to be the best fitting model for the changes in the weight of these traits with age. The estimated parameters from this model are shown in Table 3.1. The upper asymptote (C) of the fitted model was an estimate of the final weight for each trait and the point of inflection (M) along with the rate (B) was used to compare the time course of growth for each trait. The upper asymptotes of the fitted models for body weight and breast muscle weight showed significant differences ($P<0.001$) between both strains and sexes in final weight. BUT males had the heaviest body and breast muscle weight followed by the BUT females then the Spot males while the Spot females were the lightest. The time courses (B+M) of the four curves were also found to be significantly different ($P<0.001$) for both body weight and breast muscle weight. The point of inflection for body weight was similar for the males of the two lines (14.7±0.28 and 13.4±0.77 weeks respectively for the BUT and Spot male) and also for the females of the two lines (10.9±0.23 and 9.4±0.48 weeks respectively for the BUT and Spot female). This result suggested a sex difference in the time course of body growth with females reaching their maximum body growth rate before males. The point of inflection was also reached at an earlier age for the Spot compared with the BUT turkeys. The points of inflection for breast muscle weight followed a similar pattern to body weight with the females reaching this stage at an earlier age than the

males and the Spot female earlier than the BUT female. The pattern was not the same for the males with the BUT reaching the point of inflection before the Spot, however there was a large standard error associated with the Spot male curve which could account for this difference. The upper asymptotes of the fitted models for feather weight showed a significant difference ($P<0.001$) in final feather weight between the sexes but no difference between the strains. The males had a higher feather weight than the females. No significant differences were found between the rates or points of inflection of the four curves suggesting that there was no difference in the time of development of feathers in the two strains and sexes.

Figures 3.4, 3.5 and 3.6 show the change in feather lengths from the back, wing and tail respectively for male and female modern BUT Big 5 male line and traditional Nebraska Spot turkeys from 2 to 25 weeks. An exponential curve was found to be the best fitting model for the changes in the lengths of these feathers with age. The estimated parameters from this model are shown in Table 3.2. The upper asymptotes (A) were used to compare the final lengths of these feathers and the rate parameter (R) to compare the time course of the growth of each feather. Multiple regression analysis showed that there were no significant sex or strain differences in either the final lengths of the back, wing or tail feathers or in the rate of growth of these feathers.

Figures 3.7 and 3.8 show the change in feather lengths from the caudal and cranial breast regions respectively for male and female modern BUT Big 5 male line and traditional Nebraska Spot turkeys from 2 to 25 weeks. An exponential curve was

fitted to the data and the estimated parameters from this model are shown in Table 3.3. As with the other feather lengths no significant differences were found in the rate of breast feather growth between the strains or sexes, however significant differences ($P<0.01$) were found in the final lengths of these feathers. The caudal breast feather lengths were similar for the males of the two strains and also for the females of the two strains suggesting a sex but not a strain difference with males of both strains having longer feathers than the females. The cranial breast feathers showed a similar sex difference in the Nebraska Spot turkeys with the males having longer feathers than the females. However, the cranial breast feathers in the BUT turkeys were of similar lengths in the males and females and were significantly ($P<0.001$) shorter than in the Nebraska Spots. The breast feathering of a representative modern and traditional turkey is shown in Figure 3.13.

The changes with age in feather follicle density on the sternal tract of the two strains and sexes are shown in Figure 3.9. An exponential curve was fitted to the data and the estimated parameters from this model are shown in Table 3.3. Feather follicle density decreased with age in both strains and sexes. A significant ($P<0.001$) strain difference in final follicle density was observed with the Spot turkeys having a higher follicle density than the BUT turkeys. The sex difference in final follicle density was not significant. There was no significant difference in the rate parameter of the fitted curves suggesting that feather follicle density declined at the same rate in both sexes and strains.

Figures 3.10, 3.11 and 3.12 show the changes with age in back tract and breast tract length and breast tract width respectively for the two strains and sexes. An exponential curve was fitted to the data and the estimated parameters from this model are shown in Table 3.4. A significant sex and strain ($P<0.01$) difference was found in the rate of growth of the back tract length with males having a higher rate of growth than the females and Spot turkeys a higher rate of growth than the BUT turkeys. The final lengths of the back tract were also significantly ($P<0.001$) different with males being longer than females and BUT turkeys longer than Spots. A similar pattern was seen in the final lengths of the breast tract where again the males of both strains had significantly ($P<0.001$) longer breast tracts than the females. The BUT female had a significantly ($P<0.001$) longer breast tract than the Spot female, however this was reversed in the males with the Spot breast tract being longest. A large standard error was associated with the measurement from the Spot male that could account for this difference. There was no significant sex or strain difference in rate of growth for the breast tract length or width and no significant difference in final breast tract width.

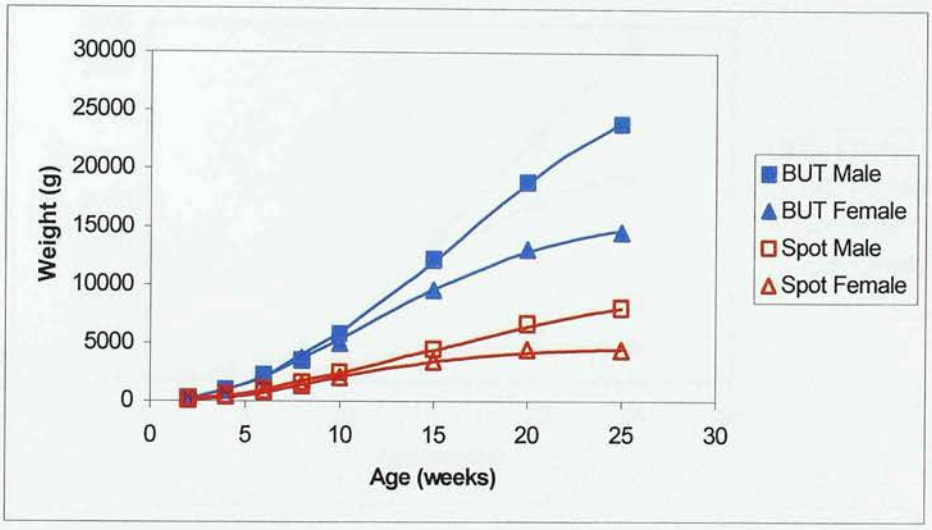


Figure 3.1 Mean body weight of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted Gompertz functions.

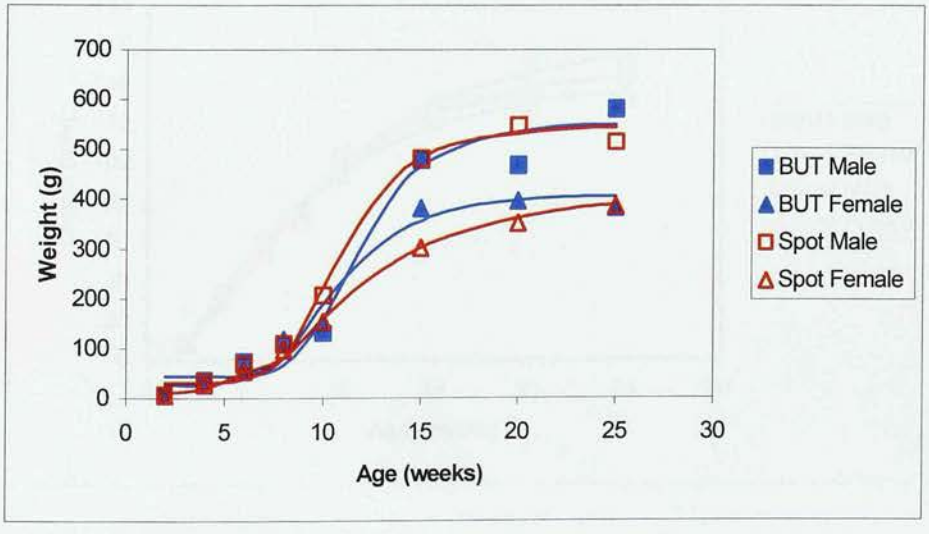


Figure 3.2 Mean feather weight of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted Gompertz functions.

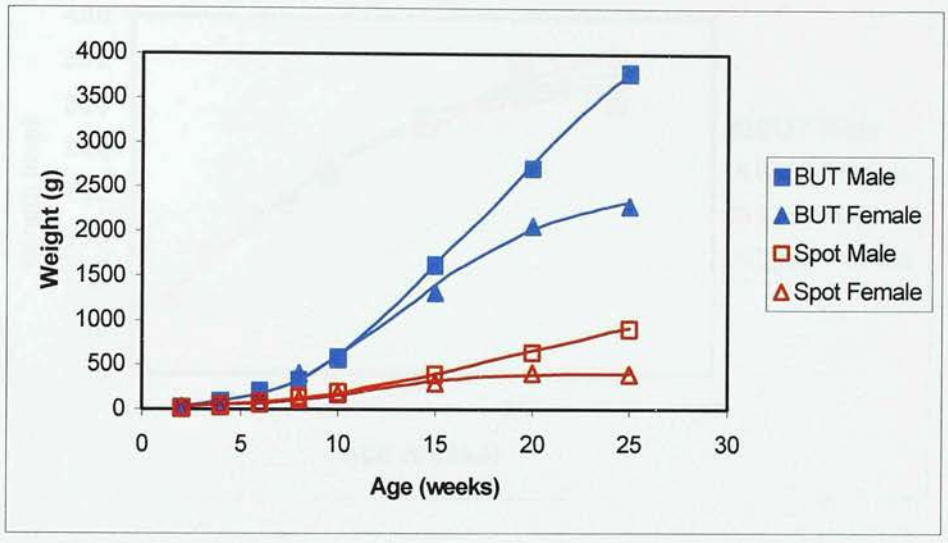


Figure 3.3. Mean breast muscle weight of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted Gompertz functions.

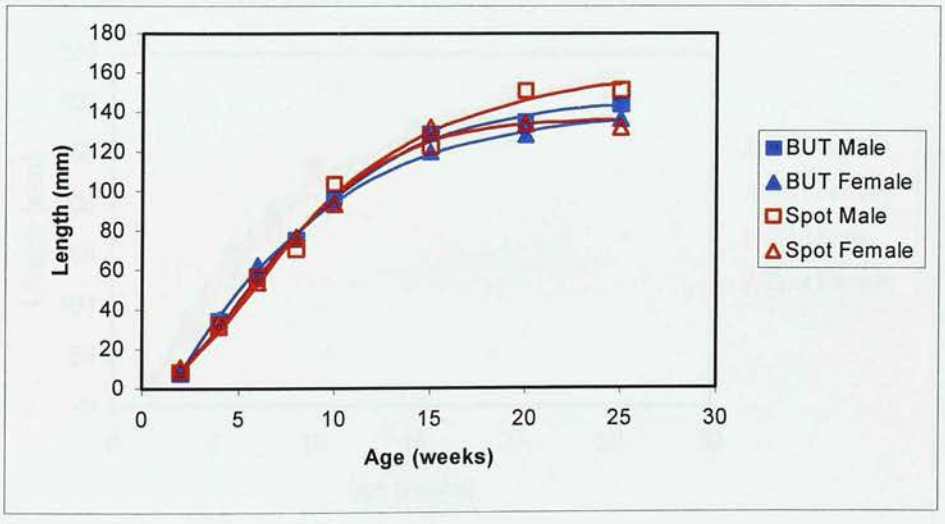


Figure 3.4 Mean back feather length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

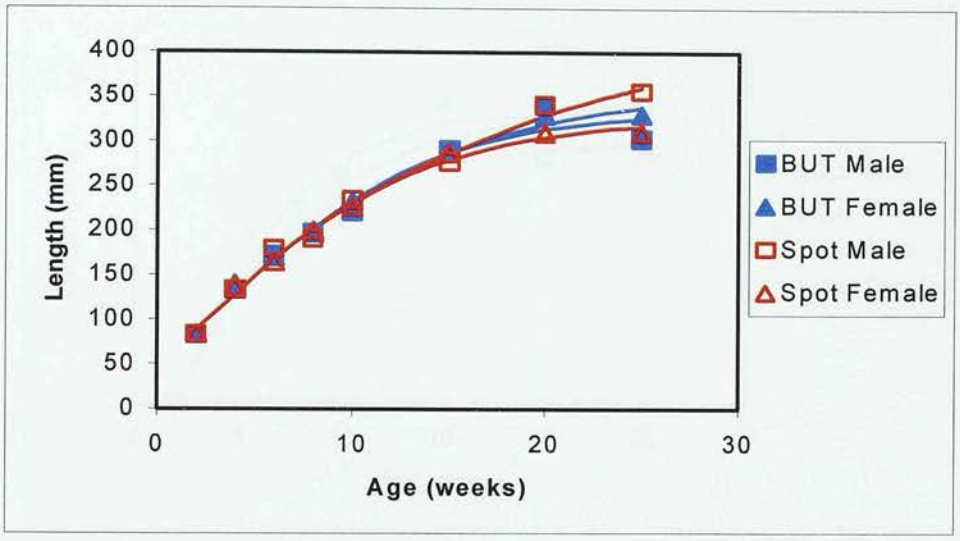


Figure 3.5 Mean wing feather length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

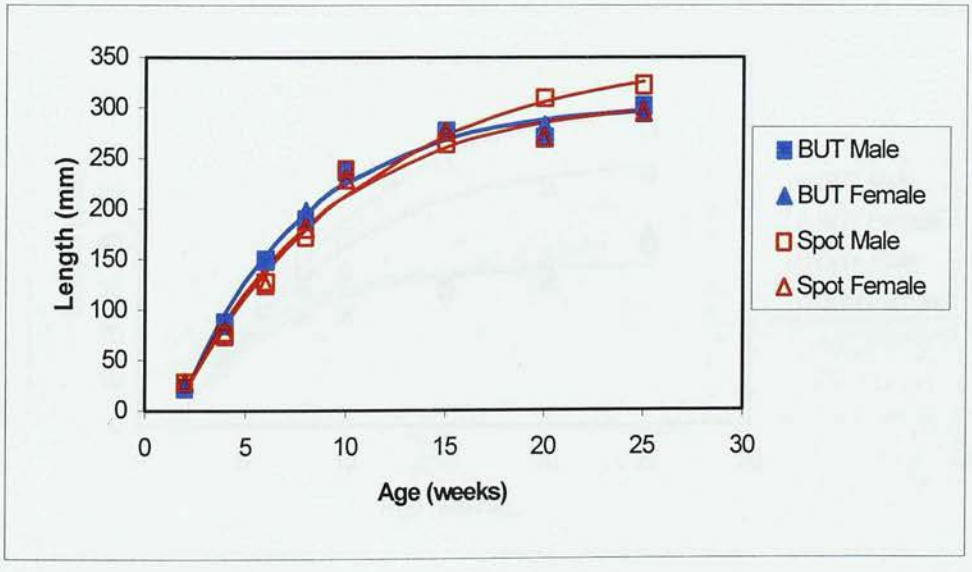


Figure 3.6 Mean tail feather length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

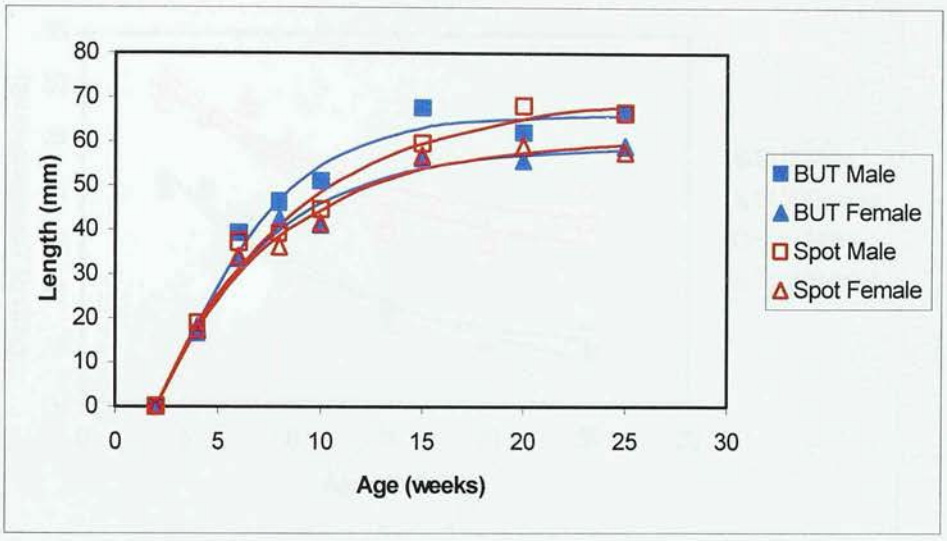


Figure 3.7 Mean caudal breast feather length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

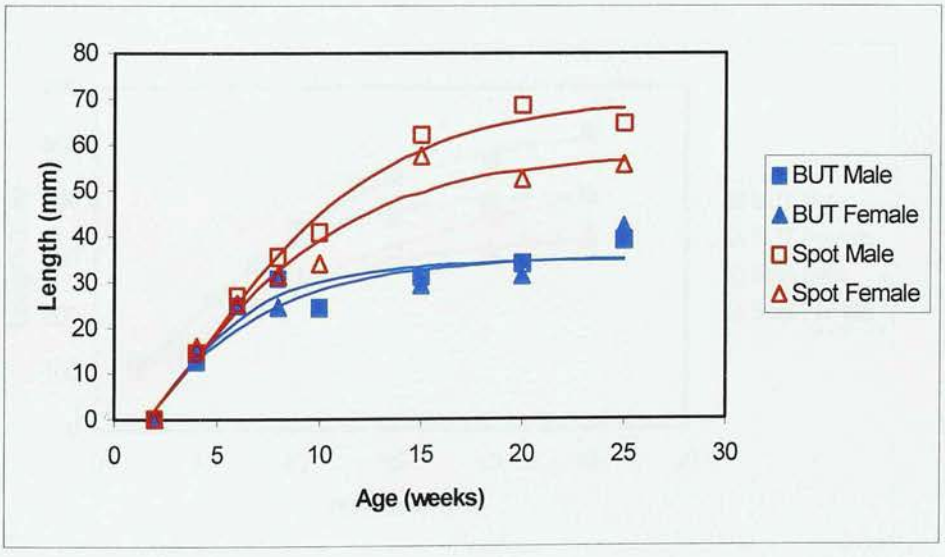


Figure 3.8 Mean cranial breast feather length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

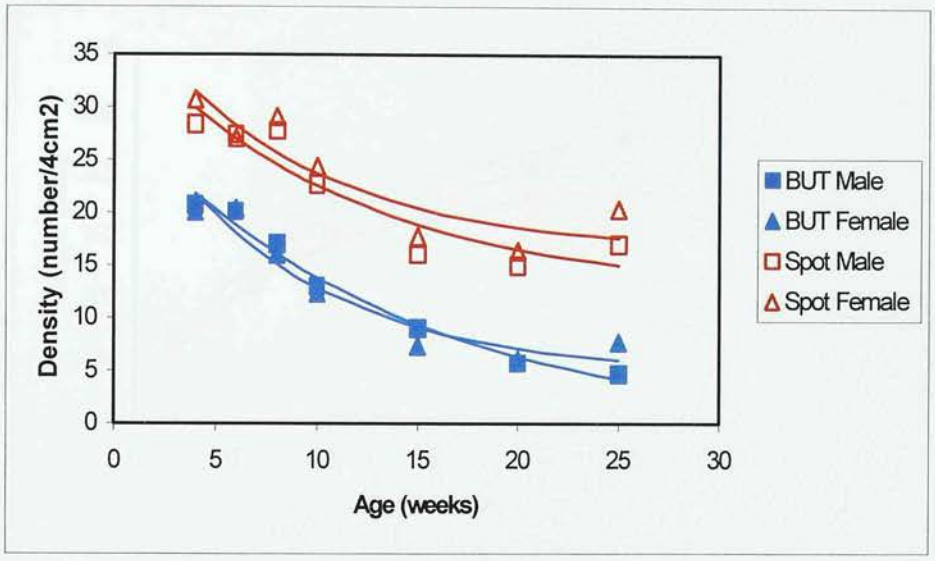


Figure 3.9 Mean breast feather-follicle density of male and female BUT Big 5 male line and Nebraska Spot turkeys from 4 to 25 weeks of age with fitted exponential curves.

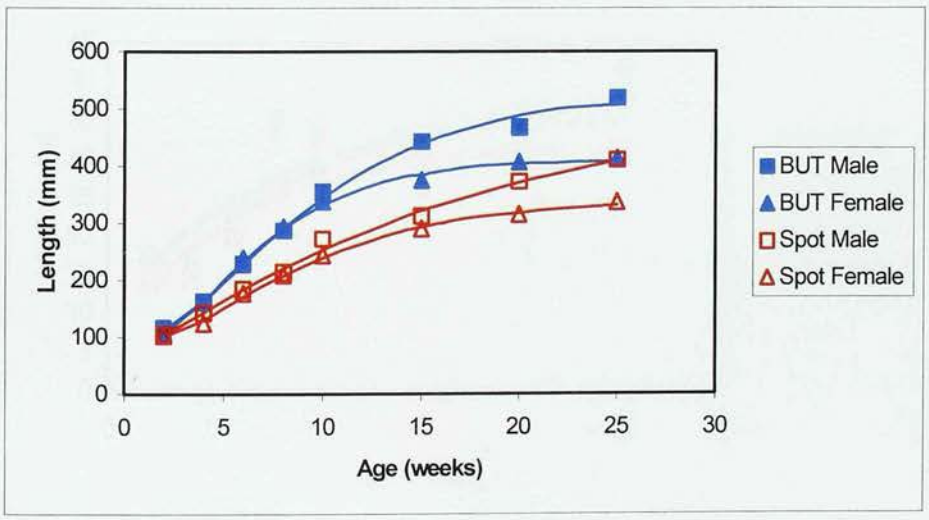


Figure 3.10 Mean back tract length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

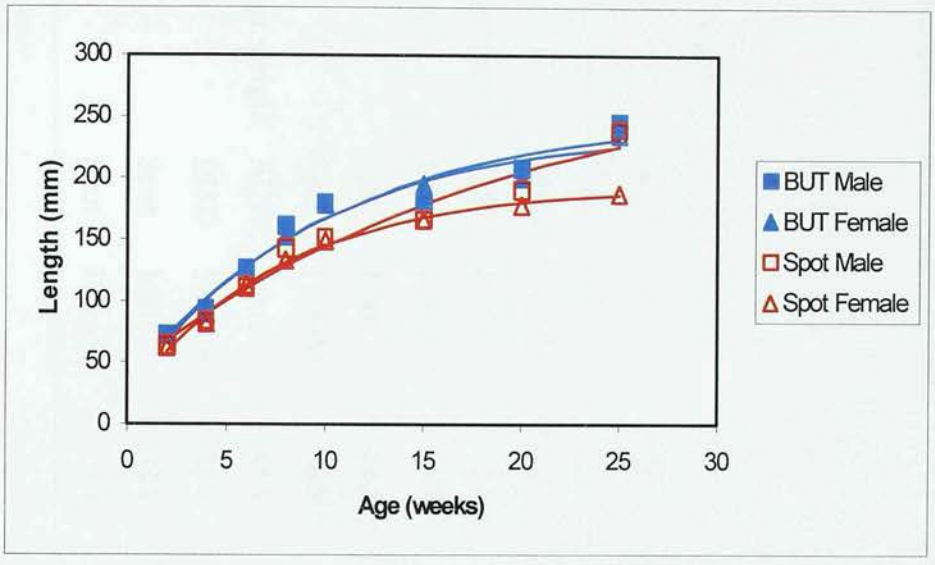


Figure 3.11 Mean breast tract length of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

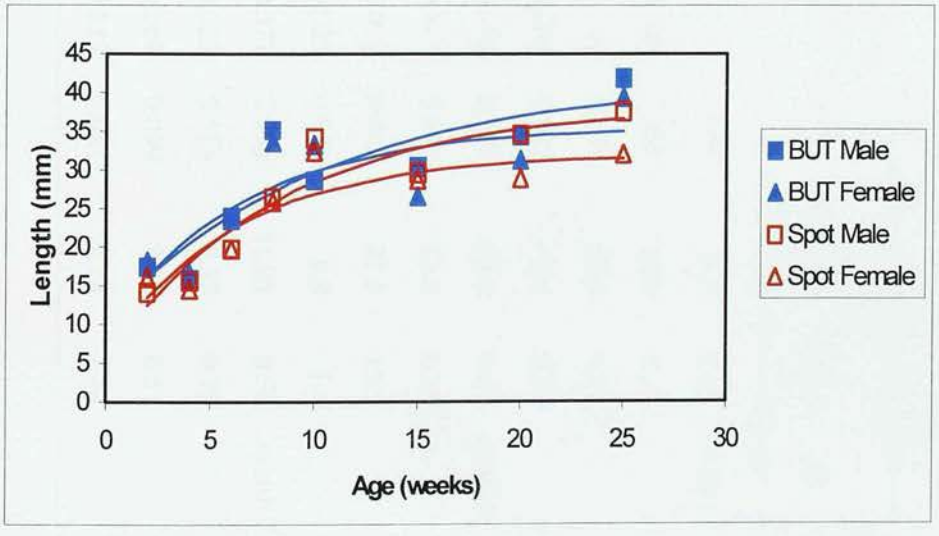


Figure 3.12 Mean breast tract width of male and female BUT Big 5 male line and Nebraska Spot turkeys from 2 to 25 weeks of age with fitted exponential curves.

Table 3.1 Estimates of fitted parameters (±s.e.) of a Gompertz growth curve for body weight, breast muscle weight and feather weight in male and female BUT and Spot turkeys. A is the constant, C is the asymptote, B is the rate parameter and M is the age at point of inflection. Significance levels (P values) shown.

Trait	Strain	Sex	A		C		B		M		significance		
			estimate (g)	s.e.	estimate (g)	s.e.	estimate	s.e.	estimate (weeks)	s.e.	A	C	B+M
Body weight	BUT	Male	-131.0	214.0	32739.0	1164.0	0.114	0.005	14.7	0.28	<0.001	<0.001	<0.001
	BUT	Female	-82.0	237.0	16707.0	581.0	0.149	0.009	10.9	0.23			
	Spot	Male	-144.0	309.0	10946.0	1210.0	0.110	0.017	13.4	0.77			
	Spot	Female	170.0	175.0	4588.0	324.0	0.205	0.032	9.4	0.48			
Breast muscle	BUT	Male	-1.6	39.9	5864.0	424.0	0.109	0.009	17.5	0.67	<0.001	<0.001	<0.001
weight	BUT	Female	34.8	35.6	2626.0	124.0	0.163	0.014	12.6	0.35			
	Spot	Male	-53.0	124.0	2386.0	2275.0	0.061	0.047	23.5	13.7			
	Spot	Female	10.9	41.2	416.2	81.3	0.205	0.089	9.8	1.4			
Feather weight	BUT	Male	45.4	19.5	501.1	37.6	0.371	0.096	11.05	0.59	<0.01	<0.001	NS
	BUT	Female	28.8	23.9	376.5	41.3	0.323	0.115	9.52	0.75			
	Spot	Male	32.2	21.5	508.2	37.8	0.368	0.099	9.93	0.51			
	Spot	Female	9.9	33.0	387.3	64.1	0.219	0.085	9.74	1.18			

Table 3.2 Estimates of fitted parameters (±s.e.) of an exponential curve for feather lengths from the back, wing and tail in male and female BUT and Spot turkeys. R is the rate parameter, B is the scaling parameter and A is the asymptote. Significance level NS not significant.

			R		B		A		significance		
Feather lengths	Strain	Sex	estimate	s.e.	estimate	s.e.	estimate (mm)	s.e.	A	B	R
Back	BUT	Male	0.8959	0.0148	-190.9	8.57	158.1	9.04	NS	NS	NS
	BUT	Female	0.8861	0.0165	-175.2	8.74	145.2	7.88			
	Spot	Male	0.9128	0.0135	-206.8	9.45	177.4	12.1			
	Spot	Female	0.8887	0.0157	-183.1	8.66	149.6	8.16			
Wing	BUT	Male	0.9026	0.0175	-336.0	18.20	353.0	21.10	NS	NS	NS
	BUT	Female	0.9147	0.0168	-348.1	20.40	375.8	26.50			
	Spot	Male	0.9367	0.0153	-398.3	35.90	436.9	45.90			
	Spot	Female	0.9009	0.0184	-319.5	18.10	341.8	20.50			
Tail	BUT	Male	0.8513	0.0185	-394.7	23.6	302.7	12.4	NS	NS	NS
	BUT	Female	0.8516	0.0185	-393.0	23.5	304.2	12.4			
	Spot	Male	0.8974	0.0152	-415.3	19.1	353.5	20.7			
	Spot	Female	0.8715	0.0176	-383.1	20.9	308.8	14.9			

Table 3.3 Estimates of fitted parameters (±s.e.) of an exponential curve for feather lengths from the caudal breast, cranial breast and follicle density in male and female BUT and Spot turkeys. R is the rate parameter, B is the scaling parameter and A is the asymptote. Significance levels (P values) NS not significant.

			R		B		A		significance		
Feather lengths	Strain	Sex	estimate	s.e.	estimate	s.e.	estimate (mm)	s.e.	A	B	R
Caudal breast	BUT	Male	0.8215	0.0212	-102.22	7.23	67.40	2.66	0.01	NS	NS
	BUT	Female	0.8256	0.0246	-86.05	7.07	58.78	2.72			
	Spot	Male	0.8652	0.0193	-91.95	5.59	70.12	3.60			
	Spot	Female	0.8454	0.0230	-83.98	6.29	60.46	3.06			
Cranial breast	BUT	Male	0.7851	0.0597	-56.40	11.40	35.04	2.95	0.001	0.001	NS
	BUT	Female	0.8292	0.0546	-48.75	8.85	35.70	3.54			
	Spot	Male	0.8917	0.0222	-94.53	6.33	74.34	6.25			
	Spot	Female	0.8775	0.0281	-77.43	6.67	60.01	5.22			
Breast tract follicle density							($n/4cm^2$)				
	BUT	Male	0.9297	0.0499	30.16	5.19	-0.75	8.52	0.001	NS	NS
	BUT	Female	0.8829	0.0591	27.91	6.21	4.74	3.42			
	Spot	Male	0.9120	0.0600	25.21	4.26	12.45	5.59			
	Spot	Female	0.8856	0.0671	24.32	5.99	16.42	3.55			

Table 3.4 Estimates of fitted parameters (±s.e.) of an exponential curve for back tract length, breast tract length and breast tract width in male and female BUT and Spot turkeys. R is the rate parameter, B is the scaling parameter and A is the asymptote. Significance levels (P values) NS not significant.

Trait	Strain	Sex	R		B		A		significance		
			estimate	s.e.	estimate	s.e.	estimate (mm)	s.e.	A	B	R
Back tract length	BUT	Male	0.9205	0.0098	-577.8	21.8	590.7	28.9	0.001	0.001	0.01
	BUT	Female	0.8656	0.0141	-443.1	19.6	428.4	12.7			
	Spot	Male	0.9456	0.0130	-476.0	48.8	525.5	59.1			
	Spot	Female	0.9141	0.0168	-335.6	19.5	372.8	25.2			
Breast tract length	BUT	Male	0.9112	0.0246	-218.9	17.9	252.7	22.8	0.001	NS	NS
	BUT	Female	0.8977	0.0255	-214.2	16.6	239.2	18.0			
	Spot	Male	0.9500	0.0241	-253.3	56.7	295.6	66.8			
	Spot	Female	0.8800	0.0327	-173.9	17.3	193.2	14.1			
Breast tract width	BUT	Male	0.9099	0.0673	-30.7	6.8	41.5	8.5	NS	NS	NS
	BUT	Female	0.8594	0.0902	-26.0	7.5	35.5	4.4			
	Spot	Male	0.8869	0.0655	-32.69	6.5	38.2	5.9			
	Spot	Female	0.8521	0.0949	-25.5	7.8	31.9	4.2			

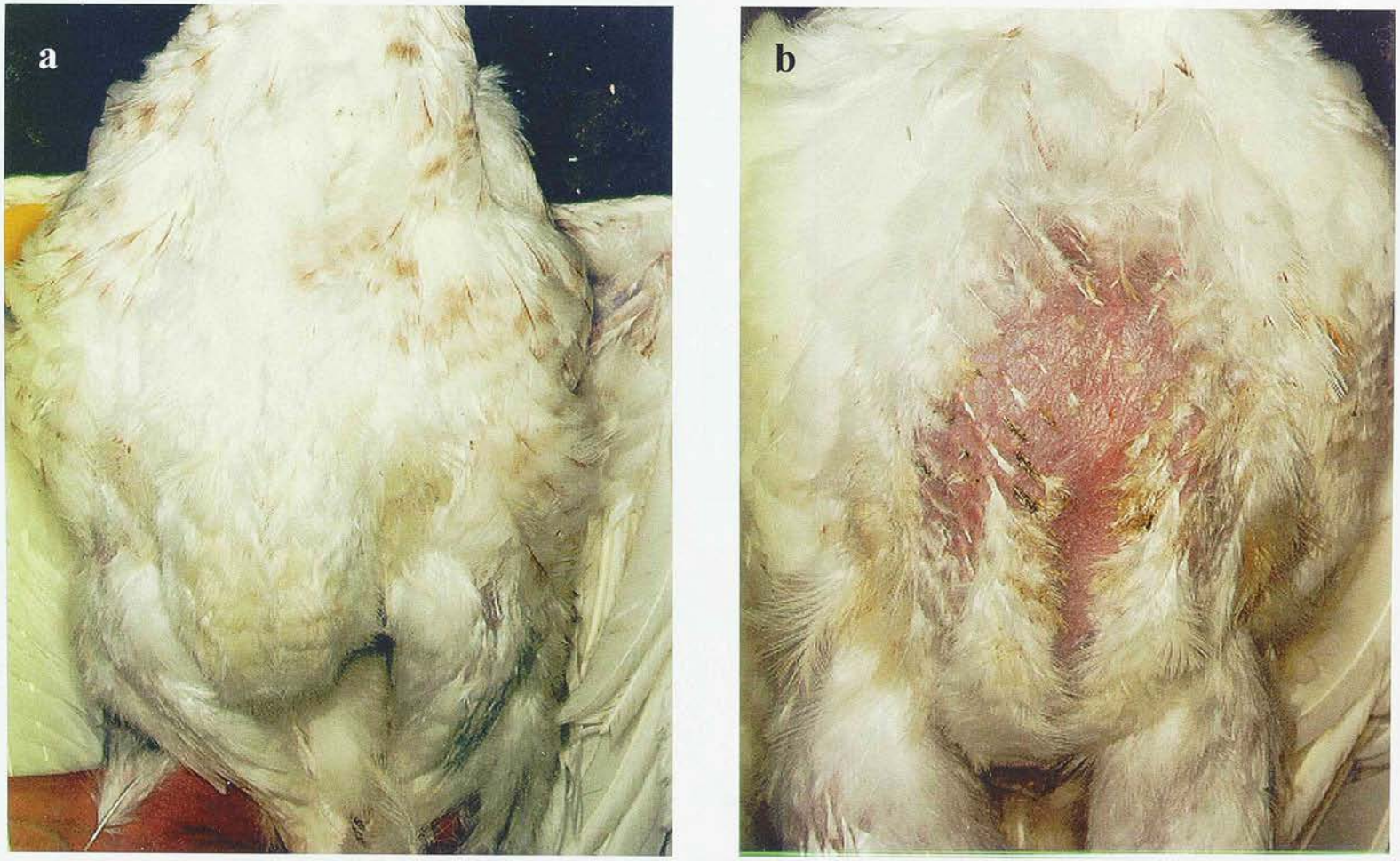


Figure 3.13 Breast feathering of a typical traditional Nebraska Spot turkey (**a**) and modern BUT Big 5 male-line turkey (**b**).

Carcass Analysis

The results of the carcass analysis showed a highly significant ($P<0.001$) interaction of strain, sex and age for total carcass protein (Figure 3.14). Total carcass protein was calculated from the analysed percentage of crude protein in the feather free carcass and the carcass weight. Carcass protein increased with age for both sexes and strains. At all ages the BUT turkeys contained more protein than the Spot turkeys and the strain difference increased with age. Male and female turkeys from the same strain had similar total carcass protein to 10 weeks of age but by 25 weeks the males of each strain had significantly more carcass protein than their respective females.

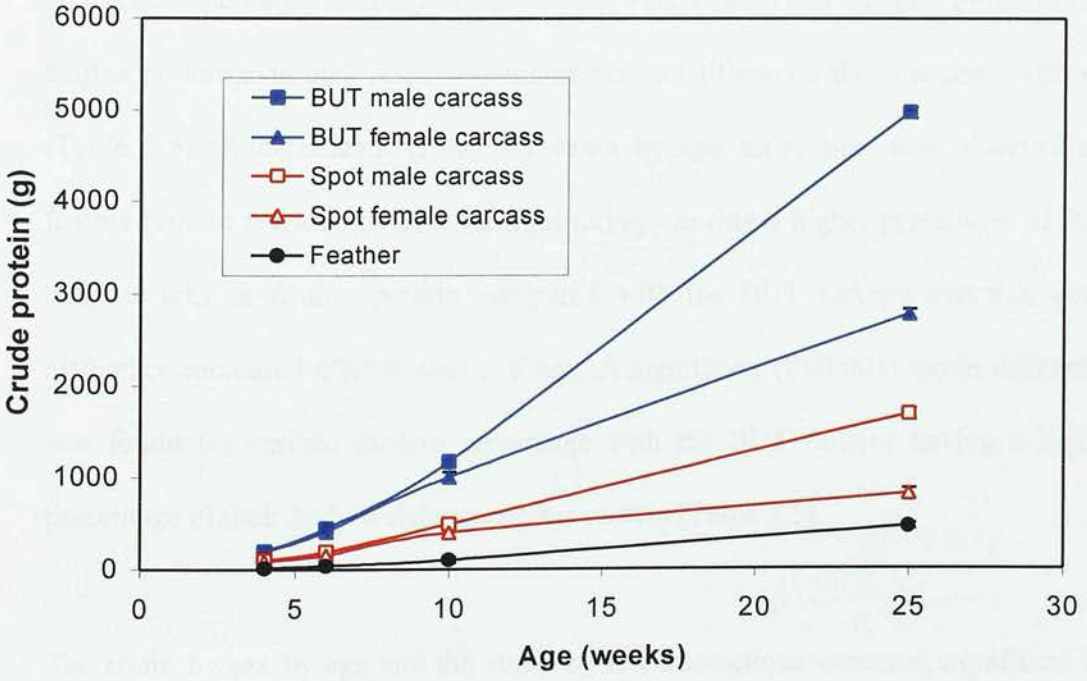


Figure 3.14 Interaction of sex, strain and age for carcass crude protein (g) for male and female BUT and Spot turkeys from 4 to 25 weeks of age (mean ±SED) and change in mean (±SED) feather protein (g) with age for all turkeys.

No strain, sex or age interactions were observed for total feather protein. Total feather protein weight was not significantly different between male and female BUT and Spot turkeys but did increase significantly ($P<0.001$) with age (Figure 3.14).

Figure 3.15 shows carcass protein and feather protein as a percentage of total body weight. The strain by sex by age and the strain by sex interactions were not significant for carcass or feather protein as a percentage of body weight and the marginal means are presented in Table 3.5. A significant ($P<0.05$) interaction of sex and age was found for both carcass and feather protein with the proportion of carcass protein in the females of the two strains being lower than the males and the differences increased with age. The females of each strain had a higher proportion of feather protein than their respective males and this difference also increased with age (Table 3.5). A significant ($P<0.001$) strain by age interaction was observed for feather protein percentage with the Spot turkeys having a higher percentage of their body weight as feather protein compared with the BUT turkeys and this strain difference increased after 6 weeks of age. A significant ($P<0.001$) strain difference was found for carcass protein percentage with the BUT turkeys having a higher percentage of their body weight as carcass protein (Table 3.5).

The strain by sex by age and the strain by sex interactions were not significant for feather dry matter, carcass moisture, fat or ash (Table 3.5). Significant interactions of sex and age were observed for moisture ($P<0.001$), fat ($P<0.001$) and feather dry matter ($P<0.05$). Females had similar carcass moisture to the males up to 10 weeks after which the moisture in the females decreased with increasing age (Table 3.5). Fat

in the females was also similar to the males up to 10 weeks after which fat increased significantly compared to the males (Table 3.5). A significant ($P<0.001$) strain by age interaction was found in carcass ash with ash being higher in the BUT turkeys at younger ages but decreasing to be lower than the Spot turkeys by 25 weeks. Ash was also significantly ($P<0.01$) different between the sexes with males having a higher carcass ash percentage than the females (Table 3.5).

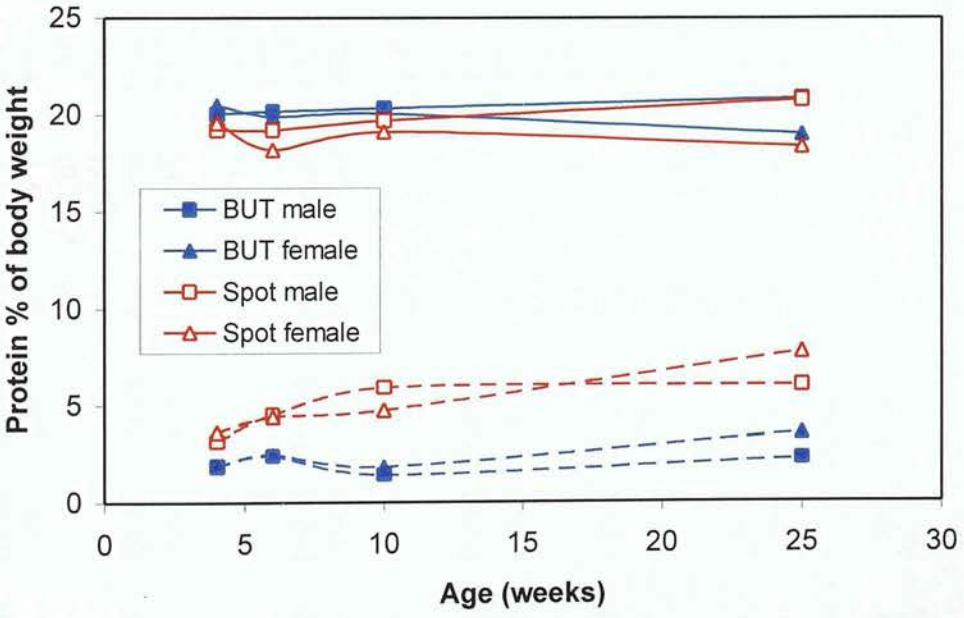


Figure 3.15 Carcass (——) and feather (-----) protein as a percentage of body weight for male and female BUT and Spot turkeys from 4 to 25 weeks of age.

Table 3.5 Mean carcass and feather composition analysis for male and female BUT and Spot turkeys from 4 to 25 weeks. Analysis of variance significance levels for model and interactions with associated SED. * P<0.05, ** P<0.01, *** P<0.001, NS not significant.

Trait	Strain	Sex	Age				Significance of model and interactions (SED)						
			4	6	10	25	Strain	Sex	Age	Strain x sex	Strain x age	Sex x age	Strain x sex x age
Feather free carcass													
% Moisture	BUT	male	71.3	69.4	68.2	64.9	*	***	***	NS	NS	***	NS
	BUT	female	70.9	69.3	67.7	58.6	(0.33)	(0.33)	(0.60)	(0.46)	(0.81)	(0.81)	(1.14)
	Spot	male	70.1	70.6	67.1	64.0							
	Spot	female	70.6	69.9	65.2	55.2							
% Fat	BUT	male	2.6	3.8	4.2	10.1	NS	***	***	NS	NS	***	NS
	BUT	female	2.6	4.2	5.6	17.7	(0.28)	(0.28)	(0.94)	(0.39)	(1.18)	(1.18)	(1.67)
	Spot	male	3.8	2.9	4.5	9.7							
	Spot	female	2.9	4.9	7.9	19.1							
% Ash	BUT	male	3.7	4.4	5.0	3.0	**	**	***	NS	***	NS	NS
	BUT	female	3.5	4.0	4.0	3.1	(0.07)	(0.07)	(0.14)	0.10)	(0.19)	(0.19)	(0.27)
	Spot	male	3.9	3.7	4.9	4.6							
	Spot	female	3.8	3.7	4.6	4.0							
Feather													
% Dry matter	BUT	male	50.9	72.8	65.7	90.0	NS	NS	***	NS	NS	*	NS
	BUT	female	54.9	71.1	59.9	89.3	(1.69)	(1.69)	(2.14)	(2.38)	(3.11)	(3.11)	(4.40)
	Spot	male	51.4	66.1	71.5	95.5							
	Spot	female	58.3	68.0	63.5	88.9							
% of body weight													
Carcass protein	BUT	male	20.0	20.2	20.4	20.8	***	***	NS	NS	NS	*	NS
	BUT	female	20.5	19.8	20.1	19.0	(0.13)	(0.13)	(0.38)	(0.19)	(0.48)	(0.48)	(0.68)
	Spot	male	19.2	19.2	19.7	20.6							
	Spot	female	19.5	18.3	19.1	18.5							
Feather protein	BUT	male	1.9	2.4	1.4	2.2	***	NS	***	NS	***	*	NS
	BUT	female	1.9	2.5	1.8	3.5	(0.27)	(0.27)	(0.29)	(0.38)	(0.44)	(0.44)	(0.63)
	Spot	male	3.1	4.5	5.9	6.0							
	Spot	female	3.6	4.4	4.7	7.7							

3.3 Experiment 2: Behaviour comparison

The modern turkey is almost three times the body weight of the traditional turkey and does not appear to be as active. If the large modern turkey spends an increased amount of time resting, perhaps for prolonged periods, then the time during which the breast muscle and skin are under pressure because of the bird's heavy body weight will be increased. Prolonged periods of pressure on the breast may cause damage to the skin, the feather follicles or to the capillaries supplying blood to these areas. This in turn may impair the growth of breast feathers. The act of resting may cause friction to the breast area resulting in feather damage and the growth of short, stubby cranial breast feathers observed in the modern turkey. The motion of alternating between a resting and standing posture may increase the friction on these feathers and the extent of the damage to the breast feathers would increase with increasing changes of posture.

The aim of this study was to quantify and compare differences between the modern and traditional turkey in the time spent resting.

3.3.1 Materials and Methods

Male and female Nebraska Spot and BUT Big 5 male line day-old turkey poults were reared in floor pens until 6 weeks of age. Twelve male and 12 female Spot and 18 male and 18 female BUT turkeys were randomly allocated to 12 pens using a

randomised block design. Birds were fed on a standard turkey starter diet from 0 to 4 weeks and then on a standard grower diet (Chapter 2).

During the week prior to testing, the birds were habituated to the presence of the observer at the front of the pens. At 6 weeks of age the behaviour of the birds in each pen was observed. Pens were scan sampled every 5 minutes for 2 hours and the number of birds resting, standing or walking (Table 3.6) in each pen recorded. The 2 hour observation period was repeated on 4 consecutive days.

As the number of birds per pen varied, the number of observations of each behaviour was calculated as a percentage of the total number of observations from each pen. Analysis of variance was used to test for differences in behaviour with effects for sex, strain and their interaction.

Table 3.6 Description of behaviours recorded

Behaviour	Description
Resting	Ventral surface in contact with the floor
Standing	Both feet in contact with the floor, no body contact with the floor
Walking	Normal gait advancing each foot alternately

3.3.2 Results

There was no significant interaction of sex and strain for any of the behaviours observed.

The modern turkey was found to be resting in 67% of the observations, significantly ($P<0.001$) more than the 25% for the traditional turkey. In contrast the traditional turkey was standing ($P<0.001$) and walking ($P<0.01$) in significantly more observations than the modern bird (Figure 3.16).

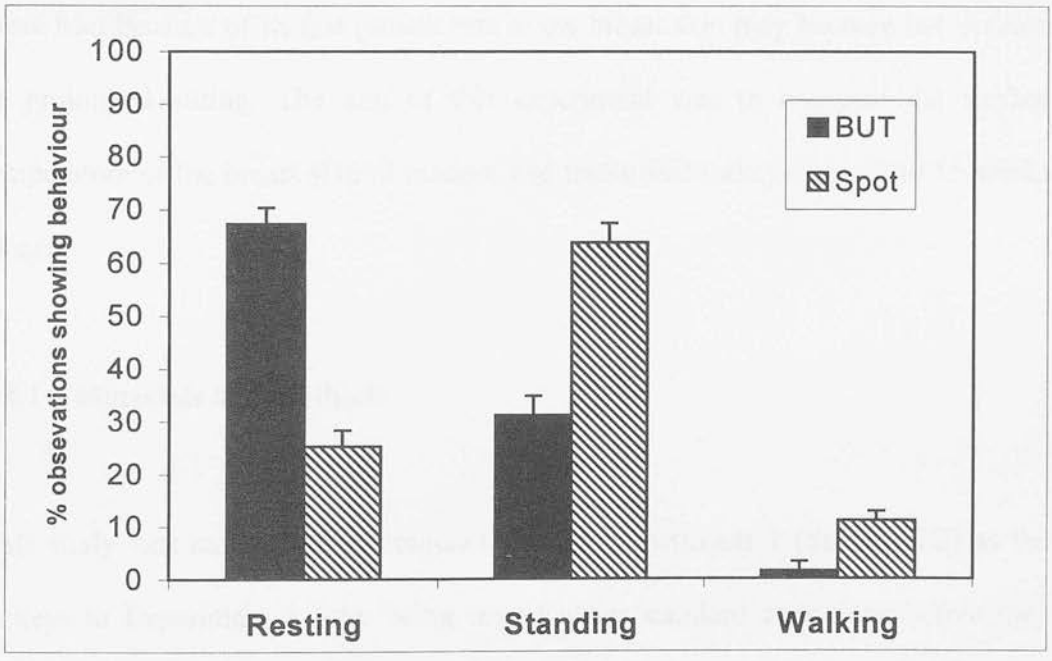


Figure 3.16 Mean number of observations (± SED) of resting, standing and walking as a percentage of the total number of observations per pen from 6 pens of Spot and BUT turkeys in 4 two-hour periods.

Significant effects of sex were found for resting and standing ($P<0.001$) with the males sitting in 67% of observations compared with 25% for the females and the females standing in 67% of observations compared with 28% for the males. There was no significant difference between the sexes in the percentage of observations of walking.

3.4 Experiment 3: Breast skin temperature comparison

Poor feathering on the breast of modern turkeys may be an adaptive response allowing more heat to be dissipated from the skin. The modern turkey may produce more heat because of its fast growth rate or the breast skin may become hot because of prolonged sitting. The aim of this experiment was to compare the surface temperature of the breast skin of modern and traditional turkeys from 2 to 15 weeks of age.

3.4.1 Materials and Methods

This study was carried out in conjunction with Experiment 1 (Section 3.2) as the turkeys in Experiment 1 were being reared under standard conditions before they were killed for feather measurements (Section 3.2.1). Each week from 2 to 15 weeks of age skin temperatures were measured from the same three birds, selected at random at the start of the trial, from each of the 12 pens used in Experiment 1 (Section 3.2). Any feathers over the breast were separated to allow direct contact with the skin and the skin temperature measured in the centre of the sternal apteria using a

Pistol-Grip infrared thermometer with circular laser sight (RS Components Ltd, Corby, Northants, UK).

Analysis of variance was used to test for differences in skin temperature in a model with effects for age, strain, sex and their interactions taking into account effects of block, pen and bird.

3.4.2 Results

The three way interaction of sex, strain and age was not significant and there were no significant effects of sex.

A significant ($P<0.01$) interaction of strain and age was observed and the data are shown in Figure 3.17. Although there did not appear to be many ages where there were clear strain differences in skin temperature the pattern showed Spot skin temperature to be consistently lower than BUT skin temperature up to 8 weeks of age. From 9 weeks onwards the skin temperature of the Spot was consistently higher than the BUT and the difference between the strains was greater. Skin temperature of both strains appeared to decline with age although this decline was more gradual for the Spot and compared with a steep decline of almost 2°C for the BUT. Both strains had a sharp decline in skin temperature at 7 weeks of age.

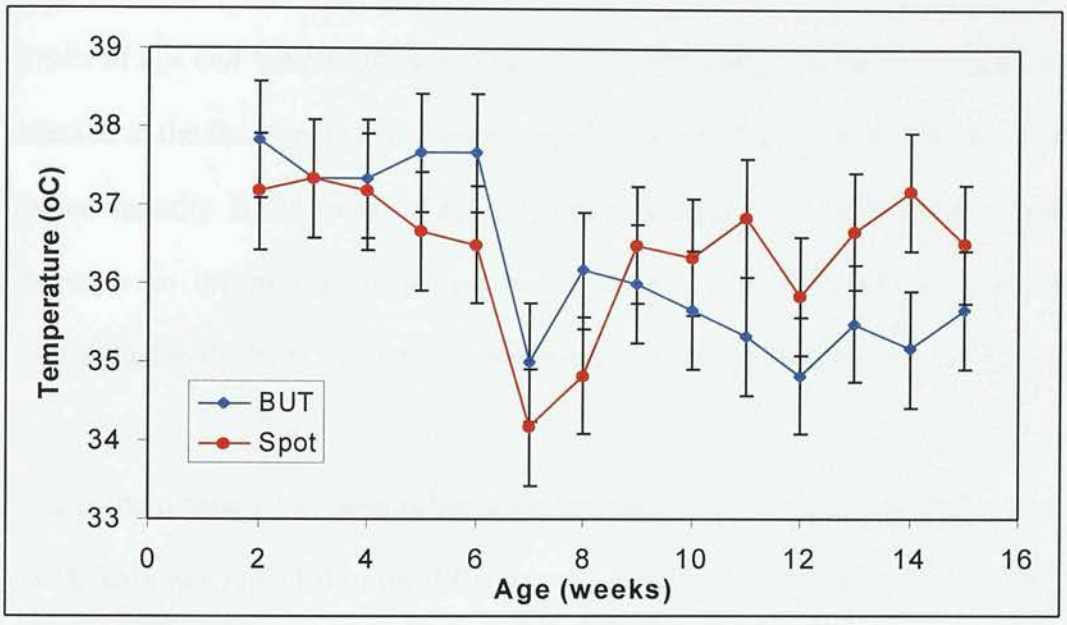


Figure 3.17 Breast skin temperature (mean ± SED) of BUT Big 5 male line and Nebraska Spot turkeys from 2 to 15 weeks of age.

3.5 Discussion

The aim of Experiment 1 (Section 3.2) was to quantify and compare feather and body growth in a modern and a traditional strain of turkey. Eight ages during the growth of the turkeys were selected for the comparison from the appearance of the first feathers to the age when the birds become sexually mature. At two weeks of age feathers were easily measured from the wing, tail and back but down was still present on the breast as feathers in the sternal tracts had only recently started to grow and were not long enough to be measured. Measuring feather follicle density at two weeks of age was not possible as the sternal tract was small and the fixed area selected for counting follicle numbers extended on to the apteria. This would have resulted in an inaccurate

assessment of the follicle density at this age. Two-week intervals were used during the period when feather growth was expected to be at a maximum between 2 and 10 weeks of age and 5-week intervals were used subsequently. Twenty-five weeks was selected as the final age to avoid any changes in growth that might be associated with sexual maturity. By 25 weeks of age only one male Spot remained because of earlier mortality so the measurements are based on only one bird and this will have contributed to the large standard errors associated with the male Spots.

The modern turkey has been selected for increased body weight and breast muscle yield. This was reflected in the difference in body weight and breast muscle weight between the modern and traditional turkey. By 25 weeks of age the modern male bird was 3 times the weight of the traditional male and yet there was no difference in the weight of feathers from these birds. This would suggest that selection for increased body weight of the modern turkey has not resulted in an increase in feather weight. Indeed because there was no difference in feather weight between the strains but such a large difference in body weight the percentage of the body weight that is feathers was much higher in the traditional turkey suggesting that relative feather cover was poor in the modern turkey. Work by Katanbaf (1989) showed that positive correlated responses to selection for body weight in chickens included proportionally larger breast but that feather weight was included in the negative responses. Selection has increased the body weight of the modern turkey but the same weight and lengths of feathers as the smaller traditional bird remain to cover the larger bird and result in relatively poor feather cover.

The rate of feather growth does not differ between the strains for any of the feathers measured so it would appear that the rate of feather development has not been influenced by the selection of the modern turkey. Feather lengths from the back, wing, tail and caudal breast were also found to be similar between the strains so it would appear that there were no differences in the growth of feathers from these areas in the modern turkey compared with the traditional turkey. The only area of feathering on the modern turkey that was found to differ from the traditional turkey was the area of the cranial breast. Here the feathers were much shorter in length on the modern turkey suggesting that feather growth on the cranial region of the breast was impaired. This result supports the observation by Hocking (1995) that feathers from the anterior portion of the breast of modern turkeys were short or absent.

Feather follicle density was found to decrease with age in both the modern and the traditional turkey. This decrease in breast feather follicle density corresponds with an increase in body weight and breast muscle weight. It is therefore likely that as the birds grew the same number of feathers were available to cover a larger area of breast resulting in a smaller number of follicles in the same fixed area. Follicle density was lower in the modern compared with the traditional turkeys at all ages. The lower follicle density on the breast tracts of the modern turkey may be caused by the breast skin stretching to cover the much larger area of muscle without an increase in the total number of feather follicles. No difference was found in the rate of decrease in follicle density between the two strains. It might have been expected that if follicle density was related to breast muscle growth then there would be a difference in the follicle density rate parameter because of the difference in breast muscle rate

parameter. However neither the breast tract length or width showed a difference in rate of increase suggesting that the breast tracts grew at the same rate in both the modern and traditional turkey and that this was not dependent on rate of breast muscle growth. The final length of the sternal tract did not differ between strains but there was a large standard error associated with the Spot male. However the shorter tract length of the Spot female might also have been expected in the Spot male and indeed this was the case up to 20 weeks of age. The sternal apteria was not measured in this experiment but it is likely that changes in the sternal apteria width would be similar to changes in the sternal tract width unless the featherless structure of the apteria made it more susceptible to stretching.

The length of the back tract was used as an indication of skeletal size relatively independent of muscle mass for further comparisons of the modern and traditional turkey. Although the final back tract measurement was found to be longer in the modern turkey the difference between the strains was small compared with the difference in body weight. It is therefore possible that feather growth is more closely related to skeletal size than to body weight.

No measurements were taken to compare feather weight with the surface area of the two strains but it would be expected that the relative surface area of the modern turkey has not increased as much as body weight. However, it would be expected that there has been some increase in surface area in the modern turkey because of the longer back tract length and breast tract length and that when related to surface area, relative feather cover would still be greater in the traditional turkey.

The total crude protein in the feather free carcass showed a similar increase with age as body weight because there was no difference in the proportion of carcass protein between the two strains. It was interesting to find that carcass protein was different between both the sexes and the strains but there were no sex or strain differences in total feather protein. This result suggests that, although the modern turkey contained more grams of crude protein than the traditional turkey, the majority of the extra protein was used for body and muscle growth with no increase in the protein used for feather growth. Feather protein did increase with age but this was expected because feathers increase in size with age. Feather protein will also change with the maturity of the feathers because as the feather matures the percentage of moisture in the feather declines. As the feather dry matter was similar between strains at all ages this would suggest that feathers from both the modern and traditional turkeys are maturing at the same rate and could indicate that moulting patterns might be similar. When carcass and feather protein were compared as a percentage of the total body weight no differences were found between the two strains in percent carcass protein. However as the traditional turkeys had a much lighter body weight and there was no strain difference in the total feather protein the feather protein makes up a higher percentage of the traditional bird's body weight. This suggests that relative to body weight the traditional turkey partitions a larger amount of protein to feather growth than the modern turkey. The females of both strains have a lower percentage of their feather free carcass weight as protein compared with the males, particularly at 25 weeks, that is associated with the increase in carcass fat with age in the females.

The results from the behaviour study confirmed observations that the large modern turkey was less active and spends a greater proportion of its time resting. The observations were made when the turkeys were 6 weeks old but with increasing age the difference in body weight between the two strains increases. It would therefore be likely that the modern turkey would spend an even greater time resting as it got older if the behaviour was related to body weight. As there were differences in the behaviour of the two strains it is possible that the differences in breast feathering may be related to this difference in resting behaviour. Resting, or the change in posture from standing to resting and vice versa could cause friction to the breast and result in the breaking of breast feathers. Prolonged periods spent sitting could also result in poor feather growth, perhaps because of increased pressure causing a reduction in blood flow, or damage to the feather follicles. In this study the length of time of each period of sitting and the number of changes of position were not recorded.

When the breast skin temperature of the two strains was compared from 2 to 15 weeks it was interesting to find that skin temperature decreased with age in both strains but at a greater rate in the modern turkey. Skin temperature also started to decline at an earlier age in the traditional turkey. Up to 8 weeks the modern turkey had a higher skin temperature but by 9 weeks the skin temperature of the modern turkey had fallen below that of the traditional turkey. These results suggest that the modern turkey was producing more heat at the younger ages that it was then losing through the skin. This might be related to the rapid early growth rate of the modern turkey. By 9 weeks of age the breast feather cover of the traditional turkey was good and this may provide insulation to the breast skin trapping heat at the skin and not

allowing it to dissipate. Conversely the breast feather cover of the modern bird was poor allowing heat to be dissipated easily perhaps resulting in the lower skin temperatures. It is possible that when turkeys rest the temperature of the breast skin increases because it is in contact with the floor. In this study it was not possible to use birds that were resting or to record posture directly before measurement because of the disturbance of the birds on entering the pen. Another reason for lower skin temperature in the modern turkey could be a reduction in the blood flow to the skin because of the pressure associated with prolonged periods of resting. A sharp decrease in skin temperature was noted in both strains at 7 weeks of age and this could be associated with a drop in the ambient temperature after the removal of supplementary heating at 6 weeks of age.

Comparing the modern turkey with the traditional turkey, it would appear that feather growth has not increased with increased selection for body weight in the modern bird. The lower follicle density on the breast tracts of the modern turkey may be caused by the breast skin stretching or growing to cover the much larger area of muscle without an increase in the total number of feather follicles. The growth of breast feathers from the cranial region of the breast tract appears to be impaired in the modern turkey and this may be related to the increased proportion of time spent resting. It is possible that the difference in carcass protein between the two strains has resulted from protein being partitioned preferentially for muscle and body growth in the modern turkey. It is also possible that the size and number of feathers may be genetically determined and is not related to genes associated with body growth. The poor breast feathering of the modern turkey may also have resulted in cooler skin

temperatures as the birds age because of decreased feather insulation or this may be an adaptive response to practical ambient temperatures. This point will be addressed in the next chapter.

CHAPTER 4: Temperature and Feeding

4.1 Introduction

Feathers provide an excellent insulative cover and assist birds in maintaining their body temperature by regulating heat loss to the environment. The major routes of heat loss are from the respiratory tract and unfeathered areas of the body.

Thermoregulation in a hot environment can be improved by sensible heat dissipation (radiation, convection and conduction). An important component of sensible heat loss is convection, which is affected by peripheral blood flow, body surface area and body covering (insulation). In a hot environment skin temperatures increase because of an increase in the blood flow to the periphery and body heat can be lost more effectively from areas with no or poor feather cover (Pech-Waffenschmidt *et al.*, 1995).

Richards (1977) compared the thermoregulatory responses of normally and poorly feathered laying hens and found that body temperature in the poorly feathered birds began to fall at a higher environmental temperature than in the normally feathered birds. The difference in heat loss between poor and well feathered birds increased as the environment became colder. Naturally poor feather cover can be seen in birds with the naked neck gene and the frizzle gene. It has been shown that heat emissions from the poorly feathered sites of these birds are significantly greater compared with

well feathered areas thus allowing improved heat loss from the body surface (Pech-Waffenschmidt *et al.*, 1995). The poor plumage of both frizzle fowl and moulting hens results in an increase in metabolic rate (Balnave, 1974). Richards (1977) reported that poorly feathered birds had a 60% higher metabolic rate compared with normally feathered birds. The heat production of defeathered cockerels is much greater than control birds and this difference increases with decreasing environmental temperature (Balnave, 1974). Lee *et al* (1983) examined the possibility of minimising heat loss of poorly feathered layers through the use of insulative jackets and found that the jackets reduced fasting heat production in these birds.

Using a modelling approach Emmans (1989) concluded that ambient temperatures above 10-12°C would reduce growth rates in turkeys from 10-18 weeks of age. In the process of digesting food and growing, heat is produced which the turkey must lose if it is to maintain constant body temperature. The unfeathered head and neck of the turkey contribute to heat dissipation at high ambient temperatures and it has been suggested that this may have been an adaptive response which has allowed these birds to survive in hot environments with reduced risk of hyperthermia (Buchholz, 1996).

Poor feather cover could result in the modern turkey losing more heat to the environment, so permitting it to achieve rapid growth rates. Feather cover might be improved in the turkey by restricting feed intake, as broilers given restricted feeding appear to have better feather growth and body cover than that of a full-fed bird. Smith (1994) suggested that because breast development was reduced and the length

of the feathers was hardly changed in restrict-fed birds, this resulted in the body being apparently better feathered and, in particular, that the breast was more fully covered. Restricted feeding of broiler hens also decreased their fasting heat production (MacLeod and Hocking, 1993) although this was largely because of the reduction in body weight. However, MacLeod *et al* (1979) found that regulating food intake in laying hens decreased metabolic rate per bird and per unit metabolic body weight.

Poor feather cover would aid the heat loss process and it is possible that reduced feather cover, particularly over the breast, is an adaptive response to practical ambient temperatures. Conversely, food restriction might result in greater feather growth to minimise heat loss to the environment.

The objective of the experiment was to test the hypothesis that loss of breast feathers is an adaptive response to practical ambient temperatures. Feather growth was compared at ambient temperatures of 26°C and 15°C. This was combined with a comparison of feather growth when birds were fed *ad libitum* or restricted quantities of food to investigate whether feather growth can be improved by restricted feeding. Fasting heat production was measured to investigate the effects of temperature and feeding on metabolic rate.

4.2 Materials and Methods

Sixty BUT Big 5 male-line male turkeys were reared on the floor at normal brooding temperature (30-32°C) with free access to a standard turkey starter diet from day old to 14 days of age. Lighting pattern throughout was maintained at 14h light:10h dark. At 14 days the birds were weighed individually and the 40 heaviest birds allocated randomly to 20 individual cages (305mm x 457mm) in each of two climate chambers. One climate chamber was maintained at 15°C and the other at 26°C. Temperature and humidity were monitored in each room using Tinytalk II dataloggers (RS Components Ltd, Corby, Northants, UK). Rectal temperature was recorded twice per day for the first week to ensure that body temperature was maintained. The cages were arranged in pairs, with birds in each pair randomly allocated to either *ad libitum* (AL) or restricted (R) feeding. The restricted feeding programme was adjusted, within each climate chamber, to maintain body weight at 50% of the *ad libitum* fed birds in that climate chamber. The birds were weighed twice a week and the mean body weight of AL and R birds at each temperature was calculated. The daily food ration given to R birds was adjusted according to the mean body weight of R birds in each chamber. Food intake of the AL birds was recorded twice each week.

At 42 days of age 12 birds from each climate chamber were killed by intravenous injection of sodium pentobarbitone (Euthatal, Rhone Merieux Ltd, Harlow, UK). Feather measurements were taken as described in Chapter 2 (Materials and Methods).

The experiment was conducted in two phases so that both climate chambers were used for the two ambient temperatures.

Four AL and 4 R birds from each climate chamber were selected at random at the start of the trial to be used in a calorimetry study. The open-circuit indirect calorimetry system described by Lundy *et al* (1978) was used to measure heat production. This experiment was designed as a Latin square using four calorimeters with each calorimeter occupied by a bird from one of the four treatments. There were therefore 4 replicates giving a total of 16 birds used in the experiment over a period of 8 days. The birds were randomly assigned to calorimeters. The calorimetry chambers were set to a temperature of 20°C and body temperature was recorded once a day using a rectal probe. Heat production was measured in KJ/bird/day over two periods of 22 hours. During this time the birds were only allowed access to water and fasting heat production was determined in the second 22h period. Birds were weighed before the start of each 22h period. To permit comparisons between birds and with other studies, results were related to metabolic body size ($kgW^{0.75}$ where W = body weight (Kleiber, 1947)). Activity level was recorded using a Doppler-radar activity measurement system (MacLeod *et al.*, 1982). Heat production (J/min) was corrected for activity level and the results expressed as KJ/ $kgW^{0.75}$ /day. After 48 hours in the calorimeter the birds were killed and feather measurements were taken as described in Chapter 2 (Materials and Methods).

Statistical Analysis

The data from the two experiments using the controlled environment chambers were analysed as four units. Feather measurements and weights, excluding those from birds used in the calorimetry study, were analysed using a split plot analysis of variance. The effects of temperature were modelled at the level of whole plots (chambers) and the effects of feeding and feeding × temperature were analysed as split plot effects. The block effects were chamber and the split plots were each pair of birds. Calorimetry data were analysed using general analysis of variance. Differences in AL food intake at the two temperatures were compared using analysis of variance with effects for day, temperature and their interaction.

4.3 Results

The information collected from the Tinytalk dataloggers showed that the temperatures in the controlled environment chambers were accurately maintained at 15°C and 26°C. There was a small amount of fluctuation around the set temperatures but this did not exceed ± 1°C. Humidity varied between 30% and 50% in both chambers although the average humidity during the experiment was 40%.

The feather, weight and calorimetry measurements showed no significant temperature and feeding interactions.

Calorimetry

Temperature had a significant ($P<0.05$) effect on activity with birds previously kept at 15°C being more active in the calorimeter than those at 26°C. Fasting heat production (KJ/bird/day) was not significantly different for birds previously kept at the two temperatures either before or after correction for activity (J/min/activity) (Table 4.1). Correcting heat production for metabolic size ($kgW^{0.75}$) resulted in a significant ($P<0.001$) difference as birds previously kept at 15°C produced significantly more heat per $kgW^{0.75}$ than those kept at 26°C. This difference remained when heat production corrected for metabolic size was also corrected for activity level (Table 4.1).

Feeding regime had no significant effect on activity level in the calorimeter. Fasting heat production was significantly ($P<0.001$) affected by feeding, with AL birds producing more heat than R birds, and when heat production was corrected for activity this significant difference remained. There was no significant difference between AL and R birds after correcting heat production for metabolic body size and for activity (Table 4.1).

Table 4.1 Mean (± SED) fasting heat production and activity measures in calorimeters at 20°C for birds previously kept at 15°C and 26°C on either restricted or *ad libitum* feeding. Analysis of variance significance levels * P<0.05, *** P<0.001

Trait	Temperature				Feeding			
	15°C	26°C	SED	Sig	*Ad Lib*	Restrict	SED	Sig
Activity count (units/min)	164	122	19.5	*	131	155	19.5	NS
Fasting Heat Production								
KJ/bird/day	1024	986	47.4	NS	1306	704	47.4	***
Activity corrected (J/min)	760	706	36.6	NS	958	508	36.6	***
$KJ/kgWt^{0.75}$/day	714	661	14.6	***	691	684	14.6	NS
$KJ/kgWt^{0.75}$/day (corrected for activity)	759	681	19.7	***	729.2	710.5	19.7	NS

Temperature

Temperature had a significant (P<0.05) effect on body weight with birds kept at 15°C being lighter than those kept at 26°C. Back tract length was significantly shorter in the birds reared at 15°C and despite this, and the difference in body size, the lengths of feathers from the tail, wing, cranial breast and caudal breast were not significantly different in the birds kept at the two temperatures. The length of feathers from the back were significantly (P<0.05) shorter in birds kept at 15°C. Total feather weight was similar in birds kept at 15°C and 26°C and the reduction in feather weight at 15°C was found to be significantly less (P<0.01) than the reduction in body weight. Temperature had no significant effect on the length and width of the sternal tract or the width of the sternal apteria. Feather follicle density was significantly (P<0.05) greater in birds kept at 15°C (Table 4.2).

Feeding

Body weight and breast muscle weight of the R birds were significantly ($P<0.001$) reduced compared with the AL birds. Feather lengths from the back, tail, wing and caudal breast were significantly ($P<0.001$) longer in the AL birds than in the R birds. However, cranial breast feathers were significantly ($P<0.01$) longer in the R birds. Feather tract measurements and feather weight were significantly ($P<0.001$) greater in the AL birds compared with R whereas feather follicle density was increased significantly ($P<0.01$) in R turkeys. The reduction in feather weight in the R birds was significantly less ($P<0.001$) than the reduction in body weight of the R birds.

A significant ($P<0.001$) day × temperature interaction was observed for daily food intake of AL fed birds. At both temperatures AL food intake increased with time but birds kept at 15°C increased their food intake to a greater extent than those kept at 26°C (Table 4.3).

Table 4.2 Mean (± SED) body and feather measurements from birds kept at 15°C and 26°C on either restricted or *ad libitum* feeding. Analysis of variance significance levels * P<0.05, ** P<0.01, *** P<0.001.

Trait	Temperature				Feeding			
	15°C	26°C	SED	Sig	*Ad Lib*	Restrict	SED	Sig
Body weight (g)	1535	1801	52.9	*	2223	1112	59.5	***
Breast muscle w (g)	148.6	150.9	1.6	NS	211.6	87.9	7.35	***
Feather Lengths (mm)								
Back	43	47	0.6	*	52	37	1.1	***
Tail	116	132	4.0	NS	136	112	2.3	***
Wing	176	183	7.8	NS	188	171	1.9	***
Cranial breast	25	26	1.0	NS	23	27	1.1	**
Caudal breast	32	33	3.5	NS	35	30	1.0	***
Feather Tracts (mm)								
Back tract length	207	224	2.7	*	244	187	3.8	***
Back tract width	47	51	6.2	NS	55	43	2.0	***
Sternal tract length	107	115	4.9	NS	125	97	2.5	***
Sternal tract width	20	22	3.4	NS	22	20	0.5	**
Sternal apteria	15	17	2.7	NS	24	8	1.2	***
Feather Cover								
Feather weight (g)	89.1	92.7	10.9	NS	102.2	79.6	5.67	***
Follicle density	21	19	0.4	*	19	22	0.8	**

Table 4.3 Mean daily food intake (g) of *ad libitum* fed birds kept at 15°C and 26°C (SED 3.9), and daily food ration (g) for restricted birds, adjusted twice weekly according to mean body weight of *ad libitum* fed birds at each temperature.

Day	15°C		26°C	
	Restricted	*Ad libitum*	Restricted	*Ad libitum*
18	30	52.1	30	50.3
21	30	57.3	30	53.2
25	40	71.8	30	67.0
28	50	84.3	40	72.3
32	50	109.2	50	79.8
35	60	122.3	60	92.3
39	80	141.5	70	103.7
42	120	153.2	100	121.5

4.4 Discussion

The use of two climate-controlled chambers did not allow for repetition of the temperature treatments so the experiment was conducted in two phases. However, the data from the two chambers and two phases were analysed as four units because there was a time difference between the phases and each chamber was used for each of the two ambient temperatures over the two phases. As a further control, the temperature and humidity within the climate chambers were accurately monitored to ensure consistency of environment in the two phases of the experiment.

Temperature had a significant effect on body weight with birds kept at 15°C being 15% lighter than those kept at 26°C. Temperature can affect body weight in two ways. Firstly an environmental temperature that is too high will depress food intake and restrict growth rate as the bird tries to lose heat to maintain constant body temperature (Emmans, 1989). Environmental temperatures above 20°C have been observed to depress growth of turkeys after 10 weeks of age but not to restrict weight gains of younger turkeys (Waibel and MacLeod, 1995). The higher temperature of 26°C was used for this study to provide a warm environment for birds at two weeks of age and to avoid severe heat stress in birds at six weeks of age. Secondly an environmental temperature that is too cold will cause increased heat production in order to maintain constant body temperature. This will result in less energy available for growth and cause an increase in food intake (Gonyou and Morrison, 1983). In the present experiment food intake was found to be greater in the birds kept at 15°C. Emmans (1989) calculated that a temperature of 10-12°C would allow sufficient heat dissipation for turkeys between 10 and 18 weeks of age. The lower temperature of 15°C was used for this study to rear birds, up to six weeks of age, in a much cooler environment than is standard practice for birds of this age but without the temperature being low enough to cause hypothermia at two weeks of age.

Rearing turkeys at 15°C did not increase feather growth compared with birds reared at 26°C. Feather lengths and feather weight were decreased in birds kept at 15°C but this decrease of less than 10% was not significant. Feather weight expressed as a percentage of body weight was 5.8% and 5.1% for birds kept at 15°C and 26°C respectively. Although feather growth was not dramatically improved in birds reared

at 15°C the results show that feather growth was at least maintained to a greater extent than body weight. This would suggest that it is physiologically important for birds to maintain feather growth at low temperatures in order to minimise heat loss even if this results in reduced body growth.

Instead of increasing feather cover to help with thermoregulation it would appear that the bird instead alters metabolic rate. No difference in fasting heat production was found between birds kept at the two temperatures, however, a more accurate comparison between treatments can be made by taking metabolic body size and activity into account. When fasting heat production was corrected for both metabolic body size and activity it was found that birds kept at 15°C produced more heat per $kgW^{0.75}$ than those at 26°C. The results suggest that the birds have adapted to the environmental temperature by altering their metabolic rate either by reducing metabolic rate at the higher temperature or increasing metabolic rate at the lower temperature. This adaptation was maintained during hours 24 to 48 in the calorimeters at 20°C. MacLeod *et al* (1979) described an adaptation of fasting heat production to high and low environmental temperatures and showed that, when the environmental temperature was changed from hot to cold, the response was complete within one day. However, when the temperature was changed from cold to hot, metabolic rate remained significantly higher than in birds acclimatised to the hot temperature, and complete adjustment took 14 days. The difference in fasting heat production may therefore be caused by a relatively high metabolic rate in birds kept at 15°C.

Heat production was corrected for activity for two reasons. Firstly because heat is produced due to activity and secondly any movement which disturbs feather cover may increase the heat loss through the escape of warm insulating air between the skin and feathers (Balnave, 1974). When comparing activity in the birds it was found that birds previously kept at 15°C showed higher activity levels in the calorimeters. This may be because the temperature in the calorimeters was 20°C and birds coming from a cooler temperature may find this more pleasant and become more active. The birds introduced from the warmer temperature may find 20°C a little cool and may reduce their activity level to reduce heat loss.

Restricted fed turkeys were successfully maintained at 50% of the body weight of the *ad libitum* fed birds. Restricting the bird's body weight resulted in a significant decrease in feather lengths from the back, tail, wing and caudal breast. However, these feather lengths were only reduced by between 10% and 30%, a much smaller reduction than for body weight. Breast muscle weight was reduced by 58% in the restricted birds. These results suggest that feather growth was maintained more than body weight or muscle weight when food supply was limited. Indeed if feather weight as a percentage of body weight was compared in birds from the two feeding regimes it can be seen that restricted fed birds had relatively greater feather weight for their body weight (7.2%) compared with *ad libitum* fed birds (4.6%).

It is interesting that back tract length was reduced by 23% in restricted birds which was a similar reduction to that seen in the feather lengths and feather weight. It is possible that if back tract length is a more accurate indication of skeletal size than

body weight, that it is a more accurate measure of the bird's size or stage of development for comparisons of relative growth. If back tract length was used then the feather lengths decrease in proportion to the size of the bird. The only feather measurement that did not follow this pattern on either a body weight or tract length basis was the cranial breast feather length. The lengths of these feathers increased significantly by 15% in the restricted fed birds compared with the *ad libitum* fed birds. The results suggest that the growth of the cranial breast feathers in *ad libitum* fed turkeys is impaired.

Feather follicle density increased in the restricted fed birds perhaps because the same total number of follicles were present over a smaller breast muscle and reduced area of breast skin. This would give the appearance that feather cover had improved but would be a consequence of the size of the bird rather than any real increase in feather cover. Smith (1994) reported that improved feather cover in broilers given restricted feeding resulted from a reduction in body weight, and in particular reduced breast development, with the length of feathers being hardly changed. In the present experiment feather weight was reduced to the same extent as feather lengths. However, Smith (1994) found the weight of feathers in restricted fed broilers to be affected more than the lengths and suggested that this reduction in feather weight was due mainly to a decrease in the structural thickness rather than length. It is possible that the decrease in feather lengths and feather weight observed in this study are similar because the turkeys maintained the structural integrity of the feathers but reduced feather length in proportion to body size. However, no measurements of structural thickness were made in the present experiment.

The results from the calorimetry experiment indicated that fasting heat production was significantly greater in birds fed *ad libitum*. The heat production in restricted birds was 50% lower than in the *ad libitum* fed birds. It would appear that this difference in fasting heat production was caused by the difference in body weight between birds on the two feeding regimes. When fasting heat production was corrected for body weight there was no significant difference between *ad libitum* and restricted birds. MacLeod and Hocking (1993) showed that controlled feeding of broiler hens reduced heat production but that this was largely because of the reduction in body weight. There was no significant difference in recorded activity between the birds in the calorimeters although the restricted birds appeared to be more active in the climate chambers. This observation could be caused by the restricted birds associating people with feeding while in the climate chambers and therefore increasing their activity in anticipation of food (Petherick and Waddington, 1991). During the calorimetry experiment the birds were visually isolated and would have no stimulus for this increase in activity.

The aim of this experiment was to investigate the possibility that breast feather growth might be improved by rearing turkeys at a low ambient temperature. The results suggest that feather growth was not increased in turkeys kept at 15°C but that it was maintained to a greater extent than body weight. The metabolic rate of these birds was increased to maintain body temperature rather than increased feather cover. Restricted feeding had a greater apparent effect on feather growth by decreasing body mass more than feather weight. Feather growth was therefore maintained in preference to muscle and body growth when food was restricted. Restricting food

intake also resulted in improved growth of the cranial breast feathers in modern turkeys.

In conclusion rearing birds at 15°C did not improve breast feather cover. It is suggested that breast feather growth in the *ad libitum* fed turkey is impaired and that restricted feeding can improve breast feather growth.

CHAPTER 5: Nutrition

5.1 Introduction

The turkey requires dietary protein for the maintenance and growth of both body mass and feathers. Growth of body tissues is the result of the balance between protein synthesis and degradation, however feather keratin is not degraded once it has been synthesised. Inadequate dietary protein will result in a reduction of growth and a withdrawal of protein from less important body tissues to maintain the functions of more vital body organs (Muramatsu and Okumura, 1985).

Feathers can represent 4 to 13 percent of body weight and have a high protein content, with reports suggesting that feather dry matter is 89-97% protein (Fisher *et al.*, 1981; Graber *et al.*, 1971) (W.K. Smith personal communication). Dr W.K. Smith (personal communication) also suggested that the protein requirement for feather growth has been underestimated because the amount of pulp produced during feather synthesis is in fact 5-10 times that which is currently assumed. Protein requirement for feather growth is therefore likely to be a significant proportion of the total protein requirement of the bird. The modern turkey has been selected for increased breast muscle yield and rapid growth rates and it is likely that protein requirements for muscle growth are also high. It is possible that the dietary protein requirements of the modern turkey are underestimated, particularly during periods of

rapid feather growth, and that this could result in competition between feather and muscle growth for dietary protein or specific amino acids.

The sulphur amino acids (cystine and methionine) are the major amino acids involved in feather growth (Wheeler and Latshaw, 1981), although feathers showing signs of amino acid deficiency have been noted in chicks fed rations deficient in arginine, valine, leucine, isoleucine, phenylalanine and tyrosine (Anderson and Warnick, 1967). Poor feather growth in the modern turkey may be caused by dietary deficiencies or imbalances of the amino acids required for feather growth.

Restricted feeding has been shown to reduce muscle growth to a greater extent than feather growth (Chapter 4). This would be a sensible strategy when energy supply is limited because, by maintaining insulation, energy can be conserved. However selection for increased muscle growth in the modern turkey might have resulted in a bird in which muscle accretion will continue when dietary protein is limited, at the expense of feather growth. If protein is preferentially partitioned to muscle growth any dietary deficiency in protein or specific amino acids may result in poor feather growth.

The purpose of Experiment 1 (Section 5.2) was to compare modern and traditional turkeys on low protein diets and the objective of Experiment 2 (Section 5.3) to assess the role of specific amino acids on feather growth by supplementing a low protein diet defined in Experiment 1 (Section 5.2).

5.2 Experiment 1: Low Protein

The aim of this experiment was to compare the effect of a low protein diet on feather and muscle growth in a modern and a traditional turkey. Feather and muscle growth were compared in birds fed on diets with decreasing protein concentration to test the hypothesis that body growth, and in particular breast muscle growth, are maintained at the expense of feather growth when protein concentrations in the diet are deficient.

5.2.1 Materials and Methods

Male and female Nebraska Spot and BUT Big 5 male-line turkeys were reared in separate floor pens until one week of age. During this period birds were fed on a standard turkey starter diet (Chapter 2). The lighting programme was maintained throughout the trial as 14h light:10h dark. After one week, 24 male and 24 female Spots and 48 male and 48 female BUTs were weighed, wingbanded and randomly allocated to 32 pens that were arranged in 2 blocks of 16 pens. Four diets were formulated, using the Uni-Mix diet formulation package (New Century Software, Format International Limited, Woking, England) to obtain crude protein (CP) concentrations of 180, 220, 260 and 300g/kg. The same ingredients were used to formulate each diet (Table 5.1). The metabolisable energy concentration (ME) of each diet was formulated at 12.0 MJ/kg. The diets were analysed for nitrogen (N) content using a protein/nitrogen analyser (LECO Corporation, Stockport, Cheshire, UK).

The experimental diets were fed to the birds from one to 6 weeks of age. Birds were weighed as a pen and the food intake per pen recorded weekly. At 6 weeks of age the birds were removed from the pens and killed by injection of sodium pentobarbitone and feather measurements recorded by the methods described in Chapter 2. The carcasses were frozen and prepared for carcass analysis (Chapter 2) after pooling carcasses within pen.

Trait measurement data from birds in each pen were pooled and the pen mean used in the statistical analysis. Analysis of variance was used to test for treatment differences in trait measurement and carcass composition data in a model with effects for sex, strain, crude protein concentration and their interactions.

The results of the analysis of experimental diets for nitrogen content were used to calculate the crude protein (N x 6.25) concentrations of each diet. The diets contained 182, 218, 259 and 296g CP/kg, approximating the formulated crude protein concentrations (Table 5.1).

Table 5.1 Composition of experimental diets (g/kg), formulated to contain crude protein concentrations of 180, 220, 260 and 300g/kg.

Ingredients	Dietary crude protein concentration (g/kg)			
	180	220	260	300
Wheat	713.0	635.3	557.7	480.0
Soya	198.0	248.7	299.3	350.0
Fish meal	10.0	40.0	70.0	100.0
Soya oil	13.5	12.8	12.2	11.5
Maize gluten meal	0	7.2	14.3	21.5
Dicalcium phosphate	43.6	34.5	25.4	16.2
Sodium chloride	3.3	3.1	3.0	2.8
Choline chloride 50%	0.4	0.4	0.4	0.4
Methionine	0.8	1.0	1.2	1.5
l-Lysine HCl	2.5	2.0	1.5	1.1
Vitamin / mineral mix[1]	7.0	7.0	7.0	7.0
Pellet binder[2]	8.0	8.0	8.0	8.0

[1] Supplied per kg diet: Cu 14 mg, I 1.4 mg, Fe 112 mg, Mn 140 mg, Zn 112 mg, Se 0.28 mg, Mo 0.7 mg; Co 0.7 mg, retinol 5.0 mg, cholecalciferol 175 μg, α-tocopherol 47 mg, menadione 4.2 mg, riboflavin 9.8 mg, thiamine 2.8 mg, nicotinic acid 70 mg, pantothenic acid 21 mg, biotin 280 μg, pyridoxine 7 mg, cyanocobalamin 21 μg, folic acid 1.4 mg.

[2] Walfolin S. (Holman Lignotech Ltd, Reading, UK).

5.2.2 Results

Food intake was not significantly affected by dietary protein concentration (Table 5.2). Both sex and strain had a significant ($P<0.001$) effect on food intake with males consuming more than females and the larger BUT turkey consuming more than the Spots.

Table 5.2 Cumulative feed intake (g/bird) of male and female BUT and Spot turkeys fed on diets containing crude protein concentrations of 180, 220, 260 and 300g/kg for weeks 3 to 6. Analysis of variance significance levels, NS not significant.

Protein concentration (g CP/kg)	BUT male	Spot male	BUT female	Spot female
180	2761	1736	2552	1459
220	2979	1799	2484	1460
260	2915	1788	2680	1500
300	3023	1813	2745	1494
SED	154.9	154.9	154.9	154.9
Significance	NS	NS	NS	NS

Feather and body traits

The three-way interaction of sex, strain and crude protein concentration was not significant for any of the traits measured. A significant interaction between strain and dietary protein concentration was observed for body weight ($P<0.001$), breast muscle weight ($P<0.001$) and feather weight ($P<0.05$) (Figure 5.1). Decreasing dietary crude

protein concentration from 300g/kg to 180g/kg reduced the body weight of the modern turkey by 44% and the traditional turkey by 19%. Breast muscle weight was affected by diet in a similar way with the muscle weight of the modern turkey reduced by 52% compared with 24% in the traditional turkey. Feather weight was also reduced in both the modern and traditional turkey by decreasing crude protein concentration, however the reduction was by 18% in the modern turkey compared with 24% in the traditional turkey.

The effect of diet on the body weight of the modern and traditional turkey has been included in figures 5.2 and 5.3 to allow for comparison with the effect of diet and strain on the other traits. The feather lengths from the cranial region of the breast showed a significant ($P<0.001$) strain by dietary protein concentration interaction (Figure 5.2). In the traditional turkey the cranial breast feathers decreased in length from 26mm to 19mm respectively when dietary crude protein was decreased from 300 to 180gCP/kg, however in the modern turkey these feathers were increased in length from 14mm to 25mm (SED 1.0). Sternal apteria width and sternal tract length also showed significant ($P<0.05$) strain by protein interactions (Figure 5.3). In the traditional turkey the width of the sternal apteria was not affected by diet whereas, for the modern turkey, reducing dietary crude protein to 180g/kg decreased the width of the sternal apteria by 36%. Sternal tract length was also decreased to a greater extent in the modern compared with the traditional turkey.

There were no strain by protein interactions for feather lengths from the back, tail, wing and caudal region of the breast. The marginal means of these feather lengths

were significantly ($P<0.001$) shorter when protein concentration was decreased (Figure 5.4). Decreasing protein concentration also significantly ($P<0.001$) reduced the length of the back tract and the width of the sternal tract (Figure 5.4). Protein concentration had no significant effect on follicle density.

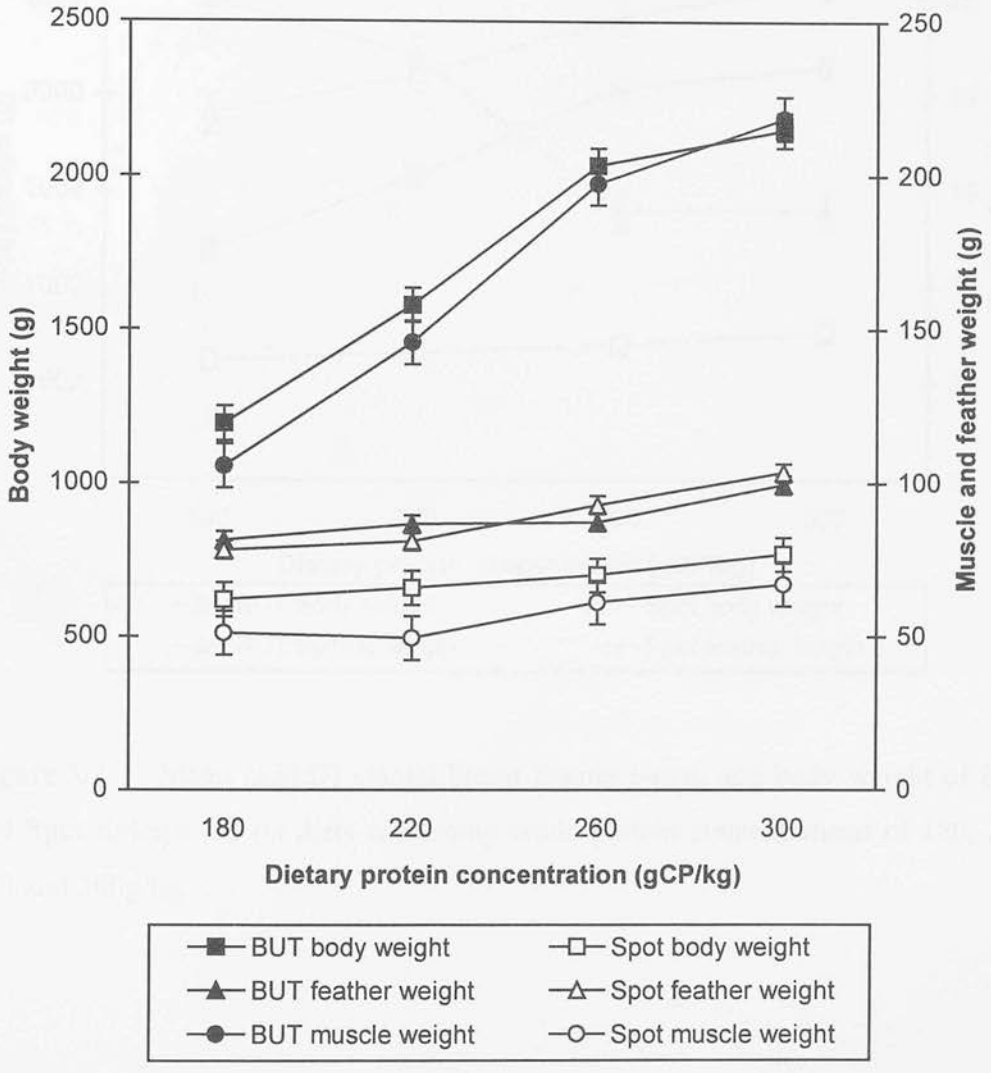


Figure 5.1 Mean (±SED) body weight, breast muscle weight and feather weight of BUT and Spot turkeys fed on diets containing crude protein concentrations of 180, 220, 260 and 300g/kg.

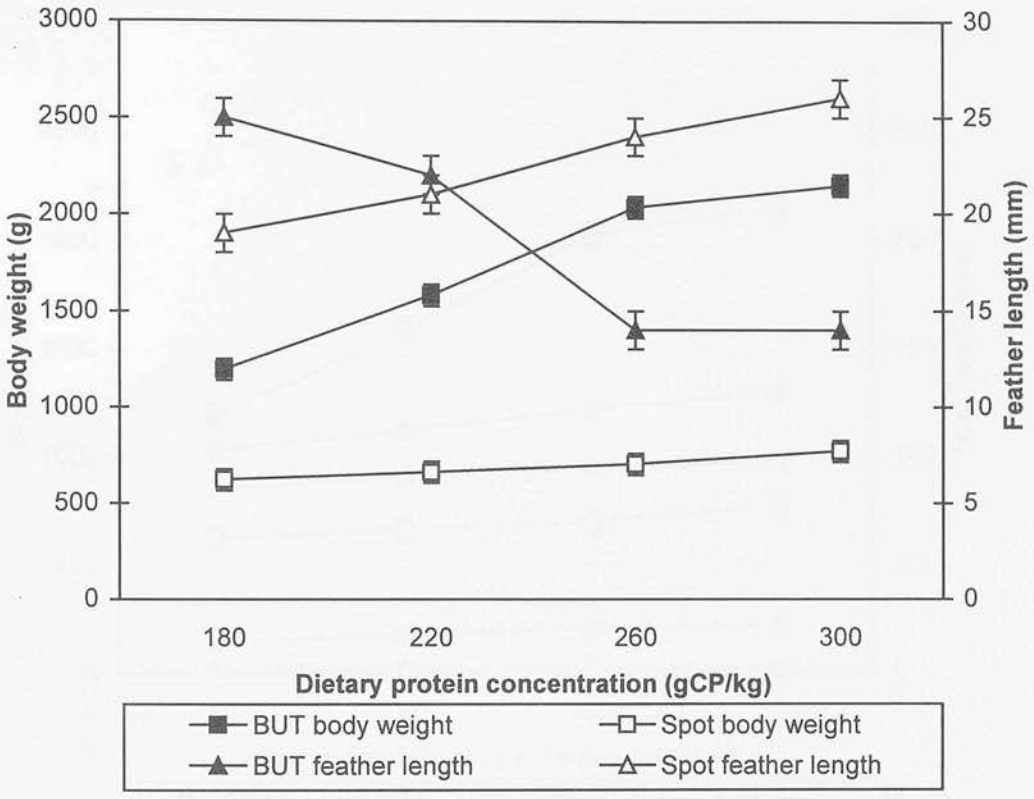


Figure 5.2 Mean (±SED) cranial breast feather length and body weight of BUT and Spot turkeys fed on diets containing crude protein concentrations of 180, 220, 260 and 300g/kg.

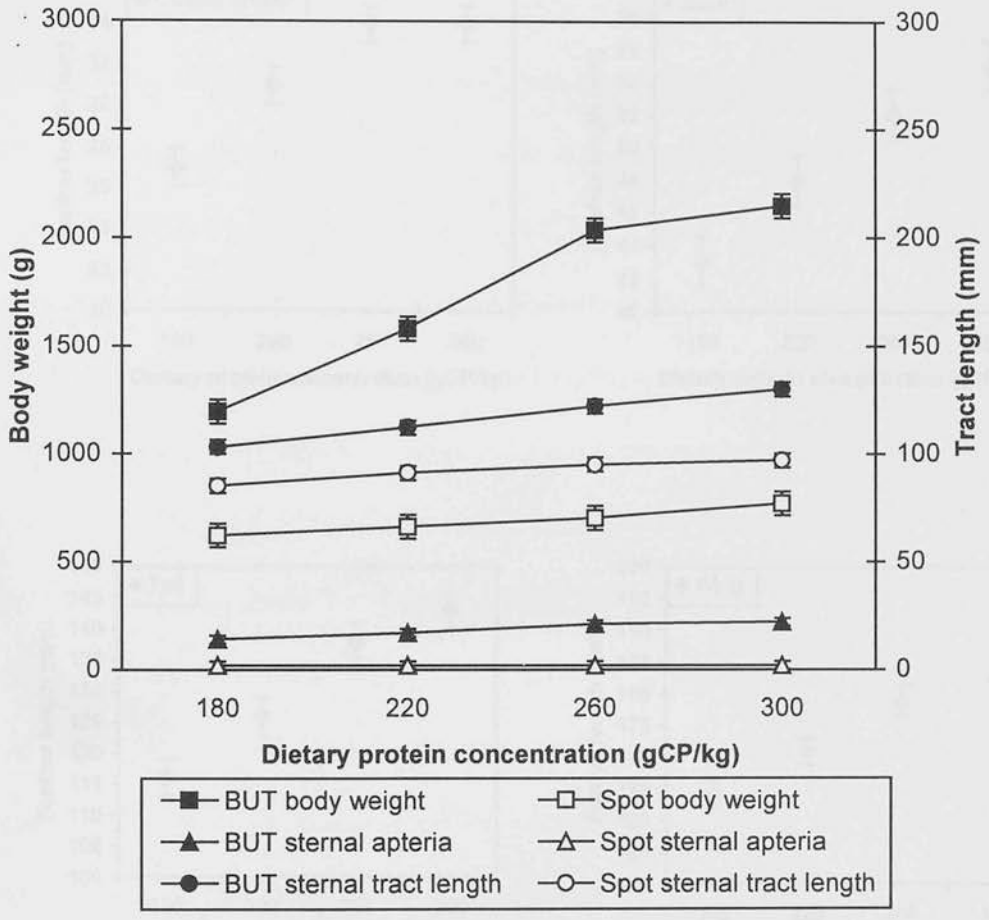


Figure 5.3 Mean (±SED) sternal tract length, sternal apteria width and body weight of BUT and Spot turkeys fed on diets containing crude protein concentrations of 180, 220, 260 and 300g/kg.

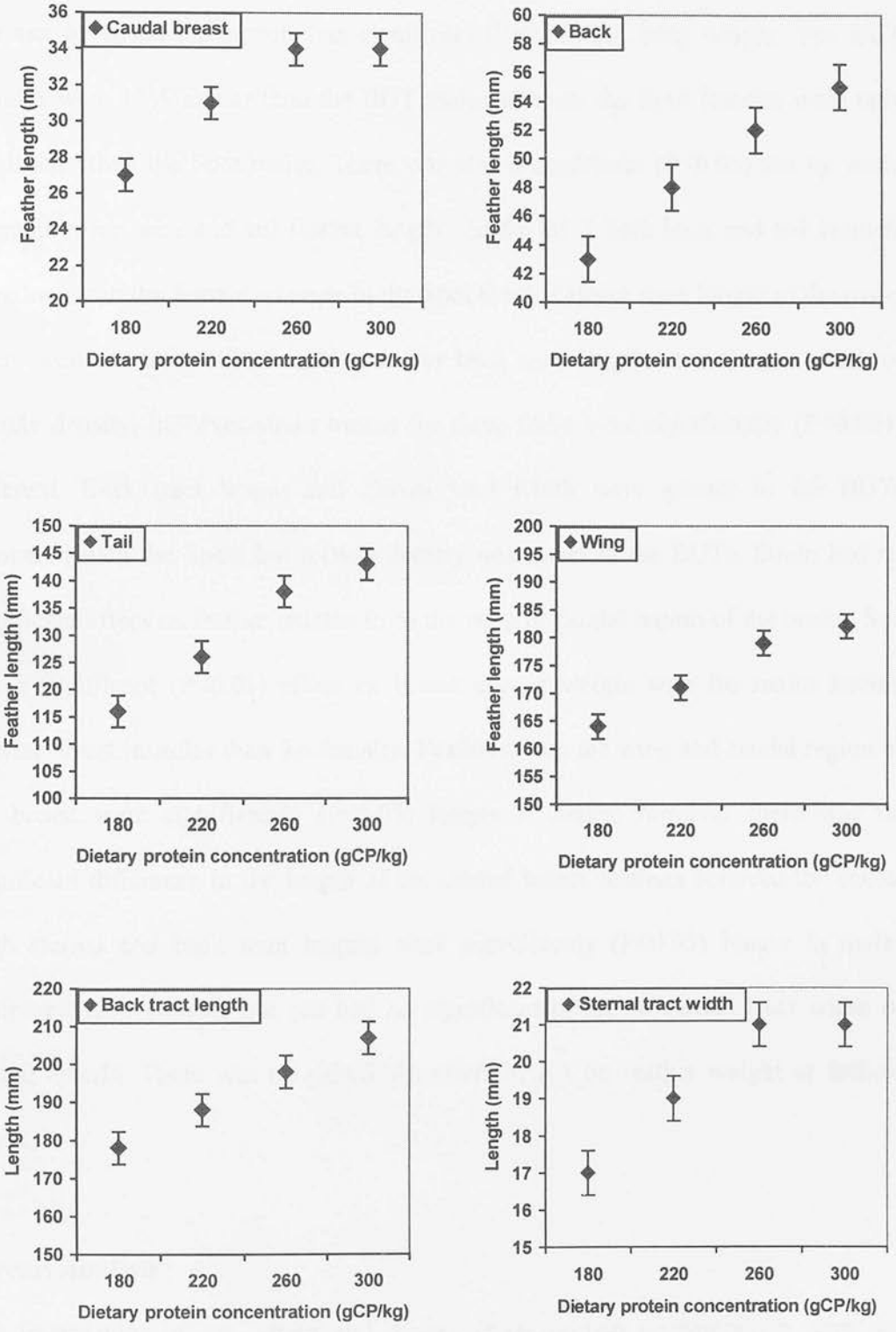


Figure 5.4 Effect of dietary crude protein concentration (180, 220, 260 and 300g/kg) on mean (±SED) feather lengths and feather tract measurements.

The sex by strain interaction was significant ($P<0.01$) for body weight. The BUT females were 13% lighter than the BUT males whereas the Spot females were only 8% lighter than the Spot males. There was also a significant ($P<0.05$) sex by strain interaction for back and tail feather lengths. In the BUT both back and tail feathers were longer in the female whereas in the Spot these feathers were longer in the male. There were no interactions with strain for back tract length, sternal tract width or follicle density, however strain means for these traits were significantly ($P<0.001$) different. Back tract length and sternal tract width were greater in the BUTs compared with the Spots but follicle density was lower in the BUTs. Strain had no significant effect on feather lengths from the wing or caudal region of the breast. Sex had a significant ($P<0.01$) effect on breast muscle weight with the males having heavier breast muscles than the females. Feathers from the wing and caudal region of the breast were significantly ($P<0.05$) longer in males, however there was no significant difference in the length of the cranial breast feathers between the sexes. Both sternal and back tract lengths were significantly ($P<0.05$) longer in males compared with females but sex had no significant effect on sternal tract width or sternal apteria. There was no significant effect of sex on feather weight or follicle density.

Carcass Analysis

The interactions of sex, strain and dietary crude protein concentration were not significant for any of the carcass composition traits measured.

The results of the carcass analysis showed that there were no significant strain differences in moisture content, crude protein content or ash content (Table 5.3). The BUT turkeys had a significantly ($P<0.01$) higher carcass fat content compared with the Spot. Feather dry matter was significantly ($P<0.01$) greater in the Spot.

Table 5.3 Mean carcass and feather composition analysis for male and female BUT and Spot turkeys at 6 weeks of age fed on diets containing protein concentrations of 180, 220, 260 and 300g/kg. Analysis of variance significance levels ** $P<0.01$, NS not significant.

	Strain			
	BUT	Spot	SED	Significance
Feather free carcass (%)				
Moisture	71.3	71.5	0.17	NS
Fat	3.7	3.3	0.13	**
Crude protein	20.6	20.6	0.22	NS
Ash	3.8	3.9	0.10	NS
Feather (%)				
Dry matter	55.7	59.6	1.48	**

Table 5.4 Mean carcass composition of male and female Spot and BUT turkeys fed on diets containing protein concentrations of 180, 220, 260 and 300g/kg. Analysis of variance significance levels (Sig) *** P<0.001, NS not significant.

	Dietary protein concentration (gCP/kg)					
	180	220	260	300	SED	Sig
Feather free carcass (%)						
Moisture	71.1	71.4	71.6	71.7	0.23	NS
Fat	4.2	3.4	3.3	3.2	0.19	***
Crude protein	20.2	20.7	20.6	20.8	0.32	NS
Ash	3.8	3.9	3.8	4.0	0.15	NS
Feather (%)						
Dry matter	59.4	58.2	57.9	58.0	2.09	NS

Decreasing dietary protein concentration had a significant (P<0.001) effect on fat content of the feather free carcass (Table 5.4). Moisture, crude protein, ash and feather dry matter were not significantly affected by dietary protein concentration.

5.2.3 Discussion

The aim of Experiment 1 (Section 5.2) was to investigate the effects on feather and muscle growth in modern and traditional turkeys offered diets containing low concentrations of crude protein. A diet containing 300gCP/kg was used as the control diet. The standard turkey starter diet that would be fed to birds from 0 to 4 weeks of age contains a CP concentration of 290g/kg (Chapter 2). Birds would then be fed on a

grower diet containing a CP concentration of 240g/kg (Chapter 2) from 5 to 8 weeks of age. Birds in this trial were fed on the experimental diets from 1 to 6 weeks of age and feeding a diet containing 300g CP/kg ensured that there was sufficient CP in the diet to meet commercial recommendations and maximise growth. Decreasing the CP concentration to 180g CP/kg between 1 and 6 weeks of age limited CP intake and growth was depressed.

The ME of each diet was formulated to be the same (12.0 MJ/kg) because dietary energy level is the main factor regulating feed intake (NRC, 1994). Feed intake was monitored and there were no significant differences in feed intake of the experimental diets over the experimental period. The effects on feather growth can therefore be attributed to the composition of the diet and not to variation in feed intake. Food intake for the first week on the experimental diets was ignored because the birds were wasting some of the food and the food intake record was inaccurate. The problem was solved after the first week by adjusting the height of the food hoppers.

Auckland (1972) reported that *ad libitum* feeding of low protein isocaloric diets increased the fat composition of turkey carcasses. The carcass composition of birds from Experiment 1 (Section 5.2) also showed that reducing dietary protein concentration resulted in an increase in carcass fat.

The body weights of modern turkeys fed on the control diet (300g CP/kg) were 2.8 times the weight of the traditional turkeys. When the dietary protein concentration

was decreased from 300 to 180 g/kg, the body weight of the modern bird decreased by 44%, however, the body weight of the traditional bird only fell by 19%. Breast muscle weight followed the same pattern but was reduced to a greater extent than body weight (respectively by 52% and 24%). The results suggest that breast muscle weight is affected more than body weight and that the modern bird is affected more by low dietary protein than the traditional bird. Low dietary protein was found by Barbour and Lilburn (1996) to have a greater effect on turkey breast muscle weight than on body weight. The strain difference in the feather weight reduction was much less dramatic (18% in the modern turkeys compared with 24% in the traditional turkeys) and feather weight in the modern bird was reduced to a much lesser extent than body and muscle weight. Muscle protein accretion is the result of the balance between synthesis and degradation. The effect of dietary protein quantity and quality on changes in weight gains of chickens were found by Nieto *et al* (1994) to be due to significant changes in the rates of degradation but not in the rates of synthesis. This would suggest that the synthesis of both muscle and feather protein continues when dietary protein is deficient however, once formed, feather protein cannot be degraded, whereas muscle protein can. The reduction in breast muscle growth of the modern bird fed a low protein diet may therefore be due to increased muscle protein degradation and a subsequent re-partitioning of the limited supply of protein to more important body tissues. The feather weight of the traditional bird was reduced to the same extent as the muscle weight and only slightly more than the body weight. This would suggest that protein is partitioned in the same way if dietary protein is deficient or not, perhaps because the smaller breast muscle of the traditional turkey does not have the same capacity for sparing protein. Reducing dietary CP in the

traditional turkey therefore resulted in an overall reduction in body weight with a proportional effect on muscle and feather weight. The reduction in feather weight of the modern bird was similar to the reduction in the body weight of the traditional bird (Figure 5.1). In the modern bird the smaller reduction in feather weight would suggest that feather growth was maintained at the expense of muscle and body growth when protein was deficient. It would be biologically sensible to maintain feather growth for flight and thermoregulation compared with disproportionate muscle growth in the modern turkey that has no significance for survival. The results suggest that if protein for feather growth is not optimal in the diet then breast muscle will not grow to the maximum potential. When formulating commercial diets protein partitioning and the priority attached to feather over muscle growth should therefore be considered.

The feathers of the traditional turkey, when compared with the modern turkey, were found to have a greater dry matter content. As the dry matter of feathers is mostly protein this would suggest that in the traditional turkey a higher proportion of the fresh feather weight was protein.

Feather weight as a percentage of body weight in the traditional bird declined from 13.4% on the 300gCP/kg diet to 12.6% on the 180gCP/kg diet. In the modern bird feather weight as a percentage of body weight increased from 4.6% on the 300gCP/kg diet to 6.8% on the 180gCP/kg diet. Reducing body weight by decreasing the dietary CP concentration therefore increased the relative feather cover in the modern bird. Even with this increase in relative feather cover, feather weight as a

percentage of body weight was only half that of the traditional turkey fed on 300gCP/kg.

Low dietary protein reduced the back tract length of both the modern and the traditional turkey to a similar extent. A large part of the difference between the two strains was associated with the difference in breast muscle size and it is possible that back tract length is a better measure of relative body size than body weight. When fed low dietary protein, muscle weight was reduced to a much greater extent in the modern bird compared with the traditional bird and this might be reflected in the larger reduction in sternal tract length. It was therefore surprising that there was no difference in sternal tract width between the strains. Sternal apteria width decreased by 36% in the modern bird fed on the 180gCP/kg diet but did not change in the traditional turkey. As the breast muscle decreased in size, the width of the apteria between the sternal tracts declined and the feathers were able to cover more of the bare area of skin giving the appearance of improved breast feathering.

The effect of low dietary protein on breast muscle weight was also reflected in the feather follicle density. Follicle density was hardly affected in the traditional bird, whereas follicle density was higher in the modern bird fed on the 180g CP/kg diet compared with turkeys fed 300g CP/kg. It is likely that the total number of feather follicles was not affected by diet but that reduced muscle weight meant that the skin was not so stretched and that there were more follicles in a given surface area. Thus follicle density may be directly proportional to breast muscle size.

It is interesting that feather lengths in both strains appear to be affected by low dietary protein in the same way as back length consistent with the suggestion that feather growth might be more closely related to body size than to body weight. Low dietary protein affected the lengths of feathers from the back, tail, wing and caudal breast in the same way for both the modern and traditional birds. Wing feathers appeared to be the least affected by decreased protein. It could be that these flight feathers are more important for survival and are maintained in preference to other feathers. The tail feathers were the next least affected with body contour feathers from the back and breast most affected. This would suggest that there could be some control over the partitioning of protein to different feathers.

Reducing dietary CP to 180g/kg decreased the length of the cranial breast feathers in the traditional bird but increased the length of these feathers in the modern bird. In the traditional bird these feathers followed a similar pattern of reduced growth in response to low dietary protein as other feathers. So why do the cranial feathers of the modern bird increase with reduced dietary protein? In Experiment 2 (Section 5.3) the hypothesis that deficiencies of specific amino acids required for feather growth may be responsible for the relatively poor growth of cranial breast feathers in modern turkeys was evaluated.

5.3 Experiment 2: Amino acid replacement

The aim of this experiment was to investigate the effect on feather and body growth of increasing the dietary concentration of amino acids that are important for feather growth. The diet from Experiment 1 (Section 5.2) with a crude protein concentration of 180g/kg was used as a basal diet and individual crystalline amino acids were added to the basal diet to increase their concentration to the level found in the diet with a crude protein concentration of 260g/kg.

The sulphur amino acids are, in combination, the most common in feather protein and a deficiency of the sulphur amino acids will result in poor feather growth or abnormal feathering (Deschutter and Leeson, 1986). Feathers showing signs of amino acid deficiency have also been noted in chicks fed rations deficient in arginine, valine and tyrosine (Anderson and Warnick, 1967). The amino acids methionine, arginine, valine and tyrosine were therefore selected for addition to the 180 diet. These amino acids were added to the 180 diet up to the concentration found in the 260 diet and not the 300 diet to minimise the effects of amino acid imbalances. The 260 diet was only marginally deficient with near maximum performance for the majority of traits and was closer to the requirements of 4-6 week old birds.

5.3.1 Materials and Methods

Two hundred male BUT Big 5 male line turkeys were reared in a floor pen until two weeks of age. During this period birds were fed on a standard turkey starter diet

(Chapter 2). The lighting programme was maintained throughout the trial as 14h light, 10h dark. After two weeks 192 birds were weighed, wingbanded and randomly allocated to 48 cages (600mm x 450mm) arranged in blocks of 24 cages in each of two rooms. Temperature was monitored to ensure that the environment did not vary between the two rooms. Six diets were prepared (180, 260, tyrosine, arginine, valine and methionine) and each randomly allocated to 4 cages per room. The diet (180) containing 180g crude protein/kg from Experiment 1 (Section 5.2) was used as the basal diet. Crystalline amino acids were added to this diet to increase the concentration of the individual amino acid to that found in the diet containing 260g crude protein/kg. The 260g crude protein/kg diet was used as a control diet (260). The amino acids used were arginine, valine, methionine and tyrosine (Sigma Chemical Company LTD, Poole, Dorset, UK). Glutamic acid (ICN Biomedicals Inc, Aurora, Ohio, USA) was added to all diets except the 260gCP/kg diet and the arginine diet to make the diets isonitrogenous. The molecular weight and number of nitrogen atoms per molecule of each amino acid were used to calculate the amounts of glutamic acid to add to each diet. The two major ingredients of the diets (wheat and soya) were reduced proportionally to incorporate the added weight of the amino acids (Table 5.5). The diets were analysed for nitrogen (N) content using a protein/nitrogen analyser (LECO Corporation, Stockport, Cheshire, UK) and amino acid composition was determined commercially (Roslin Nutrition, Roslin, Midlothian, UK). The crude protein and amino acid analysis of the six diets is shown in Table 5.6. The metabolisable energy (ME) concentration of each diet was formulated at 12.0 MJ/kg.

The experimental diets were fed to the birds from two to 6 weeks of age. Birds were weighed as a cage and the food intake per cage recorded weekly. At 6 weeks of age the birds were removed from the cages and killed by injection of sodium pentobarbitone and feather measurements recorded by the methods described in Chapter 2.

The experiment was a randomised block design. Analysis of variance was used to test for treatment differences in trait measurement data. Regression analysis was carried out using body weight as a covariate to evaluate the effects of diet on feather growth independently of body weight.

Table 5.5. Composition of experimental diets (g/kg). Diets 180 and 260 are the diets containing crude protein concentrations of 180 and 260g/kg respectively. The diets Tyrosine, Arginine, Methionine and Valine are the 180gCP/kg diet from Experiment 1 (Section 5.2) with the appropriate amino acid added to the level of the 260gCP/kg diet.

Ingredients	Diet (g/kg)					
	180	Tyrosine	Arginine	Methionine	Valine	260
Wheat	698.0	697.0	709.0	698.0	699.0	557.7
Soya	194.0	194.0	197.0	194.0	194.0	299.3
Fish meal	10.0	10.0	10.0	10.0	10.0	70.0
Soya oil	13.5	13.5	13.5	13.5	13.5	12.2
Maize gluten meal	0	0	0	0	0	14.3
Dicalcium phosphate	43.6	43.6	43.6	43.6	43.6	25.4
Sodium chloride	3.3	3.3	3.3	3.3	3.3	3.0
Choline chloride 50%	0.4	0.4	0.4	0.4	0.4	0.4
Glutamic acid	19.0	13.3	0	16.1	14.5	0
Arginine	0	0	5.0	0	0	0
Valine	0	0	0	0	3.5	0
Tyrosine	0	6.7	0	0	0	0
Methionine	0.8	0.8	0.8	3.7	0.8	1.2
l-Lysine HCl	2.5	2.5	2.5	2.5	2.5	1.5
Vitamin / mineral mix[1]	7.0	7.0	7.0	7.0	7.0	7.0
Pellet binder[2]	8.0	8.0	8.0	8.0	8.0	8.0

[1] Supplied per kg diet: Cu 14 mg, I 1.4 mg, Fe 112 mg, Mn 140 mg, Zn 112 mg, Se 0.28 mg, Mo 0.7 mg; Co 0.7 mg, retinol 5.0 mg, cholecalciferol 175 μg, α-tocopherol 47 mg, menadione 4.2 mg, riboflavin 9.8 mg, thiamine 2.8 mg, nicotinic acid 70 mg, pantothenic acid 21 mg, biotin 280 μg, pyridoxine 7 mg, cyanocobalamin 21 μg, folic acid 1.4 mg.

[2] Walfolin S. (Holman Lignotech Ltd, Reading, UK).

Table 5.6 Analyses of the amino acid composition and crude protein concentration of experimental diets (g/kg).

Analysis (g/kg)	Diet					
	180	Tyrosine	Arginine	Methionine	Valine	260
Tyrosine	5.7	9.7	5.9	5.6	6.3	7.7
Arginine	12.2	11.2	15.5	12.0	12.2	16.1
Methionine	3.3	3.6	3.9	5.5	3.8	5.0
Valine	8.4	8.0	8.7	8.5	11.1	10.7
Glutamic acid	53.0	50.6	42.9	50.7	53.1	50.3
Lysine	10.6	10.0	11.2	10.4	10.8	13.7
Crude protein	195	195	205	200	195	259

5.3.2 Results

Food intake was not significantly affected by the composition of the diets (Table 5.7).

Table 5.7 Cumulative feed intake (g/bird) of experimental diets for weeks three to six.

Diet	Cumulative intake
180	3762
Tyrosine	3851
Arginine	3746
Methionine	3792
Valine	3772
260	3923
SED	161.7

Diet had a highly significant ($P<0.001$) effect on both body weight and breast muscle weight. The diets supplemented with amino acids resulted in birds having a significantly higher body weight and breast muscle weight than birds fed on the 180 diet but the body and muscle weights did not increase to those of the birds fed on the 260 diet (Figure 5.5). Diet also had a significant ($P<0.001$) effect on feather weight. Increasing tyrosine resulted in a significantly reduced feather weight ($P<0.05$) compared with the 180 diet while valine, methionine and arginine had no significant effect on feather weight. Although not significantly different to the 180 diet, both arginine and methionine appeared to increase the weight of feathers to a similar

weight as the 260 diet. Follicle density was significantly ($P<0.05$) affected by diet with the 260 diet having a lower follicle density than the other diets.

The lengths of all feathers, except the tail feathers, were significantly ($P<0.001$) affected by diet (Figure 5.6). Tyrosine and valine had little effect on the lengths of the back feathers in contrast to arginine and methionine that increased the length of the back feathers compared with the 180 diet. The length of the wing feathers was increased by the addition of tyrosine. The birds fed on the arginine, methionine and valine diets had wing feather lengths comparable with those fed on the 260 diet. Feathers from the caudal region of the breast were longer in birds fed on the tyrosine and valine diets than those fed on the 180 diet. Birds fed on the arginine and methionine diets had caudal breast feathers of a similar length to those fed on the 260 diet. Although there was no significant difference, the length of the tail feathers from birds fed on the different diets followed the same trend as the caudal breast feathers. Cranial breast feathers were longest from the birds fed on the 180 diet and shortest from birds fed on the 260 diet. The diets tyrosine, arginine, methionine and valine increased the length of the cranial breast feathers compared with the 260 diet but not to the same length as the 180 diet.

Diet had a highly significant ($P<0.001$) effect on back tract length, sternal tract length, sternal tract width and sternal apteria (Figure 5.7). The effect of diet was similar for these four traits, with the shortest measurements for the 180 diet and the longest for the 260 diet. Diets tyrosine, arginine, methionine and valine resulted in a

similar increase in these trait measurements compared with the 180 diet but the feathers were not as long as on the 260 diet.

The regression coefficient for body weight was significant ($P<0.05$) for all traits. Regression analysis showed that treatment differences in breast muscle weight, wing feather length, caudal breast feather length, sternal apteria width and feather follicle density were due to the differences in body weight between birds fed on the different diets. Body weight alone was not responsible for the treatment differences in back feather length, cranial breast feather length, back tract length, sternal tract length, sternal tract width and feather weight. The differences due to the diets remain significant ($P<0.05$-$P<0.001$) when these traits are corrected to the same body weight (Table 5.8).

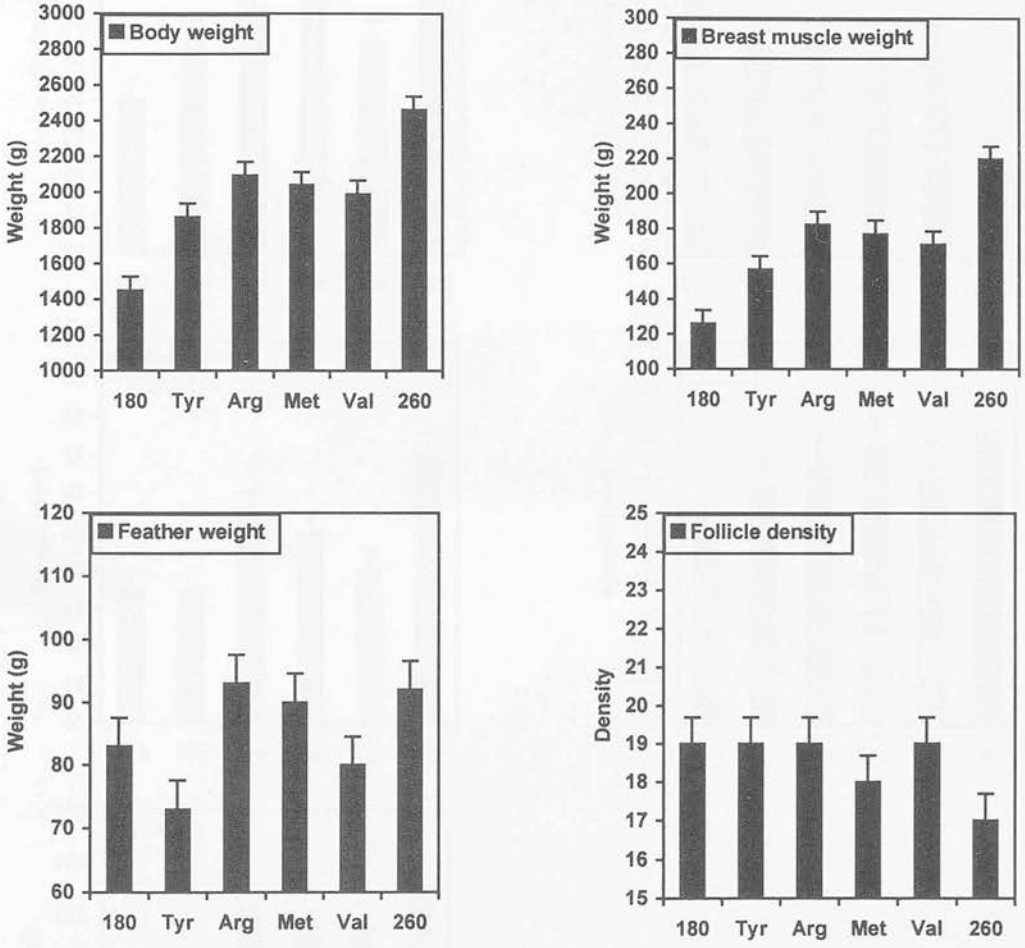


Figure 5.5 Effect of diet on mean (± SED) body, breast muscle and feather weights and feather follicle density in modern turkeys at 6 weeks of age. A basal diet of 180g CP/kg (180) was supplemented with tyrosine (Tyr), arginine (Arg), methionine (Met) or valine (Val) to the level of a diet containing 260g CP/kg (260).

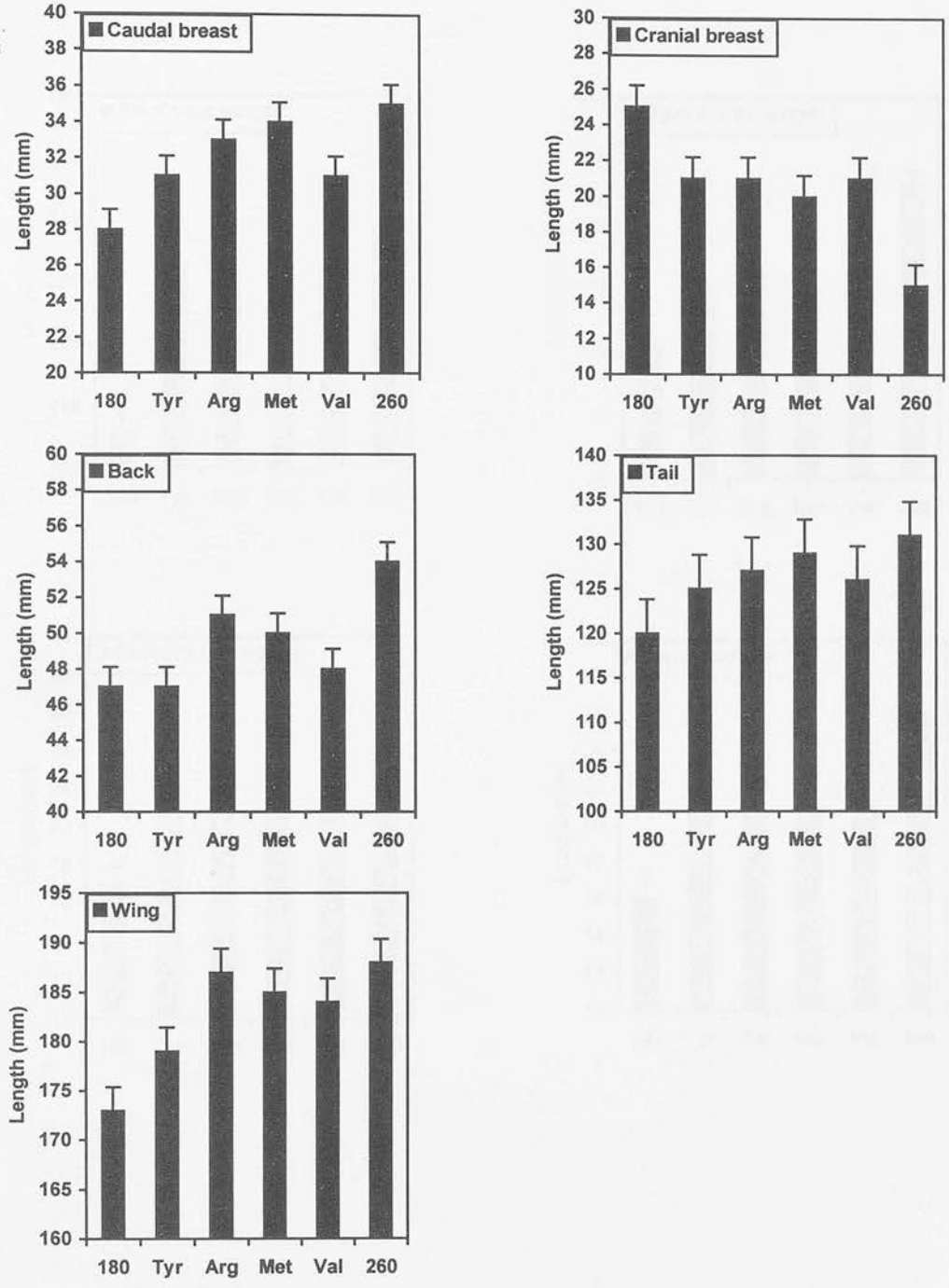


Figure 5.6 Effect of diet on mean (± SED) feather lengths in modern turkeys at 6 weeks of age. A basal diet of 180g CP/kg (180) was supplemented with tyrosine (Tyr), arginine (Arg), methionine (Met) or valine (Val) to the level of a diet containing 260g CP/kg (260).

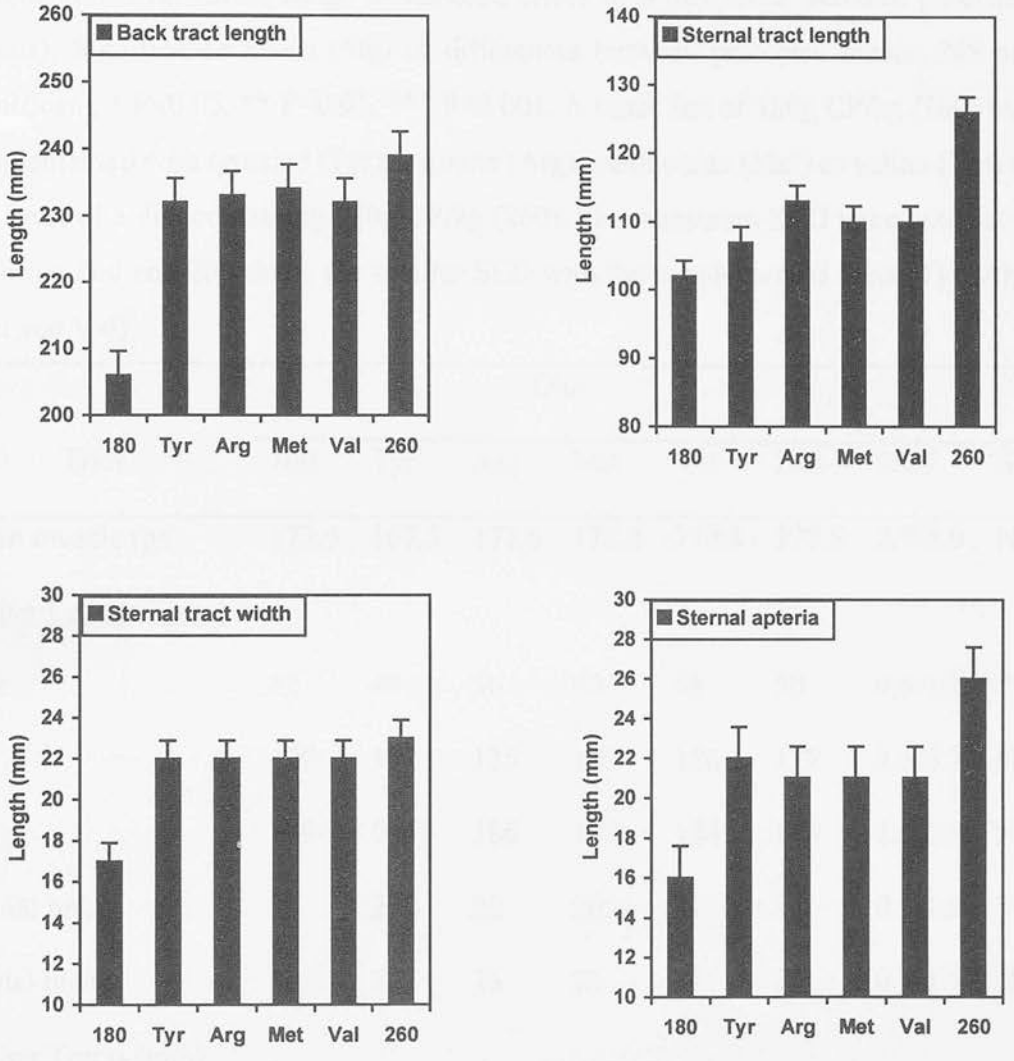


Figure 5.7 Effect of diet on mean (± SED) feather tract measurements in modern turkeys at 6 weeks of age. A basal diet of 180g CP/kg (180) was supplemented with tyrosine (Tyr), arginine (Arg), methionine (Met) or valine (Val) to the level of a diet containing 260g CP/kg (260).

Table 5.8 Predicted values for traits based on a mean (experimental) body weight of 1981g. (SED, range of standard errors of a difference between predicted means). Significance levels (Sig) of differences between predicted means, NS not significant, * P<0.05, ** P<0.01, *** P<0.001. A basal diet of 180g CP/kg (180) was supplemented with tyrosine (Tyr), arginine (Arg), methionine (Met) or valine (Val) to the level of a diet containing 260g CP/kg (260). The maximum SED were associated with the 180 and 260 diets; the smaller SED with the supplemented diets (Tyr, Arg, Met and Val).

	Diet							
Trait	180	Tyr	Arg	Met	Val	260	SED	Sig
Breast muscle (g)	172.5	167.5	172.6	172.2	170.4	177.5	2.7-5.0	NS
Feather Lengths (mm)								
Back	52	48	50	49	48	50	0.6-1.1	**
Tail	129	127	125	128	126	122	2.6-4.7	NS
Wing	179	180	186	185	184	183	1.6-2.9	NS
Cranial breast	26	21	20	20	21	14	0.8-1.5	**
Caudal breast	31	32	33	33	31	33	0.7-1.4	NS
Feather Tracts (mm)								
Back tract length	226	236	229	232	232	221	1.6-2.9	***
Sternal tract length	111	109	111	109	110	118	1.3-2.5	**
Sternal tract width	18	22	22	22	22	22	0.6-1.1	*
Sternal apteria	19	23	21	21	21	24	1.1-2.1	NS
Feather Cover								
Feather weight (g)	100	77	89	88	80	77	2.8-5.2	***
Follicle density	18	19	19	18	19	18	0.5-0.9	NS

5.3.3 Discussion

The aim of Experiment 2 (Section 5.3) was to test the hypothesis that increasing specific amino acids in a protein deficient diet would increase feather growth in preference to muscle growth.

When the dietary protein concentration was decreased in diet 180 compared with diet 260 the body weight of the turkeys decreased by 41% which was similar to the 42% decrease observed in Experiment 1 (Section 5.2). Addition of individual amino acids to the 180 diet resulted in increased body weights compared with the 180 diet but these were lower than the body weight achieved on the 260 diet. The increase in body weight varied depending on the amino acid added. As body weight was affected by addition of amino acids a regression analysis was used to correct for body weight so that treatment effects on feather growth could be disentangled from effects on body weight. This allowed a direct comparison of traits between turkeys on the different diets.

Breast muscle weight followed the same pattern as body weight and, when corrected for body weight, no treatment differences in muscle weight remained. The diets affected body weight and breast muscle weight proportionally but this was not the case with feather weight. Measured feather weight was reduced by 10% in turkeys fed on diet 180 compared with diet 260. The addition of tyrosine to the 180 diet resulted in a further reduction in feather weight. Valine had no effect on feather weight whereas arginine and methionine increased feather weight to that achieved on

the 260 diet. When feather weight was corrected for body weight it was interesting to find that relative feather cover was highest in turkeys fed on the 180 diet and lowest in turkeys fed on the 260 diet. Addition of tyrosine or valine did not improve relative feather cover compared with the 260 diet. Both arginine and methionine improved relative feather cover but not to the same extent as the 180 diet. The results suggest that individual amino acids affect body growth and feather growth in different ways. As with Experiment 1 (Section 5.2) a dietary protein deficiency was found to improve feather cover in relation to body weight suggesting that feather growth was maintained at the expense of body growth. Addition of tyrosine had a detrimental effect on feather growth although body weight was increased. It is possible that the addition of tyrosine created an amino acid imbalance that resulted in poor feather growth. The addition of valine resulted in increased body and muscle weight but no increase in feather weight suggesting that valine is used for muscle growth and body growth and not feather growth. It is interesting that increasing either arginine or methionine to the 260 concentration increased actual feather weight to the same weight as with the 260 diet. Body and muscle weight were also increased most with the arginine or methionine diets but only to 85 and 83% of the 260 body weight and 83 and 81% of the 260 muscle weight. Addition of arginine and methionine to the 180 diet therefore increased feather growth to the 260 level but improved relative feather cover more so because of the depression in body weight. The results suggest that both arginine and methionine are required for feather growth and were used for feather growth in preference to body and muscle growth.

Diet was found to affect the tail, wing and caudal breast feathers in the same way as in Experiment 1 (Section 5.2) with low dietary protein concentrations resulting in a small decrease in the lengths of these feathers and the tail and wing feathers being least affected. Addition of each amino acid to the 180 diet increased the lengths of these feathers although only supplementation with arginine and methionine resulted in the feathers growing to the same length as with the 260 diet. When feather measurements were corrected for body weight the diets were found to have no effect on the lengths of the tail, wing or caudal breast feathers suggesting that these feathers were maintained in proportion to body weight. Feathers from the back were also reduced in length with low dietary protein, however, only the addition of arginine and methionine increased these feather lengths and the feathers remained shorter than with the 260 diet. These results support the conclusion from Experiment 1 (Section 5.2) that the flight feathers are maintained in preference to body contour feathers. An effect of diet on back feather length was found after correction for body weight and this is probably because the diets tyrosine and valine did not increase the length of the back feathers compared with diet 180 but did increase body weight.

Cranial breast feathers were shown in Experiment 1 (Section 5.2) to decrease in length with increased body weight. The same result was obtained in the present experiment and when corrected for body weight the same pattern remains. This suggests that cranial breast feather length did not have a direct relationship with body weight. Cranial breast feathers were longest on the low protein diet and, unlike other feathers, the addition of each amino acid reduced feather growth in this area. Instead of using the added amino acids for increased cranial breast feather growth as was

found with other feathers, the increased body weight was associated with impaired growth of cranial breast feathers. Correcting for body weight did not take into account the size of the breast muscle when the measurements were taken. It is possible that the increase in breast muscle size with increased body weight has had some effect on feather growth in this area. Turkeys fed on diet 180 had the smallest breast muscle weight and the longest cranial breast feathers, whereas the turkeys fed on the 260 diet had the largest breast muscle weight and the shortest cranial breast feathers. With supplementary amino acids the breast muscle increased in weight but not to the same extent as the 260 diet. Cranial breast feathers were intermediate in length being shorter in turkeys fed the 180 diet but longer than those of birds fed on the 260 diet. The results suggest that cranial breast feathers were not affected by body weight *per se* but by the size of the breast muscle. The rapid growth of large breast muscles may impair the growth of cranial breast feathers. The increase in cranial breast feather length with additional amino acids relative to the 260 diet may be an effect of the reduced breast muscle size rather than supplementing the supply of amino acids available for growth of cranial breast feathers. The results suggest that a physical impairment of feather growth associated with rapid breast muscle growth may underlie the poor growth of cranial breast feathers in modern turkeys rather than a specific nutrient deficiency.

Sternal tract length and width were also affected by breast muscle size as increased breast muscle weight was associated with a longer and wider sternal tract. Diets that increased the size of the breast muscle also increased the length and width of the sternal tract. Correcting for body weight did not remove this effect because the

relative size of the breast muscle was not taken into account. Dietary differences in back tract length remained after correction for body weight suggesting that birds had grown proportionally longer instead of putting on muscle weight.

Follicle density was not affected by diet but was associated negatively with body weight. This suggests that total follicle number was not affected by diet but that the density was affected because the same number of follicles covered a larger area of breast skin. This stretching effect would appear to be proportional to body weight.

The results from the two experiments suggest that although nutrition affects feather growth and amino acid deficiencies resulted in poor or abnormal feather growth it was body weight and muscle weight that were affected first. Growth of feathers was not affected as much as muscle weight relative to body weight in deficient birds. Diet 180 resulted in the best relative feather growth and the addition of arginine and methionine increased feather growth to a greater extent than body growth. This suggests that the amino acids arginine and methionine were used preferentially for feather growth. Excess amino acids that are not required for feather growth such as tyrosine and valine were used for increased body growth and therefore relative feather cover decreased. Feather growth was maintained as much as possible at the expense of body growth.

5.4 Conclusion

The aim of the two experiments was to investigate protein partitioning between feather and body growth and to identify specific amino acids involved in feather growth. The results suggest that protein was preferentially partitioned to feather growth and that the amino acids arginine and methionine were used for increased feather growth rather than muscle growth. The impaired growth of cranial breast feathers would appear to be caused by the rapid growth of breast muscle in the modern turkey and not because of a deficiency of specific dietary amino acids.

CHAPTER 6: Breast Skin and Feathers

6.1 Introduction

Lack of feather cover on the breast of the modern turkey might be due to two factors. Firstly the breast feathers, and in particular the feathers from the cranial breast region, are much shorter than in the traditional bird suggesting that feather growth in this area is impaired (Chapter 3). Secondly the modern turkey has been selected for increased breast muscle growth and it is possible that the skin has simply stretched to cover this larger area of muscle without a proportional increase in feather numbers. This would suggest that the number of feather follicles on the sternal tracts have not increased in proportion to the larger area that the feathers from these follicles have to cover. This hypothesis is supported by the changes in follicle density observed when breast muscle size was changed, as in the low protein experiment described in Chapter 5. The majority of the feather follicles of a bird are formed during embryo development and persist throughout life (Lucas and Stettenheim, 1972). It is therefore unlikely that the number of feather follicles can be altered after hatch.

If the breast skin of the modern turkey has stretched with selection for increased breast muscle, differences in the structure of the skin might be found when compared with the traditional turkey. It is possible that the skin or the feather follicles could be damaged as the bird and the breast muscles grow. The skin may also be structurally

thinner resulting in weak skin that might be more susceptible to damage or not strong enough to support feathers as they grow.

The main protein component of dermal connective tissue is collagen and this is involved in maintaining the structure of the skin (Pines *et al.*, 1996). Skin tearing in broilers has been found to be related to tensile strength and can be affected by factors such as strain, sex and diet (Granot *et al.*, 1991). Ramshaw *et al* (1986) concluded that skin tensile strength was a direct function of the collagenous dermal layer of the skin. Collagen is therefore an important determinant of skin strength. It is possible that any change in the structure or strength of the breast skin due to stretching would result in a change in the collagen content of the skin. The collagen concentration of connective tissue may be determined by assessing the hydroxyproline content as the amino acid hydroxyproline is found in very few proteins other than collagen (Creemers *et al.*, 1997).

Though not examined in this study the number and type of intermolecular covalent cross-links formed among collagen molecules also determine structural integrity of skin (Pines *et al.*, 1996). In addition, collagen has a directive role in developing tissue and is involved in feather follicle development providing a stabilised base on which the follicles form (Mauger *et al.*, 1982).

Structural damage to the skin because of stretching could impair feather growth but there may also be damage to the feathers and follicles because of the prolonged resting of the modern turkey as discussed in Chapter 3. The modern turkey has been

selected to be broad breasted and if the breast is in contact with the floor when the turkey is resting the breast skin and feathers may be under increased pressure compared with unselected turkeys. Pressure on the skin might have an adverse effect on the growth of feathers in this area. Sosnicki *et al* (1991) suggested that the lack of activity and prolonged resting of the modern turkey may lead to muscle ischemia because of alterations in capillarity and reduced vasodilation in the muscle. It was suggested that prolonged and direct pressure on a muscle was likely to reduce the arteriovenous pressure gradient and therefore reduce capillary blood flow. It is possible that pressure when resting could also alter the capillary blood flow in the breast skin of the modern turkey and might result in impaired feather growth.

This chapter investigates firstly, the possibility that the breast skin of the modern turkey has been stretched without an increase in the number of feather follicles. Secondly that stretching might have resulted in structural damage to the skin. Thirdly the possibility that the breast skin and feather tracts might be under abnormal pressure when the modern turkey is resting and, finally, the possible effects of this pressure on the blood supply to the skin.

6.2 Breast Feather Follicles

The aim of the study was to test the hypothesis that the number of breast feather follicles has not increased in proportion to the increased body size of the modern turkey. This was done by counting the total number of feather follicles on the sternal tracts in the modern BUT and the traditional Nebraska Spot turkey.

6.2.1 Materials and Methods

Twelve male and female BUT and Nebraska Spot turkeys were reared to maturity and then killed by injection of sodium pentobarbitone. The feathers from the sternal tracts were removed by cutting with scissors close to the skin. A line was drawn round the tracts from the base of the crop around the breast muscle to the base of the keel bone using a marker pen. Any feather follicles within this area were marked with a pen as they were counted (Figure 6.1). Separate counts were made for the left and right tracts.

Analysis of variance was used to test for differences in follicle numbers with effects for sex, strain, tract (left and right) and their interactions.

6.2.2 Results

There were no significant interactions of sex, strain and tract. Feather follicle numbers were not significantly different between the males and females, between the left and right sternal tracts or between the two strains. The mean numbers of feather follicles from the sternal tracts were 308 for the modern and 311 (SED 1.7) for the traditional turkeys (Figure 6.2).

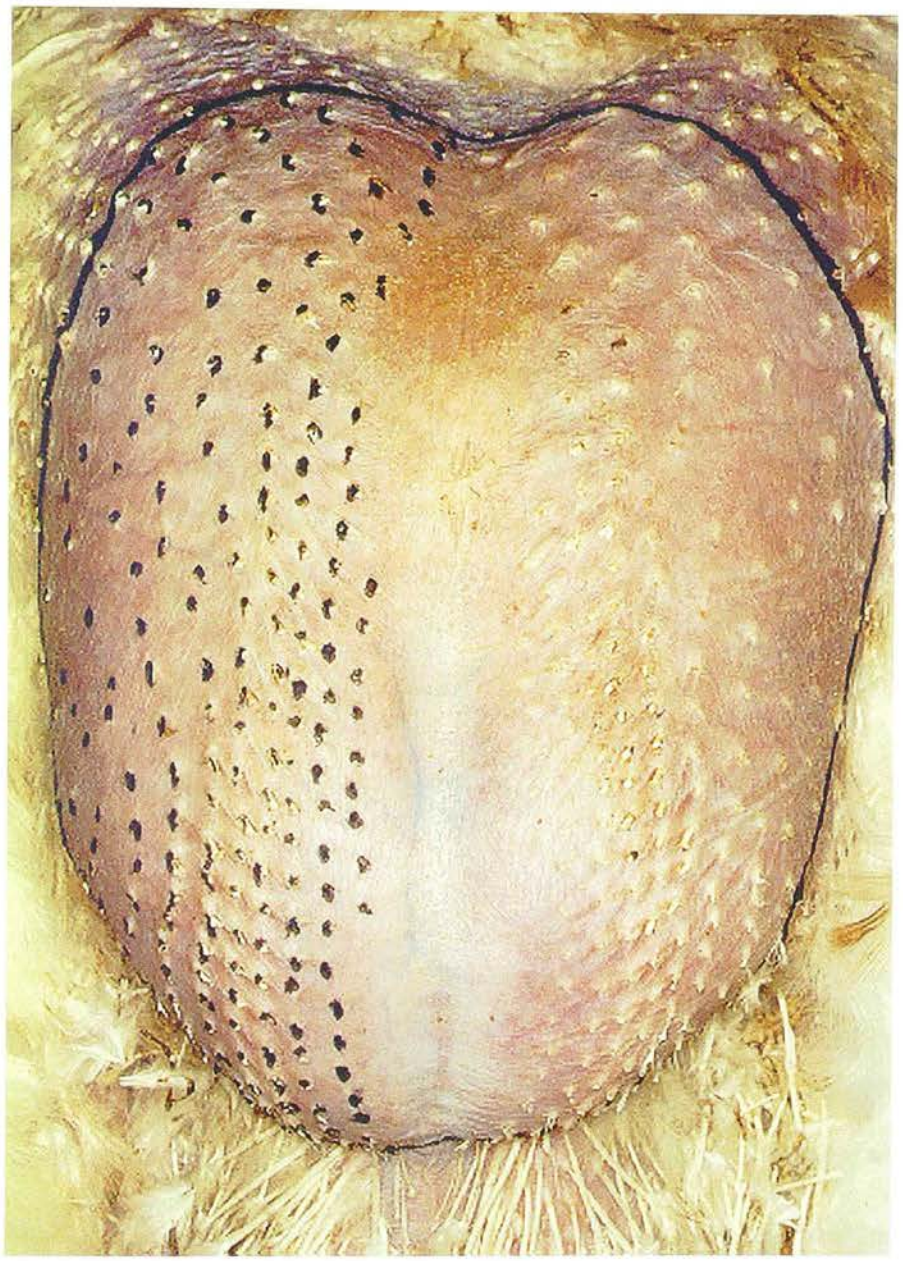


Figure 6.1 Method used for counting the total number of feather follicles from the left and right sternal tracts. A line was drawn round the tracts from the base of the crop around the breast muscle to the base of the keel bone. Follicles were marked as they were counted.

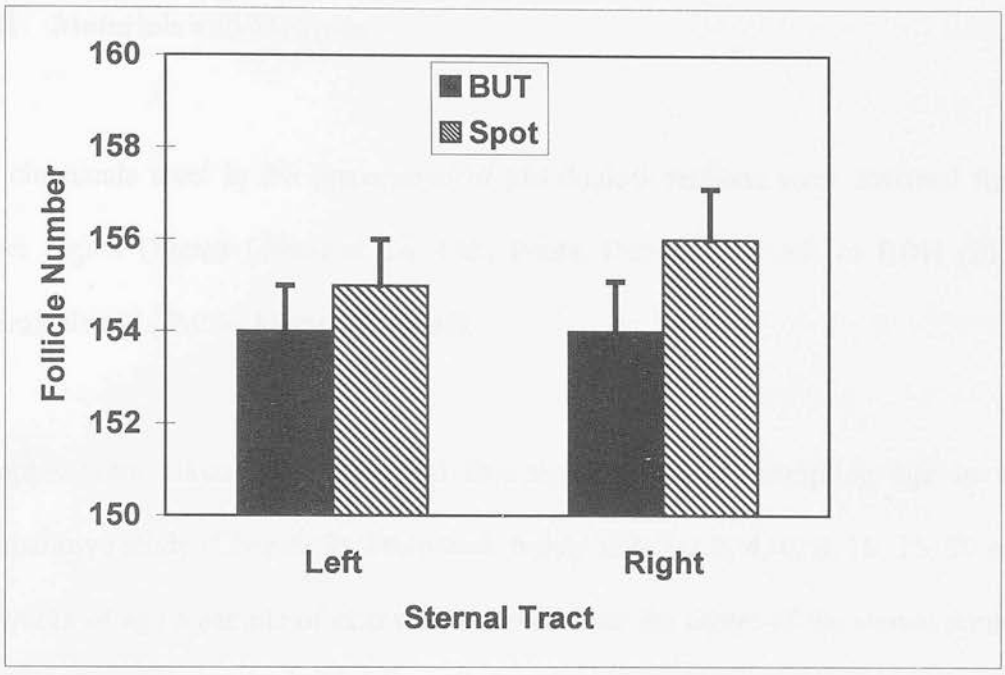


Figure 6.2 Mean (± SED) feather follicle numbers from the left and right sternal tracts for the modern BUT turkey and the traditional Nebraska Spot

6.3 Histology of the Skin

The objective of the study was to compare the structure of the breast skin in the modern and traditional turkey to detect any signs of damage to the skin of the modern bird. Skin sections were also examined for any unusual or abnormal structures and any differences in the structure of the feather follicles. Feathers from the caudal and cranial region of the breast of the modern and traditional turkey were examined under the microscope for evidence of damage and breakage.

6.3.1 Materials and Methods

All chemicals used in the preparation of histological sections were obtained from either Sigma (Sigma Chemical Co. Ltd., Poole, Dorset, England) or BDH (BDH Chemicals Ltd., Poole, Dorset, England).

Samples were taken for histological investigation at each sampling age in the comparative study (Chapter 3). From each turkey killed at 2, 4, 6, 8, 10, 15, 20 and 25 weeks of age a sample of skin was removed from the centre of the sternal feather tract. Further skin samples were taken from male BUT turkeys at 6 weeks to examine follicles from the cranial and caudal regions of the tract. The skin was pinned to balsa wood to keep it flat. Samples were placed in individual Tissue Tek Mega-cassettes (Diagnostics Division, Miles Inc. USA) and fixed in buffered neutral formalin (BNF) for a minimum of 2 weeks. Fixed samples were dehydrated through ascending alcohol concentrations using a Shandon Hypercentre XP tissue processor (Shandon Scientific Ltd. Astmoor, Runcorn, UK) and embedded in paraffin wax. Sections were cut (5μm) and mounted on albumin coated microscopy slides before staining with haemotoxylin and eosin or Van Gieson's stain. Haemotoxylin and eosin stains nuclei blue and cytoplasmic and intercellular structures pink, while Van Gieson's stains nuclei brownish black, cytoplasm and muscle yellow and collagen bright red.

Feather samples from the caudal and cranial region of the breast were removed, using tweezers and avoiding damage to the feather barbs, from turkeys killed at the 6 week

sampling age. The feathers were sealed in plastic bags and later examined using a light microscope.

Haemotoxylin and Eosin

Sections were deparaffinised in the clearing agent xylene for 5 minutes and hydrated in descending alcohol concentrations to water. Sections were stained in haemalum for 5 minutes and rinsed in water for 5 minutes before being stained in 2.5% eosin for 2 minutes. Sections were then dehydrated, cleared in xylene and mounted in DPX.

Haemalum

Solution A	Haematoxylin 1g
	Absolute alcohol 40ml
	Distilled water 360ml
Soultion B	Sodium iodate 0.2g
	Potassium alum 48g
	Chloral hydrate 48g
	Distilled water 600ml

Solution B was heated gently and solution A was added. After 48 hours 1ml of acetic acid was added.

Van Gieson's

Sections were deparaffinised in the clearing agent xylene for 5 minutes and hydrated in descending alcohol concentrations to water. Sections were stained in Weigert's haematoxylin for 10 minutes with the solution being made up fresh as below. The sections were washed, differentiated in 1% acid alcohol followed by lithium carbonate and then washed again. Sections were stained in fresh Van Gieson solution for 3 minutes before being dehydrated, cleared in xylene and mounted in DPX.

Weigert's haematoxylin

Solution A Haematoxylin 1g
Absolute alcohol 100ml

Solution B 30% Ferric chloride 4ml
Distilled water 95ml
Hydrochloric acid 1ml

Equal parts of A and B.

Van Gieson solution

Saturated aqueous picric acid 100ml
1% acid fuchsin 10ml

6.3.2 Results

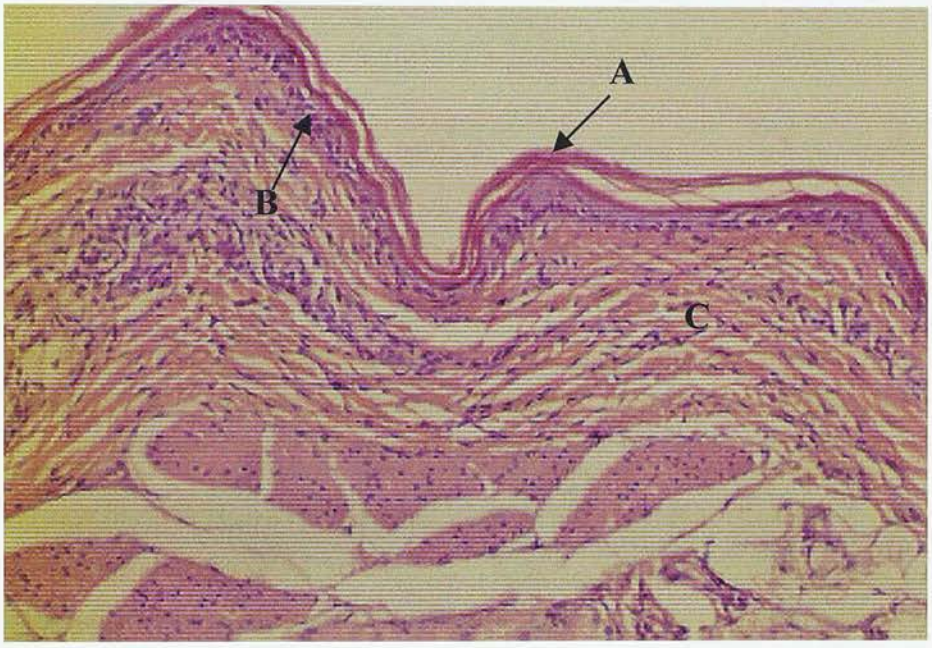
Figures 6.3 and 6.4 show representative skin cross-sections from modern (a) and traditional (b) 6-week old male turkeys prepared with haematoxylin and eosin or Van Gieson's stain. The epidermis and the dermis (C) were clearly visible. The epidermis of feathered skin is very thin and composed of a *stratum corneum* (A) and *stratum germinativum* (B). Figure 6.5 shows feathers in representative skin cross-sections from modern (a) and traditional (b) turkeys prepared with haematoxylin and eosin. The main structures of the developing feather can be seen including the feather sheath, the developing barbs and barbules and the epidermis of the feather follicle.

No differences were observed between the modern and traditional turkeys in the histological sections prepared with either haematoxylin and eosin or Van Gieson's stain from skin samples at any age. The samples taken from the cranial and caudal

regions of the sternal tract of the modern turkeys at 6 weeks did not show any differences in the structure of the feather follicles.

Figure 6.6 shows cranial breast feathers representative of the modern (a) and traditional (b) turkeys. The barbs of the traditional turkey feather were clearly seen and there was no evidence of damage to the feather. It was not clear if there was damage to the cranial breast feathers of the modern turkey because they were dirty. The dirt clogged the barbs and resulted in the structure of the feather being obscured. A similar result was found when the caudal breast feathers were examined.

(a)



(b)

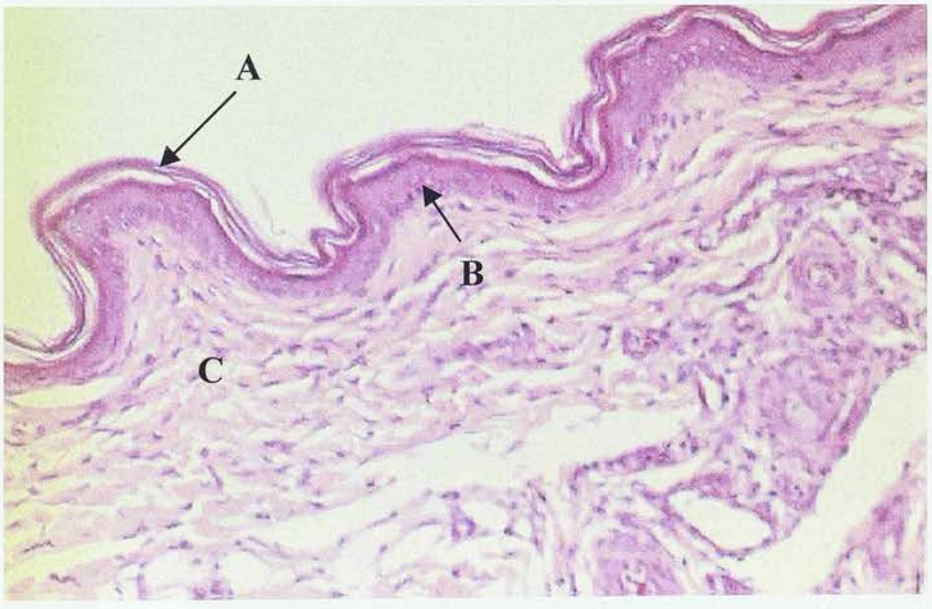
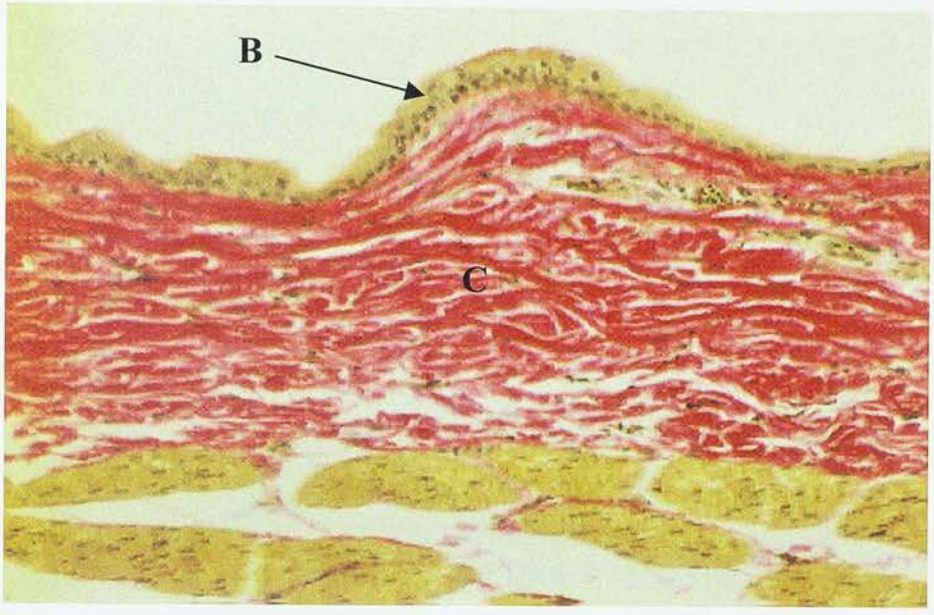


Figure 6.3 Cross section of breast skin from a male 6-week old modern turkey (a) and traditional turkey (b) prepared with haematoxylin and eosin stain (x 64). A *stratum corneum*, B *stratum germinativum* and C dermis.

(a)



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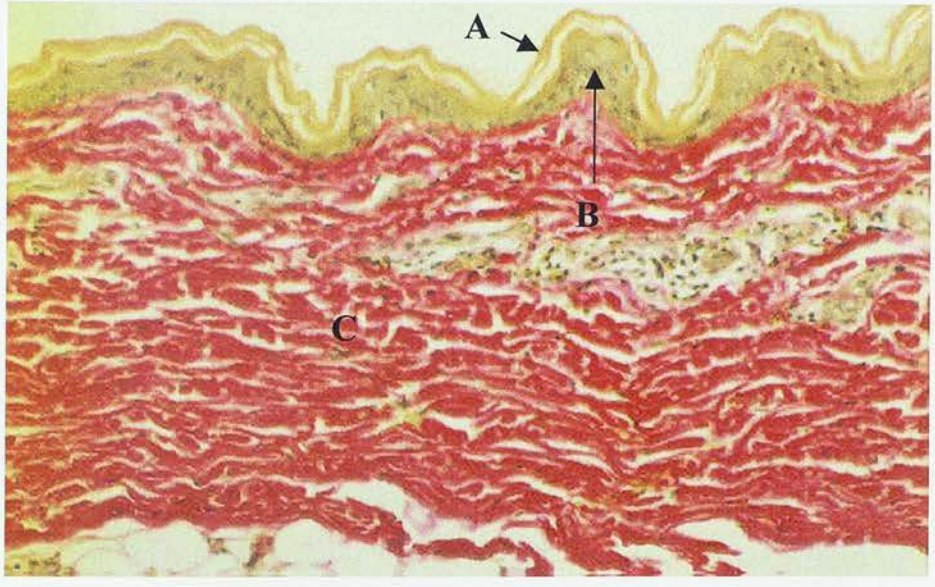
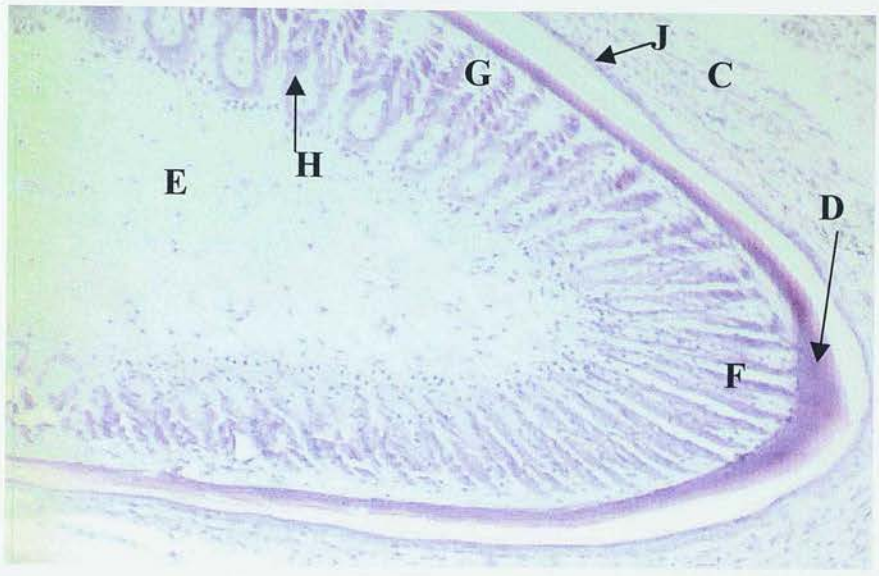


Figure 6.4 Cross section of breast skin from a male 6-week old modern turkey (a) and traditional turkey (b) prepared with Van Gieson's stain (x 64). Collagen, stained red, can be seen in the dermis. A *stratum corneum*, B *stratum germinativum* and C dermis.

(a)



(b)

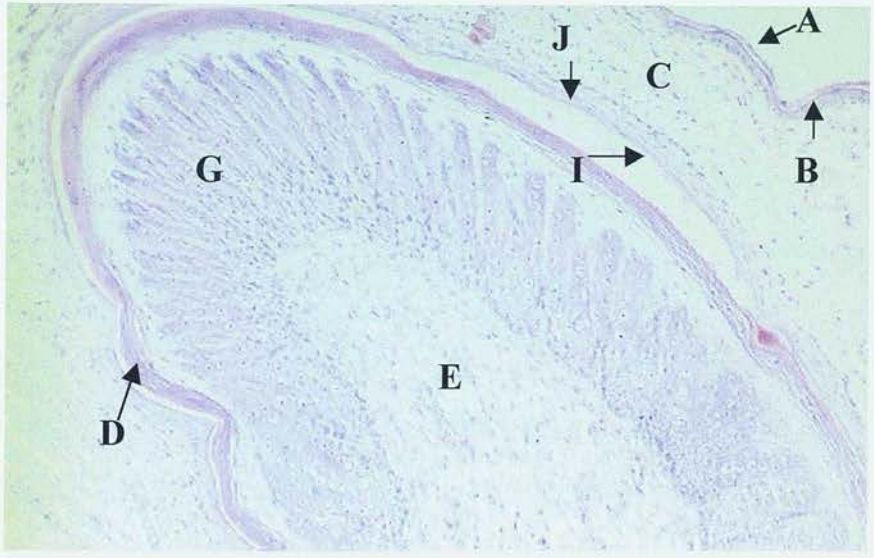


Figure 6.5 Cross section of breast skin with feather from a male 6-week old modern turkey (a) and traditional turkey (b) prepared with haematoxylin and eosin stain (x 40). A *stratum corneum* (skin), B *stratum germinativum* (skin), C dermis, D feather sheath, E feather pulp, F barbs, G barbules, H cells of barb stem, I *stratum corneum* (follicle), J *stratum germinativum* (follicle).

(a)

(b)

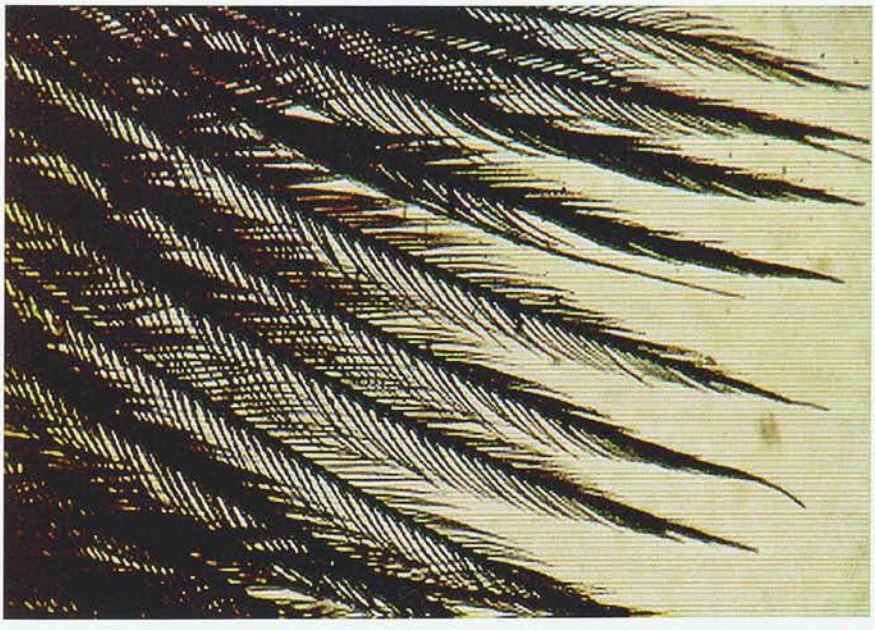


Figure 6.6 Cranial breast feathers from a male 6-week old modern turkey (a) and traditional turkey (b) (x 10). The barbs of the traditional turkey can be clearly seen with no evidence of damage to the feather. The barbs of the modern turkey are clogged with dirt, disrupting the structure of the feather.

6.4 Skin Collagen

The aim of the experiment was to compare skin collagen in the modern and traditional turkey as an indication of a possible difference in the structure of the skin of the modern turkey. The measurement of the hydroxyproline content of the skin using the colorimetric microassay based on Ehlrich's reaction as described by Creemers *et al.* (1997) was used to estimate collagen content indirectly.

6.4.1 Materials and Methods

Twelve male BUT Big 5 Male line and 12 male Nebraska Spot turkeys were used in this study. The birds were reared in floor pens on standard turkey diets (Chapter 2) until 6 weeks of age and then killed by injection of sodium pentobarbitone. Samples of skin were immediately dissected from the sternal pterylae and apteria and frozen at –20°C for subsequent analysis of hydroxyproline content. Further skin samples were dissected to compare the dry matter content between the two strains of turkey by weighing skin samples accurately before and after freeze-drying.

Hydroxyproline assay

Duplicate skin samples of approximately 100-150mg from both the sternal pterylae and apterium were weighed accurately into 1.5ml screw cap assay tubes (Greiner Labortechnik Ltd, Gloucestershire, UK). Using a pipette, 1ml of 6M HCl was added to each tube and the tubes tightly capped before being placed in the oven at 107°C for 24h. Samples were placed under vacuum in a desiccator with sodium hydroxide

pellets in the base before drying in the oven at 37°C for 7 days. When samples were completely dry they were resuspended in 0.5ml deionised water. Resuspended samples were centrifuged at 1000 **g** for 1 min and an aliquot of the supernatant diluted 1:800 with deionised water. Standards containing 6.55, 3.275, 1.638, 0.818, 0.409 and 0.205µg/ml were prepared using stock hydroxyproline (65.5µg/ml) diluted with deionised water. Aliquots of 60µl of each blank (deionised water), standard and diluted sample were transferred in duplicate into a 96-well microplate. To each well, 20µl of assay buffer (*n*-propanol, distilled water and stock buffer in a ratio of 3:2:10, with the stock buffer consisting of 0.24M citric acid, 0.88M sodium acetate trihydrate, 0.88M anhydrous sodium acetate, 0.21M acetic acid and 0.85M sodium hydroxide, pH 6.1) and 40µl chloramine-T reagent (0.282g chloramine-T, 1ml *n*-propanol, 1ml deionised water and 8ml stock buffer) were added. Microplates were incubated at room temperature for 15 min before 80µl DMBA reagent (2g dimethylaminobenzaldehyde dissolved in 1.25ml *n*-propanol and 2.75ml perchloric acid) was added and mixed with a multichannel pipette. The microplate was secured to a rack in a gently shaking water bath, with the under-surface of the microplate in contact with water, for 20 min at 60°C. The plate was then cooled on ice and read on a microplate reader (MR5000, Dynatech Laboratories, Billingshurst, West Sussex, UK) at 560nm. The collagen content was estimated by multiplying hydroxyproline concentration by 7.25 (Christensen *et al.*, 1994).

6.4.2 Results

Mean skin collagen concentrations in the modern BUT turkey and the traditional Nebraska Spot are shown in Figure 6.7. The collagen content of the skin was significantly ($P<0.001$) lower in the modern turkey than the traditional turkey. The skin from the pterylae had significantly ($P<0.01$) lower collagen than the skin from the apterium (Figure 6.8). The dry weights, expressed as a percentage of the wet weight, of the skin from the modern and traditional turkey are shown in Table 6.1. There was no significant difference in the percentage dry weight of the skin between the modern and traditional turkey or between the pterylae and the apterium.

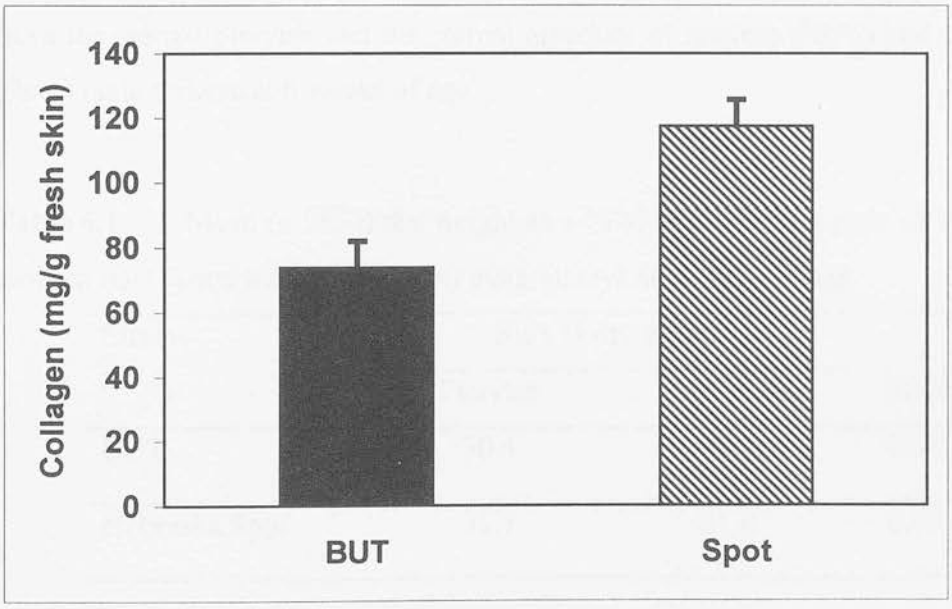


Figure 6.7 Mean (± SED) skin collagen concentration (mg/g fresh tissue) for modern (BUT) and traditional (Spot) male turkeys at six weeks of age.

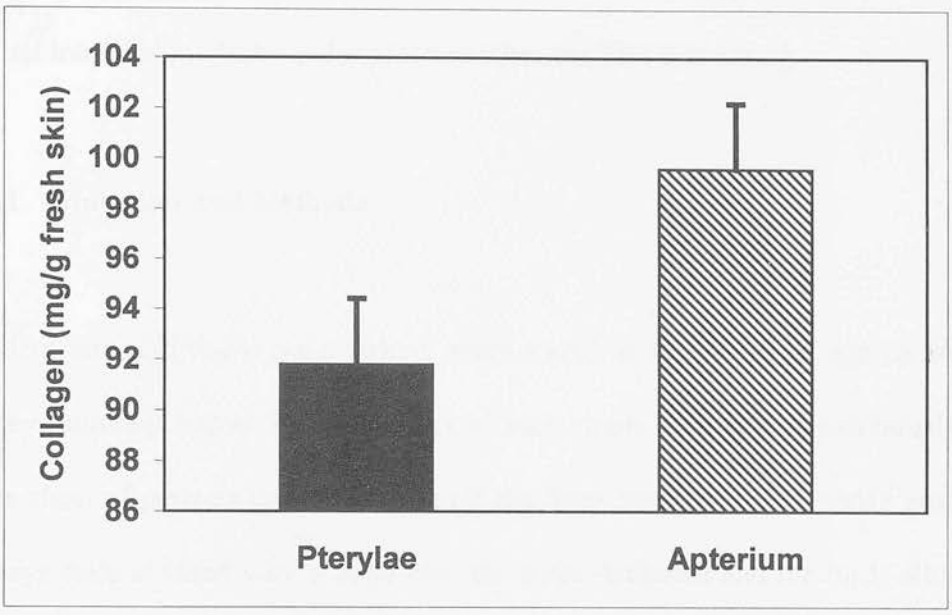


Figure 6.8 Mean (± SED) collagen concentration (mg/g fresh tissue) for skin from the sternal pterylae and the sternal apterium of modern (BUT) and traditional (Spot) male turkeys at 6 weeks of age.

Table 6.1 Mean (± SED) dry weight as a % of tissue fresh weight of the skin in modern (BUT) and traditional (Spot) male turkeys at 6 weeks of age.

Strain	Skin % dry matter		
	Pterylae	Apterium	SED
BUT	30.4	30.7	0.69
Nebraska Spot	31.7	31.6	0.69

6.5 Pressure on the Sternal Tracts

This study examined the points of contact of the breast skin with the floor when modern and traditional turkeys were resting. The objective was to examine

differences between the two strains in the areas of skin and particularly areas of the sternal tract that might be under pressure when the bird was sitting.

6.5.1 Materials and Methods

Modern and traditional male turkeys were reared to six weeks of age on standard turkey rations (Chapter 2). Six turkeys of each strain were placed individually on a large sheet of perspex that was raised off the floor, supported on wooden posts. The turkeys were covered with a large box, the room darkened and the birds allowed to settle. When the turkeys had been sitting in what appeared to be a normal and relaxed posture without movement for a short time a photograph of the underside of the turkey was taken through the perspex.

6.5.2 Results

Figures 6.9a and 6.9b respectively show representative examples of the undersides of a resting modern and traditional turkey. Points of contact with the perspex on the keel (Z), hocks (X) and feet (Y) can be clearly seen in both strains. It would appear that the traditional turkey supported its weight on the legs and keel bone with no contact of the breast with the perspex. The legs and keel bone also supported the modern turkey, however the different positioning of the legs resulted in the breast muscle resting directly on top of the feet. No direct contact was observed between the breast muscle and the perspex but the feet were positioned between the perspex and the cranial region of the sternal tracts.

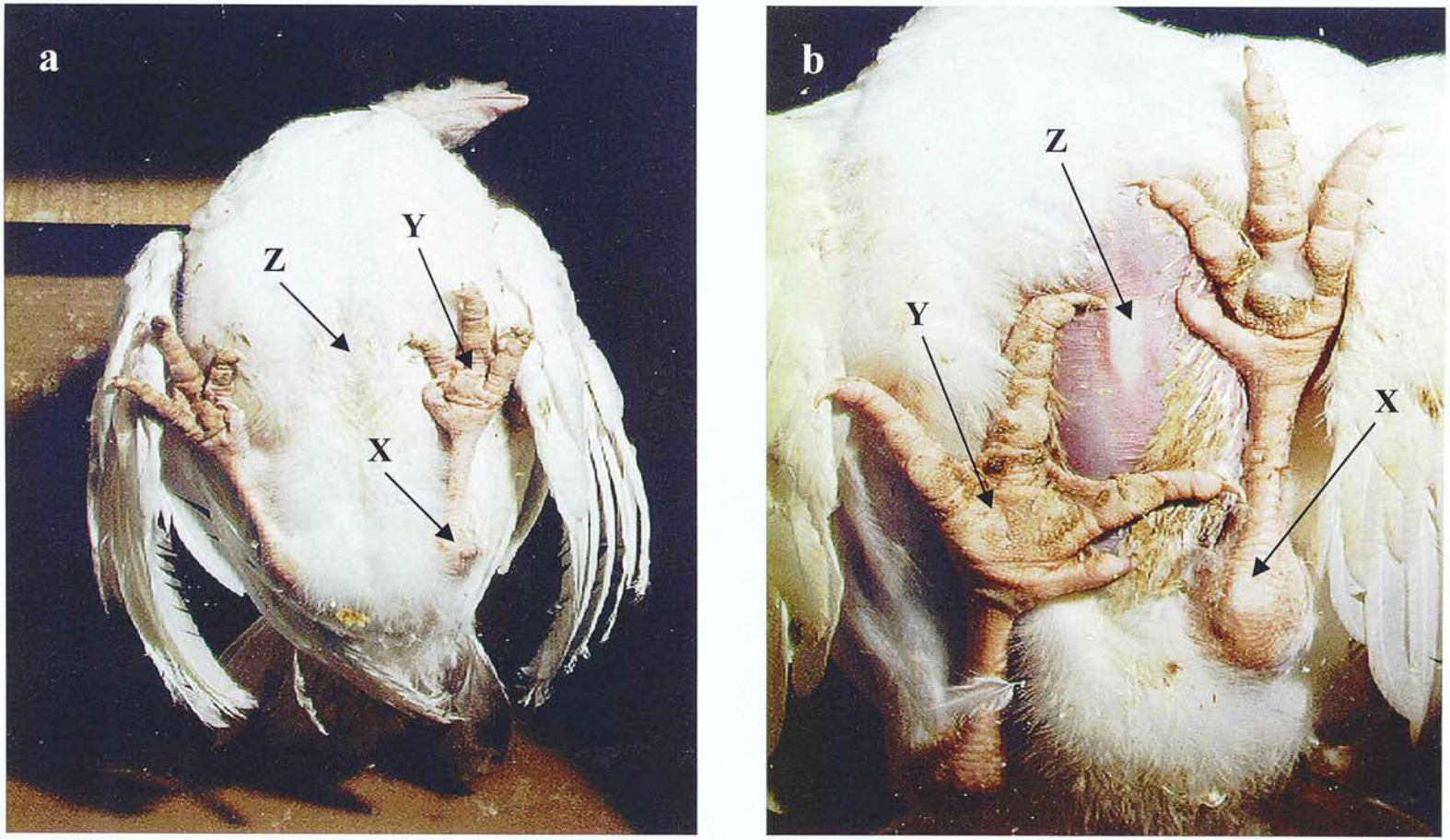


Figure 6.9 Traditional Nebraska Spot (**a**) and modern BUT Big 5 male-line (**b**) male turkeys at six weeks of age sitting on perspex. Areas of pressure can be seen on the hock (X), the feet (Y) and the keel bone (Z).

6.6 Skin Capillary Supply

The aim of this study was to investigate the capillary supply to the skin in modern and traditional turkeys using immunofluorescent staining and confocal microscopy. Indirect immunofluorescence was carried out on skin samples using antiserum to the endothelial marker von Willebrand factor (vWf), which is a marker of endothelial cells in all cutaneous blood vessels (Gu *et al.*, 1995).

6.6.1 Materials and Methods

Twelve male BUT Big 5 Male line and 12 male Nebraska Spot turkeys were used in the experiment. The birds were reared in floor pens on standard turkey diets (Chapter 2) until 6 weeks of age, killed by injection of sodium pentobarbitone and samples of skin removed from the sternal feather tract. The skin was pinned to balsa wood to keep it flat. Samples were placed in individual Tissue Tek Mega-cassettes (Diagnostics Division, Miles Inc. USA) and fixed in buffered neutral formalin (BNF) for a minimum of 1 week. After fixing, the feathers were either trimmed to the level of the skin with a pair of scissors or the feathers were removed from the skin samples by pulling the feather shaft from the follicle using a pair of tweezers. The blood capillaries in the skin were stained using the following protocol.

Capillary Staining

Fixed skin samples were washed in 0.001mol/l (pH 7.2) phosphate buffered saline (PBS) (Sigma Chemical Co. Ltd., Poole, Dorset, England) overnight at room

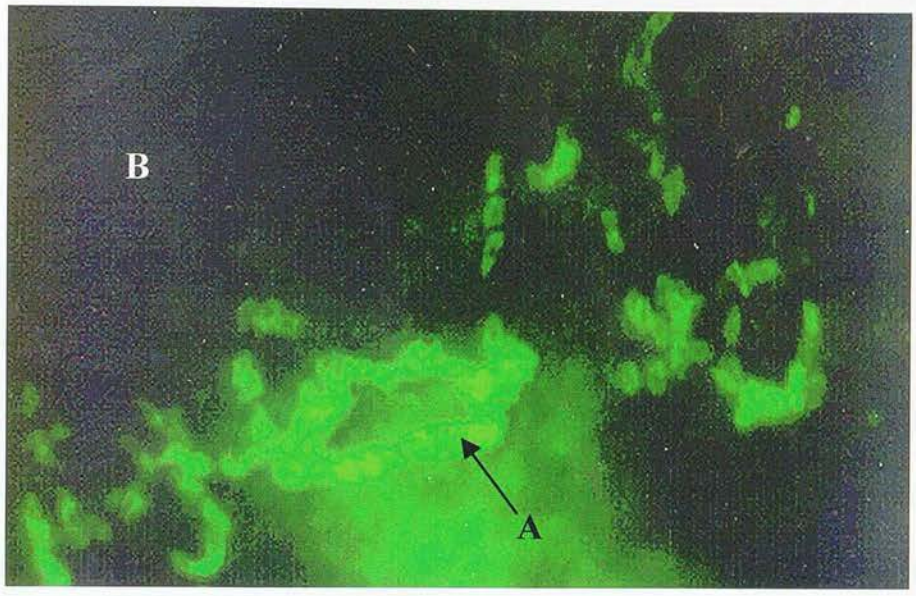
temperature followed by 0.02% Triton X-100 (BDH Chemicals Ltd., Poole, Dorset, England) in PBS for 8 hours at room temperature before being washed in PBS, again over night at room temperature. Samples were dehydrated through graded alcohol concentrations (4-5 hours in each) followed by clearing for 1-2 hours in xylene and then hydrated. Samples were incubated overnight at room temperature in normal goat serum (SAPU, Scottish Antibody Production Unit, Carluke, UK) diluted in PBS (1:30) and then for a minimum of 72 hours at room temperature in vWB antiserum-mouse (Vector Laboratories Ltd, Peterborough, UK) diluted in PBS (1:400). After being rinsed in several changes of PBS over 10-12 hours the samples were incubated with goat anti-mouse IgG antibody conjugated with FITC (Fluorescein Isothiocyanate) (Sigma Chemical Co. Ltd., Poole, Dorset, England) for 24 hours at room temperature and then rinsed again in several changes of PBS over 10-12 hours. Finally the skin samples were flattened on to glass slides and mounted with glycerine (BDH Chemicals Ltd., Poole, Dorset, England).

Stained skin samples were examined using an MRC 500 confocal laser scanning attachment (Bio-Rad, Hertfordshire, UK) mounted on an Optiphot-2 microscope (Nikon, Kingston, Surrey, UK).

6.6.2 Results

Figure 6.10 shows a projection of 30 images of the capillary network within the breast skin of a modern (a) and a traditional (b) turkey at 6 weeks of age. Capillaries could be seen round the base of the feather but it was not possible to quantify the capillary supply to the skin or feather follicles or to show any qualitative differences between the two turkey strains.

(a)



(b)

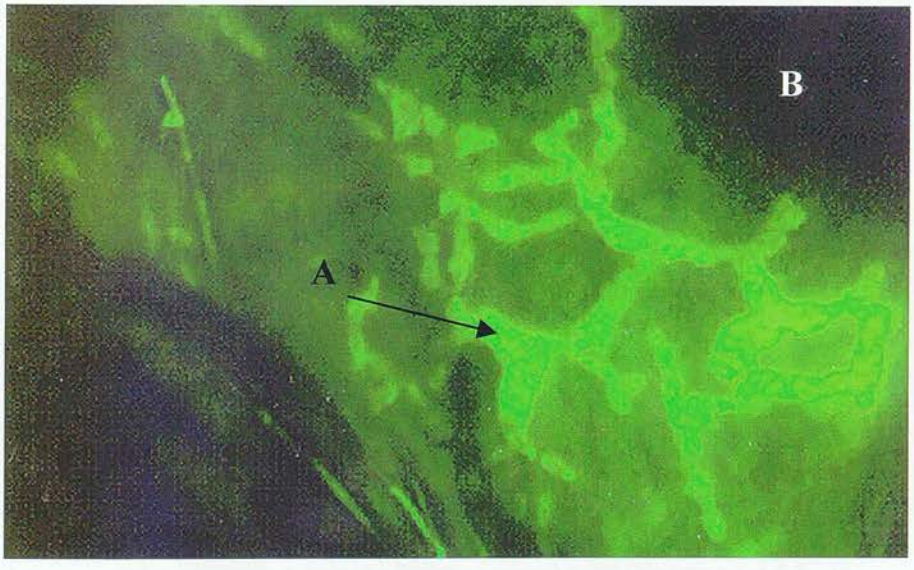


Figure 6.10 Capillaries stained with fluorescein isothiocyanate in the breast skin of representative modern (a) and traditional (b) male turkeys at 6 weeks of age. A blood capillaries, B position of the feather shaft. Projection of 30 images, each taken at 8μm intervals (x 30).

6.7 Discussion

The aim of the first experiment was to test the hypothesis that the number of breast feather follicles has not increased in proportion to the increased body size of the modern turkey. When the follicles on the sternal tracts were counted it was found that both the modern and the traditional turkey had a similar number of feather follicles. This confirms that there were the same numbers of feather follicles to produce feathers to cover a larger area of breast muscle. Follicle number is fixed at the embryo stage (Lucas and Stettenheim, 1972) so the number of follicles is unlikely to increase after hatch. In order to change the number of follicles on the modern turkey, studies identifying genetic variation between and within strains would be required. If genetic variation was found and follicle number had reasonably good heritability it might be possible to select for increased follicle number if it was not associated with a detrimental effect on selection for increased growth and muscle conformation. The lower follicle density in the modern turkey will contribute to the poor breast feather cover as will impaired growth of the cranial breast feathers described in Chapter 3.

As there was no difference in breast feather follicle numbers between the two strains this would suggest that the skin has been stretched in the modern turkey and selection for breast muscle mass has not been associated with an increase in the number of feathers over the breast muscle. This is supported by the results from Chapters 4 and 5 showing that follicle density was lower in turkeys with a higher breast muscle weight compared with turkeys in which breast muscle growth was restricted.

Stretching of the skin could lead to structural damage of the skin or feather follicles that might impair breast feather growth.

Histological investigation of the skin did not reveal any differences in the structure of the skin from the modern and traditional turkeys at the level studied. Structures in the skin appeared to be normal, however there may be differences in ultrastructure that could not be seen using light microscopy. Although there were no obvious defects in the skin, the sections of skin used for histological study were from a small sample of the centre of the tract. Samples of skin were also taken from the cranial and caudal regions of the modern turkey breast tract at 6 weeks of age to examine in more detail the structure of the feather follicles. Again no differences were visible in the follicle structure from the two areas of tract.

Skin sections proved difficult to cut especially samples from older turkeys and when cutting through the feathers. If the feathers had been removed the skin could have been damaged or follicular structures disrupted. Skin thickness was not measured in this study and no obvious differences in skin thickness were observed.

Differences in collagen content could not be quantified from the histological staining for collagen. In order to compare collagen content between the two strains of turkey skin samples were analysed for hydroxyproline content. A lower collagen content was found in the skin of the modern turkey suggesting that the skin of the modern turkey is weaker (Ramshaw *et al.*, 1986). The hydroxyproline assay measured collagen in a sample of skin, however collagen content may be reduced if there were

increased numbers of other structures present in the sample of skin. Feather follicles have muscles associated with them that could reduce the collagen in a skin sample from the feather tract compared with a sample from the apterium that does not contain these muscles. This might explain the difference in the collagen content of samples from the apterium and pterylae. However, fewer follicles were present in the samples of modern turkey skin and it would therefore be expected that the modern turkey skin should have contained more collagen whereas the opposite was found to be the case. The reduction in collagen content in the skin of the modern turkey suggests that the skin had a thinner dermal layer (Ramshaw *et al.*, 1986). This result would support the hypothesis that the skin of the modern turkey has been stretched although there was no histological evidence of damage associated with this stretching.

The final hypothesis tested in this chapter was that the two strains of turkey would show differences in the areas of skin and particularly areas of the sternal tract that were under pressure when the birds were resting. Photographs of the underside of the two strains of turkey showed the strains to be resting in different positions. It is possible that the sitting posture of the modern turkey had an adverse effect on feather growth. A possible reason for the difference in sitting posture could be that the breast muscle has become too large to allow the feet to be positioned on either side of the chest as the traditional turkey does. If the length of the legs of the modern turkey have not increased in proportion with the breast muscle the legs may be too short to be positioned in the usual way and this could also have resulted in the altered sitting

position. As well as damage to feathers the change in sitting posture may also cause damage to the feet and legs because of increased pressure.

As the feet of the modern turkey were in contact with the breast feathers, friction on the feathers could be increased especially during the change of position from standing to sitting. Breast feathers were examined under the microscope for signs of damage and the feather structure appeared normal in the traditional turkey. The structure of the breast feathers from the modern turkey was difficult to determine as the feathers were very dirty. It was not possible to look for signs of damage in these feathers. The dirt was firmly adhered to the barbs of the feather and could only be removed by stringent washing that may have damaged the feather or disrupted the feather structure. Dirty feathers may be caused by the modern turkey resting on the litter for a greater proportion of time or may suggest a lack of preening activity although this was not measured. The feather tract was also likely to be under an abnormally high degree of pressure that could have resulted in damage to the skin capillary supply or perhaps simply have cut off the blood supply to the skin or feather follicles for periods of time. A large area of white skin could be seen over the keel bone of the modern turkey where there was decreased blood flow to the skin. It was shown in Chapter 3 that the modern turkey spends a larger proportion of its time resting and prolonged sitting is thought to cause damage to capillary supply in muscle (Sosnicki *et al.*, 1991) and there may also be collateral damage to the capillary supply to the skin.

Skin under the feet must be under considerable forces and it would be interesting to examine the forces applied to the skin when the modern turkey is resting. Forces are likely to be high because the feet provide only a small surface area for the breast muscle to rest on compared with direct contact with the floor where the body weight would be spread over a larger area.

The investigation into the vascular supply to skin and follicles was successful in staining the capillaries but was not good enough to provide information on differences in capillary supply between the two strains of turkey. It was shown that capillaries were present in the skin around the feather follicles of both strains of turkey. Apparent breaks in the capillaries may represent damage but could also be a result of the stain not completely penetrating the skin sample. The technique used involved the staining of the capillary wall but this would not show any blockage in the capillary network or if blood was flowing through the capillaries. The examination of capillaries in a skin sample would give no information as to the origin of the vessels. Removal of the feathers from the skin resulted in the follicles being difficult to find but if the feathers were left in they prevented the confocal laser from adequately penetrating the skin sample. It was also difficult to make any quantitative measures of capillary supply.

The results from these studies suggest that the skin of the modern turkey had been stretched although no obvious signs of damage could be seen in the structure of the skin or the feather follicles. It would also appear that the modern turkey sits in a position that has resulted in the feet being in contact with the sternal tract. Although

it was not possible to obtain any evidence, this sitting position could have resulted in increased pressure being applied to the sternal tract and subsequent damage to the skin and follicle capillary supply. This in turn could have resulted in impaired breast feather growth. The stubby cranial breast feathers are consistent with the suggestion that friction caused by changes from resting to standing and *vice versa*, or movement while resting, could also lead directly to feather damage.

CHAPTER 7: General Discussion

The aims of this thesis were to compare the growth of feathers in a modern line of commercial turkey with that of an unrelated traditional turkey and to investigate the causes of the lack of breast feathers in the large modern bird.

Breast lesions are a common problem in modern commercial turkeys. Detailed information on the current commercial incidence of breast lesions is lacking, but it is thought that such lesions result in substantial economic loss to the turkey industry (Tilley *et al.*, 1996). Breast lesions have been observed in chickens and turkeys for many years and although much work has been done to investigate causes of breast lesions little progress has been made in reducing their incidence. It is unlikely that there is a single factor responsible for the development of breast blisters and buttons, however, several factors that cause irritation or mechanical damage to the breast skin may interact, with the eventual outcome being the development of lesions. Feathers offer natural protection to the skin from mechanical damage but this protection is reduced or lacking over the breast of the modern turkey. Improving breast feathering may lead to a reduction in the incidence of breast lesions in commercial turkeys.

In order to improve feather cover on the breast of the modern turkey it was first necessary to investigate reasons why the feathering is so poor. Work has been done to examine factors that affect feather growth such as nutritional requirements, hormones and genetic influences but not specifically to study breast feathering in the turkey.

Feather Growth in Modern and Traditional Turkeys

On initial examination the breast skin of the modern turkey is typically exposed with few feathers covering the breast area. The lack of feather cover appears to be caused by two factors. Firstly the feathers from the cranial breast region are short, stubby and do not have a fully developed structure, suggesting that their growth is impaired. Secondly the feathers that are present are not sufficient in number to be able to cover the skin and, in particular, the sternal apteria. The poor feather growth of the cranial breast region was quantified in Chapter 3. Comparisons of a modern turkey with a traditional turkey showed that feather growth from the cranial breast region was greatly reduced in the modern bird. These results are consistent with the preliminary findings of Hocking (1995) who suggested that breast feather growth in the modern turkey was poor.

It was predicted that feather growth would be poor on the breast of the modern turkey and it was suggested that there would be other differences in feather growth between the modern and the traditional turkey. In Chapter 3 it was interesting to discover that the total weight of feathers had not increased in proportion to the greater body weight of the modern bird. Indeed there were no differences found between the two strains in the lengths of the feathers measured except for the short cranial breast feathers in the modern turkey. It would appear that selection for increased body weight and breast muscle weight has not been accompanied by an increase in feather weight. This observation was similar to the findings of Katanbaf (1989) who showed that selection for body weight in chickens was associated with a proportionally larger breast but that feather weight was included in the negative responses. The length to which an individual feather grows is likely to be controlled genetically (Lucas and Stettenheim, 1972) and may be related to the body size of the bird. The relatively small size of the first feathers

in the follicles is not surprising because the young bird clearly cannot support the development of a large, adult sized feather (Lucas and Stettenheim, 1972). The increase in feather weight of successive feather generations suggests a specific and well organised mechanism that regulates the growth of individual feathers in accordance with the size of the bird and its stage of development (Smith, 1994).

Moulting patterns complicate the study of feather growth and a description of moulting in the modern turkey was used to select an age when moulting was minimal to reduce any possible effect in the experiments presented in this thesis (Appendix 2). Taken with the results of feather moisture content described in Chapter 3 the data suggested that the pattern of feather development and moult were similar in the modern and traditional turkey.

Experimental Results

Three hypotheses to explain the differences in breast feathering between the modern and traditional turkey were proposed in Section 1.3. First, the number of feathers and feather follicles may not have increased with selection for increased breast muscle in the modern turkey. Second, there may be competition between muscle and feather growth for essential nutrients such as amino acids or minerals. Third reduced feather growth may be an adaptive response by the modern turkey to its increased heat production.

The first hypothesis was evaluated by counting numbers of feather follicles in the breast tracts of the modern and traditional turkey. When the body weight of the modern turkey was restricted to 50% of the *ad libitum* fed body weight a significant increase in follicle density was observed (Chapter 4). Feeding a low protein diet (Chapter 5) had the same

effect of increasing the follicle density by decreasing body weight. It was predicted that the increased follicle density occurred because the same numbers of follicles were required to cover a reduced area of breast skin over a smaller breast muscle. As the number of feather follicles is fixed during embryo development (Lucas and Stettenheim, 1972) it is perhaps not surprising that any change in the area of skin that these follicles cover will result in a change in follicle density. In Chapter 3 feather follicle density on the breast was found to decrease with age in both the modern and the traditional turkey and at all ages the follicle density was found to be higher in the traditional compared with the modern turkey. The higher follicle density on the breast of the smaller traditional bird added weight to the hypothesis that the two strains had the same number of feather follicles on the breast. This prediction was confirmed by the results presented in Chapter 6 that showed that counts of the feather follicles in the sternal tracts of the modern and traditional turkey did not differ.

The second hypothesis to explain poor breast feathering in the modern turkey was that there is competition between body and feather growth for essential nutrients. The modern turkey has been selected for increased breast muscle yield and fast growth rates and it is likely that the protein requirements for both muscle and feather growth are high. If dietary protein was partitioned to muscle growth in preference to feather growth this could result in the impaired growth of feathers on the breast of the modern turkey. In Chapter 4 the effect of restricted feeding on muscle and feather growth in the modern turkey was examined. Cranial breast feathers were found to increase in length when the modern turkey was restricted to 50% of *ad libitum* fed body weight. It was also suggested that feather growth was maintained in preference to muscle and body growth when food intake of the modern turkey was restricted. This observation may have been

due to the reduction of energy intake, as it would seem a sensible strategy to maintain insulation to conserve energy when energy supply is limited. It was thought that a reduction in dietary protein without any change in energy intake would result in muscle accretion continuing at the expense of feather growth. The experiments presented in Chapter 5 did not support this hypothesis. It was found that reducing the dietary protein concentration resulted in breast muscle weight being adversely affected by low dietary protein more than body weight and that the modern turkey was affected more than the traditional turkey. There was a smaller strain difference in the feather weight reduction and feather weight in the modern bird was not reduced as much as body and muscle weight. In the modern bird the small reduction in feather weight compared with body weight suggests that feather growth was maintained at the expense of muscle and body growth when protein was deficient. Reducing dietary protein concentration decreased the length of the cranial breast feathers in the traditional turkey but increased the length of these feathers in the modern turkey. The supplementation of specific amino acids was not found to increase cranial breast feather growth in preference to muscle growth in the modern turkey. In fact the addition of each amino acid reduced feather growth and increased breast muscle mass in this area. The pattern of cranial breast feather growth in the modern turkey, observed in the experiments in Chapter 5, did not support the hypothesis that competition between body and feather growth for essential nutrients favoured muscle growth in the modern turkey. Instead, the increased length of the cranial breast feathers when muscle weight was reduced suggested that the impaired growth of cranial breast feathers was caused by the rapid breast muscle growth of the modern bird. It is therefore perhaps not surprising that supplementation of zinc, which was not found to effect body weight, had no affect on cranial breast feather growth (Appendix 1).

The third hypothesis to explain poor breast feathering in the modern turkey was that reduced feather growth was an adaptive response by the modern turkey to increased heat production. Rapidly growing modern turkeys produce large quantities of body heat that they must lose in order to maintain constant body temperature. Poor breast feathering could allow for increased heat dissipation. In Chapter 3 the breast skin temperatures of the modern and traditional turkey were compared. The modern turkey was found to have a higher skin temperature than the traditional turkey up to 8 weeks of age, suggesting that the modern turkey was producing more heat at younger ages. After this age the skin temperature of the modern turkey was cooler than the skin of the traditional turkey probably because of the decreased feather insulation on the breast of the modern bird. The results presented in Chapter 4 did not show that the cranial breast feathering of the modern turkey was affected by the temperature in which the birds were reared. Instead it was found that the modern turkey altered its metabolic rate to maintain body temperature in different thermal environments.

Of the three initial hypotheses proposed to explain differences in the breast feathering between the modern and the traditional turkey, the experiments presented in this thesis have only shown the first to be correct. Selection for increased breast muscle in the modern turkey has not been accompanied by an increase in the number of breast feather follicles. The question as to the cause of impaired cranial breast feather growth in the modern turkey remains. The experiments presented in this thesis were not able to support the original hypotheses proposed for causes of poor breast feather growth, however the results of these experiments did suggest further hypotheses.

A New Hypothesis

Results presented in Chapter 5 suggested that impaired cranial breast feather growth in the modern turkey may be caused by the rapid growth of the larger breast muscle. If the breast skin of the modern turkey had stretched with selection for increased breast muscle it was possible that the skin, feather follicles or capillary supply might have been damaged and result in poor feather development. The hypothesis that the breast skin of the modern turkey has stretched was supported by the finding in Chapter 6 that follicle number had not increased in the modern bird. The results of a histological investigation of the skin and feather follicles in Chapter 6 showed that there was no evidence of damage to the breast skin or its associated structures in the modern turkey. Cranial breast feathers were found to grow longer when breast muscle growth was reduced. This suggests that the follicle is not inherently defective and is capable of producing a normal feather, and it is therefore not surprising that no evidence of damage to the follicle was found. Evidence of a difference in the structure of the skin of the modern turkey was shown in Chapter 6 when collagen content of the breast skin was determined and found to be lower when compared with the traditional turkey. The lower collagen content suggests that the skin of the modern turkey is weaker than that of the traditional turkey and may be more susceptible to mechanical damage (Ramshaw *et al.*, 1986).

In Chapter 3 a comparison of the behaviour of the modern and traditional turkey showed that the large modern bird spent a greater proportion of time resting. The turkey rests on the breast and the results in Chapter 6 suggest that the cranial breast feathers may be under increased pressure in the modern bird. It would be interesting to examine the pressure that is applied to the breast skin when the modern turkey is resting to gain insight into ways in which this pressure may be alleviated. The photograph of the ventral

aspect of a resting modern turkey (Chapter 6) suggested that the posture of the bird may cause an increase in the pressure applied to the breast skin and particularly to the sternal pterylae. It is possible that the resting posture of the modern turkey may be affected by the structure of the legs and hips resulting in a change in the positioning of the feet under the bird compared with the traditional turkey. The broad breast muscle of the modern turkey means that the legs are much further apart than in traditional birds and the morphological modifications produced by selection have affected the modern bird's gait (Abourachid, 1991). The modern turkey may have altered the resting posture in order to maintain functional balance or to remain comfortable. Studies of the resting posture and pressures exerted on the cranial breast tract of the modern turkey may show differences when compared with a traditional bird that underlie the defective breast feather growth of the modern turkey.

Pressure on the breast skin in the area of the cranial feathers may result in damage to the capillary network supplying the feather follicles or blood flow to the follicles may be reduced during periods of resting. Lucas and Stettenheim (1972) states that the blood supply to the skin is from a branch of the artery supplying the skeletal muscle. If this is the case the blood supply to the breast skin may be reduced because of damage occurring in the breast muscle. The rapid growth of the large breast muscle combined with prolonged resting is thought to cause damage to the capillary supply in muscle (Sosnicki *et al.*, 1991). A suitable technique for examining blood flow to the skin is required to test this hypothesis and was beyond the remit of the present research.

The change in posture from resting to standing could result in increased friction at the surface of the skin and affect the feather follicles of the cranial breast region. It was

mentioned above that the large breast muscle of the modern bird has affected balance (Abourachid, 1991) and because of this it is possible that the movement from standing to resting has also been altered. If the breast of the modern turkey is moved forwards to the floor as the bird sits and then back along the floor or feet as the bird lowers itself onto the hocks there could be increased friction applied to the breast feathers. It would be interesting to study the movements made by the two strains of turkey when changing posture from standing to resting. No conclusive evidence of breakage of the cranial breast feathers was found but the breast feathers of the modern turkey were dirty (Chapter 6). Dirty feathers may suggest a lack of preening activity in the modern turkey. Preening is required to maintain the integrity of the feather structure and to keep it supple and a lack of preening may result in the feathers being more brittle. Nutrient deficiencies are also known to result in brittle feathers (Anderson and Warnick, 1967; Supplee *et al.*, 1958). Although no evidence of specific nutrient deficiencies was found (Chapter 5, Appendix 1) it is possible that reduced blood flow to the cranial breast feather follicles would result in a local deficiency of nutrients required for normal feather growth. Feather strength is likely to be an important assessment of feather quality. Differences in feather strength may occur even when there are no differences in feather length. The feather may still grow when nutrients are deficient but the composition of the feather may be different or weaknesses might occur that causes the feathers to break more easily when friction is applied to them. Further studies of feather growth should include the assessment of feather strength as a measure of the effect of a treatment, especially in nutritional studies.

Human Homologies of Breast Lesions in Turkeys

An increase in the time spent resting and friction to the skin may also have direct implications for the incidence of breast lesions in the modern turkey. Breast lesions and in particular breast buttons may have similar etiologic factors to the condition known as bed sores or pressure ulcers in hospitalised and bed-ridden patients. Human pressure ulcers are localised areas of tissue necrosis that result from unrelieved pressure and tend to develop when soft tissue is compressed between a bony prominence and an external surface for a prolonged period of time (Kanj *et al.,* 1998). The main etiologic factors in pressure sore development are pressure, shearing forces, friction and moisture (Kanj *et al.,* 1998). These are similar to the proposed mechanism for the development of breast lesions in turkeys. Pressure is the most important factor in ulcer formation with prolonged mechanical load closing capillaries and hindering diffusion of oxygen and metabolites to the cells (Goossens *et al.*, 1997). Increased pressure over the breast muscle and keel bone of the modern turkey may have a similar effect in increasing the incidence of ulcer like breast buttons. It has been shown in humans that although externally applied pressure alone is more effective than shear in reducing skin arteriolar blood flow, vascular occlusion is enhanced if both factors are combined (Kanj *et al.,* 1998). Shearing forces result from the sliding and relative displacement of two opposing surfaces and this may be increased over the breast of the modern turkey due to the change in posture from standing to sitting and the alteration in the bird's centre of gravity. Friction will also be increased during these postural changes and may affect the development of breast buttons as well as damaging breast feathers. Friction has been found to reduce the amount of pressure needed to produce ulcers in humans (Kanj *et al.,* 1998). Friction is the force that resists relative motion between two surfaces in contact. By damaging the protective stratum corneum, the skin barrier is compromised and skin

ulceration is enhanced (Kanj *et al.*, 1998). Reuler and Cooney (1981) demonstrated that a long-term moist environment resulting from perspiration or faecal or urinary incontinence can increase the risk of human pressure ulcer formation five-fold. Unless litter quality is good it is likely that prolonged resting will mean that the breast skin of the modern turkey will be in prolonged contact with a moist surface. Furthermore, if feathering is poor, prolonged periods of resting will increase exposure of the breast skin to noxious materials in the litter such as ammonia and faecal bacteria. These factors are responsible for diaper dermatitis in humans (Berg *et al.*, 1986; Buckingham *et al.*, 1986). Selection for increased breast muscle may have resulted in an increased susceptibility to breast buttons and poor breast feathering due to prolonged resting in the modern turkey.

It would appear that the lack of breast feathering in the modern turkey is related to selection for rapid breast muscle growth. No evidence of similar feathering problems could be found for other species. Turkeys have been selected over long periods of time within controlled environments and it is possible that these selection environments have reduced the importance of feather growth for maintenance of body temperature. Wild bird species will require feathers for thermoregulation in a more variable environment and it is likely that natural selection would favour adequate feather growth. It is therefore perhaps not surprising that similar feathering problems are not associated with wild bird species.

Improving Feather Growth

One final question remains: can breast feather cover be improved? In order to improve feather cover it would be necessary to increase the number of feather follicles on the breast and to prevent the defective growth of the cranial breast feathers.

It has been reported earlier that the number of feather follicles is fixed during embryo development (Lucas and Stettenheim, 1972). After hatch it is unlikely that any management practice will be able to alter the total number of feather follicles. It has been shown in this thesis that restricted feeding increased the follicle density but this is not likely to be a suitable commercial solution. The embryonic development of feather follicles has been used as a model for understanding the basis of pattern formation (Chuong, 1993; Noramly and Morgan, 1998; Song *et al.*, 1994). Various factors such as homeobox (Hox) genes (Chuong, 1993), fibroblast growth factors (FGF) (Widelitz *et al.*, 1996) and bone morphogenetic proteins (BMP) (Noramly and Morgan, 1998) have been shown to play a role in the formation and positioning of feather follicles. Widelitz *et al* (1996) showed that local application of exogenous FGFs to cultured skin explants could cause the formation of feather buds in normally apteric regions of skin and in the defective pterylae of the scaleless mutant, which do not normally make feathers. It is possible that in the future, with more understanding of the genes and other factors involved in the morphogenesis of feather follicles and their spacial arrangement within the pterylae that the number of feather follicles on the breast could be artificially increased. Another way of increasing the number of breast feather follicles would be to look for genetic variation in the total number of feather follicles on the breast of the modern turkey. It may then be possible to select turkeys with increased feather follicle number. Abubakr (1994) observed strain and genotype differences in follicle number and pattern in different broiler chicken and laying hen lines. The follicles in the breast tract were shown to be arranged in discrete lines and variation was found in the number of lines of follicles (Abubakr, 1994). The results of follicle number counts presented in Chapter 6 found little variation in the total number of follicles either within or between the modern and traditional turkey strains. This lack of variation may suggest that it

would be difficult to make genetic progress to increase the number of breast follicles through selection for follicle number. It would be difficult and time consuming to count total numbers of breast feather follicles in turkeys at the time of a commercial selection although counting lines of feathers may be an alternative. The success of any selection programme would also depend on the heritability of follicle number.

Further Research

There are several points raised by the present study that merit further investigation. First, it would be of interest to investigate the pressure applied to the breast during resting and to examine the possible correlation between pressure and resting posture. Second, further investigation of the capillary supply and blood flow to the breast feather follicles and also the origin of the blood supply to the integument are required. The results presented in this thesis have not suggested a solution to reduce the defective growth of the cranial breast feathers. Selection for improved breast feather cover may help to reduce the incidence of breast lesions in the modern turkey but this is likely to be detrimental to selection for increased breast muscle (Appendix 3).

In conclusion, it would appear unlikely that a simple change in the management of the modern turkey could result in improved breast feathering. Feather growth is an important component of the modern turkey and environmental factors that might affect feather growth should not be ignored.

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APPENDIX 1: Zinc

A1.1 Introduction

A dietary zinc deficiency is known to result in brittle feathers and poor feather growth in poults (Supplee *et al.*, 1958), chickens (O'Dell *et al.*, 1958), quail (Fox and Harrison, 1964) and pheasants (Scott *et al.*, 1959). The modern turkey has been selected for rapid growth and the recommended requirements for zinc may not be adequate. Poor feather growth on the breast of turkeys may result from a zinc deficiency.

The objective of this study was to compare feather growth in modern turkeys with increased zinc concentrations in the diet.

A1.2 Methods

Three turkey diets were prepared and titanium dioxide (TiO_2) (BDH Chemicals Ltd., Poole, Dorset, England) added as an indigestible marker. Diet 1 was the basic turkey starter formulation (Chapter 2) with no supplementary zinc. Diets 2 and 3 were the basic turkey starter with respectively 100mg/kg and 200mg/kg of zinc added in the form of zinc oxide (ZnO) (BDH Chemicals Ltd., Poole, Dorset, England). Diet samples were taken for analysis of their zinc contents. The diets were allocated to pens using a randomised block design. A total of 120 day-old male poults (BUT Big

5 male line) were randomly allocated among twelve pens. Birds were weighed and the food intake recorded weekly for four weeks. At 4 weeks of age, 6 birds per pen were selected randomly and the lengths and widths of the sternal feather tracts were measured using a ruler. The lengths of one feather from each of the cranial and caudal regions of each of the two tracts were measured (4 feathers per bird). Droppings from one bird per pen were collected at 4 weeks by placing the turkeys in isolation boxes and collecting the excreta produced over 4 hours. Blood samples were then taken from these birds before returning them to their home pen. To obtain a blood sample birds were restrained and the brachial (wing vein) exposed by removing a few feathers. About 5ml of blood was withdrawn with the syringe needle (25G) pointing away from the body. Pressure was then applied to the vein to prevent haematoma. Blood samples were immediately transferred to heparin coated sample tubes and chilled on ice. Samples were centrifuged at 1600 **g** for 10min and the supernatant (plasma) removed and stored at –20°C until required for analysis.

Zinc Assay - Plasma

A colourimetric zinc analysis kit (Wako Chemicals GmbH, Neuss, Germany) was used for the quantitative determination of zinc in plasma. Zinc binds to the sodium salt of 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol, forming a reddish-violet chelate. Masking agents suppress reaction with iron, copper, cobalt and nickel.

Standards containing 0.25, 0.5, 1 and 2μg/ml zinc were prepared using the kit standard solution (2μg/ml) and diluting with distilled water. 100μl of each standard,

blank and plasma sample were transferred to centrifuge tubes and 100µl of deproteinizing reagent (7% trichloroacetic acid) added. Samples were vortexed and centrifuged at 2500 **g** for 10min. The resulting supernatant was removed and 40µl transferred in duplicate to a microplate. Colour reagents A and B were mixed in the ratio 4:1 and 200µl of the resulting solution added to each well of the microplate. The plate was left for 10min and then read on a microplate reader (MR5000, Dynatech Laboratories, Billingshurst, West Sussex, UK) at 560nm.

Zinc Assay - Excreta

Excreta samples were weighed accurately into pre weighed pots and were then dried at 100°C for 4 days. Samples were cooled in a desiccator before being re-weighed and then ground to a fine powder. Duplicate samples of 1.0000g of each ground excreta sample were weighed into beakers and ashed overnight in a muffle furnace at 550°C. Ashed samples were allowed to cool in a desiccator before being re-weighed. The ash was dissolved in 10ml of 6N HCl (BDH Chemicals Ltd., Poole, Dorset, England) and extracted thoroughly by evaporating to dryness on a hotplate. Once dry, the precipitate was dissolved by adding 10ml of deionised water and heating on a hotplate. The solution was filtered through a 12.5cm No1 ashless filter paper (Whatman International Ltd., Maidstone, England) into a 100ml volumetric flask and the beaker washed with not less than 50ml of deionised water. The solution was then made up to 100ml with deionised water and mixed well by inversion. Standards containing 0, 0.25, 0.5, 0.75, 1.0 and 1.25µg/ml zinc were prepared. Zinc content of standards and samples was determined by Atomic Absorption Spectrophotometry at a

wavelength of 213.9nm. A curve of the standards was plotted and the zinc concentration of the samples determined.

Zinc Assay - Feed

The assay for feed followed the same protocol as for excreta but using 2.0000g of the ground feed sample for ashing.

Titanium Dioxide Assay

The dried and ground feed and excreta samples used for zinc determination were also used to determine the concentration of TiO_2. The ground samples were weighed accurately in duplicate into beakers using about 3g of diet and 1g of excreta and ashed overnight in a muffle furnace at 550°C. Ashed samples were then allowed to cool in a desiccator. One sodium sulphate (Na_2SO_4) tablet (Sigma Chemical Co. Ltd., Poole, Dorset, England), 10ml concentrated sulphuric acid (H_2SO_4) (BDH Chemicals Ltd., Poole, Dorset, England) and some anti-bumping granules (Sigma Chemical Co. Ltd., Poole, Dorset, England) were added to the ash. The beakers were then covered and heated to boiling point on a hotplate until the solutions turned clear. The beakers were allowed to cool before adding 40ml distilled water. The solutions were then transferred with washings into 100ml volumetric flasks and diluted to volume with distilled water. The proportion of TiO_2 in the samples was measured in duplicate at a wavelength of 420nm by a Traacs 800 (Bran and Luebbe) using a range of TiO_2 standards, 4% hydrogen peroxide (H_2O_2) (BDH Chemicals Ltd., Poole, Dorset, England) as the reagent and a 10% H_2SO_4 solution (BDH Chemicals Ltd., Poole, Dorset, England) as the sample wash.

Statistical Analysis

Zinc and TiO_2 concentration in feed and excreta were used to calculate availability of zinc using the equation:

$$\text{Availability} = \frac{\text{Zn in feed} - (\text{Zn in excreta } (TiO_2 \text{ in feed} / TiO_2 \text{ in excreta}))}{\text{Zn in feed}}$$

Analysis of variance of the effect of diet was used to test for treatment differences in food intake, feather and body measurements and zinc concentrations.

A1.3 Results

The addition of zinc oxide to the basic turkey starter resulted in three experimental diets with zinc levels of 127.3, 232.8 and 328.0mg zinc/kg diet. The results of increasing dietary zinc on the experimental variables are shown in Table A1.1.

No differences were found in body weight or in the cumulative food intake to four weeks of age between birds fed on the different diets. The size of the sternal tract and lengths of the feathers did not differ with increasing concentration of zinc in the diet. No significant differences in plasma zinc were found. The concentration of zinc in the excreta did increase with increasing dietary zinc concentration but because of a large standard error the differences were not found to be significant. Zinc availability

was found to be about 70% and was not significantly different in birds fed on the different diets.

Table A1.1 Treatment means (± SED) for body weight, cumulative food intake, sternal tract measurements, breast feather lengths, plasma zinc, excreta zinc and zinc availability of male BUT Big 5 male line turkeys at 4 weeks of age fed on diets 1, 2 and 3 containing 127.3, 232.8 and 328.0mg zinc/kg respectively. Analysis of variance significance level NS, not significant.

Measurement	Diet			SED	Significance
	1	2	3		
Body weight (kg)	1.07	1.08	1.09	0.131	NS
Cumulative food intake (kg)	1.45	1.55	1.51	0.229	NS
Sternal tract length (mm)	120	116	117	1.6	NS
Sternal tract width (mm)	14	14	14	0.5	NS
Cranial breast feather length (mm)	22	20	21	1.7	NS
Caudal breast feather length (mm)	24	25	25	1.4	NS
Plasma zinc (μg/ml)	5.26	5.33	5.55	0.306	NS
Excreta zinc (mg/kg dry matter)	131	312	438	171.8	NS
Zinc availability	0.776	0.724	0.626	0.1092	NS

A1.4 Discussion

According to published values the concentration of zinc in the basal diet was adequate for growth (NRC, 1994). The hypothesis that selection for growth of the modern turkey had resulted in an increased requirement for dietary zinc was not

supported by the results of this experiment. One of the symptoms of zinc deficiency is anorexia (Wedekind *et al.*, 1992) however there was no increase in food intake with supplemental zinc suggesting that the basal diet was not zinc deficient. As there was no significant difference in food intake for turkeys fed on the 3 diets, the zinc consumed should reflect the dietary concentration without any confounding effect of food intake.

Increasing dietary zinc did not increase the length or width of the sternal feather tracts. However, the sternal tract size is likely to be affected by the growth of the breast muscle and although the weight of the breast muscle was not recorded, no difference in body weight was found. A change in body weight might have been reflected in a change of breast muscle size.

Feather cover on the breast of turkeys was not improved by increasing the zinc concentration in the diet. It would appear that a general dietary zinc deficiency is not the cause of poor breast feather growth. However it is possible that if the capillary network or blood flow to the feather follicles is impaired, because of pressure on the breast muscle and skin, then zinc could be deficient in this specific area resulting in poor feather growth (see Chapter 6). Zinc deficiency results in brittle feathers and a comparison of the strength of feathers from the breast area would give an indication of the adequacy of zinc for growth of these feathers. Feather strength was not measured in this study.

The availability of zinc was estimated from the zinc and TiO_2 concentrations in the feed and excreta. No attempt was made to measure the endogenous mineral losses in the excreta as Weigand and Kirchgessner (1979) suggested that endogenous mineral losses were likely to be small in comparison with net retention. The zinc availability from the 3 diets was found to be similar and this reflected the increase in excreta zinc with increasing dietary zinc concentration. In the present study the average zinc availability of the 3 diets was 0.71. Zinc oxide is less available than other forms of zinc such as zinc sulphate or zinc-methionine (Wedekind *et al.*, 1992). Bioavailability estimates of zinc also depend on the diets used and the chelating effects of the feedstuffs (Underwood, 1981). Uptake of zinc might not differ if the turkey is absorbing only the zinc it requires or because of some factor limiting the uptake. Addition of higher dietary zinc concentrations would therefore have little effect on improving breast feathering.

Feather growth on the breast region was not improved by the addition of up to 200mg zinc/kg feed. It is unlikely that the NRC recommended dietary zinc requirements are too low. It is however possible that zinc availability to the feather follicles of the breast region is impaired. Feather strength measurements should be conducted to confirm that the structure of the breast feathers in the modern turkey is not impaired.

APPENDIX 2: Moulting Patterns

A2.1 Introduction

During the rearing period the feathering of turkeys changes appearance several times because of the replacement of feathers (Lucas and Stettenheim, 1972). Feathers change in shape, length and weight as they develop and mature before the feather is lost and the next feather generation repeats the development process. At any one time a bird may have feathers derived from more than one generation of feathers and the feathers present will be at varying stages of development (Lucas and Stettenheim, 1972). The moulting process therefore complicates the study of feather growth. A description of the moulting pattern of the chicken was given by Lucas and Stettenheim (1972) but no details of the moulting pattern of the turkey could be found. It is possible that the moulting pattern of the turkey might differ to that of the chicken. Factors that affect the progress of moult will alter the appearance (shape, length, weight) of feathers at any one age. The stage of moult should be considered when examining feather growth.

The aim of this field study was to record the stage of growth of feathers from the wing (primary remiges), tail (rectrices), breast (sternal tract), back (dorsopelvic tract) and thigh (femoral tract) of modern turkeys throughout the rearing period in order to obtain a description of the progress of moult.

A2.2 Methods

The study was carried out on BUT farms in Cheshire. Male and female BUT Big 5 turkeys were examined in flocks ranging in age from 2 to 24 weeks. Ten turkeys of each sex were selected at random from a flock at each age (week). The primary remiges (1-10) of the right wing were examined individually and scored 0-4 depending on the stage of development of the feather (Table A2.1). The rectrices (1-9) on the right side of the tail were individually scored using the same method. The feather tracts of the right breast, back and right thigh were examined and scored according to the stage of development of the majority of the feathers in a line down the centre of the tract.

Table A2.1 Scoring criteria for stage of feather growth.

Score	Description of scoring criteria
0	No feather present in follicle.
1	Early immature feather (pin feather). Feather tightly furled inside the sheath, conical shape with a blunt tip.
2	Mid immature feather. Feather starting to emerge from sheath, pulp (seen as a pink area) present in ensheathed portion.
3	Late immature feather. Feather lengthening and emerged half way from the sheath with pulp receding.
4	Mature feather. Feather vanes entirely free of sheath and pulp no longer visible.

A2.3 Results

The scores of the stage of development of the primary remiges, rectrices and feathers in the breast, back and thigh tracts were used to describe the pattern of growth and moult in these areas. From each area the score occurring most frequently in the 10 birds examined was plotted. Figures A2.1, A2.2 and A2.3 respectively show the feather score in the primary remiges, rectrices and tracts of the male (a) and female (b) birds at weekly intervals from 2-24 weeks.

The primary remiges showed a distinct pattern of development and moult with little difference between males and females. Each primary was found to develop at a different time so that the pattern of development was staggered. At two weeks of age the innermost primary (primary 1) was mature and the stage of maturity of the remaining 9 primaries decreased towards the tip of the wing with primary 10 missing. This pattern of growth continued with the first primary the centre of development and the wave of maturation and subsequent moults originating from this follicle and moving out towards the wing tip (primary 10). Two generations of mature feathers were observed between 2 and 24 weeks of age (Figure A2.1).

The primary rectrices also showed a distinct pattern of development and moult with little difference between males and females. The individual rectrices, like the remiges, each developed at a different time so that the pattern of development was staggered. The pattern of growth originated from the centre of the tail with the first rectrix the centre of development and the wave of maturation and subsequent moults

originating from this follicle and moving out towards rectrix 9. Three generations of mature feathers were observed between 2 and 24 weeks of age (Figure A2.2).

Two generations of mature feathers were observed in the breast tract between 2 and 24 weeks of age. From 2 to 5 weeks the first generation of feathers were immature but reached maturity by 6 weeks of age. The next moult occurred between 9 and 10 weeks with the second observed generation of feathers maturing by 15 weeks of age (Figure A2.3). The pattern of feather development in the back and thigh tracts showed differences between males and females in the number of generations of mature feathers. Females were found to have more generations of mature feathers than males between 2 and 24 weeks of age (Figure A2.3).

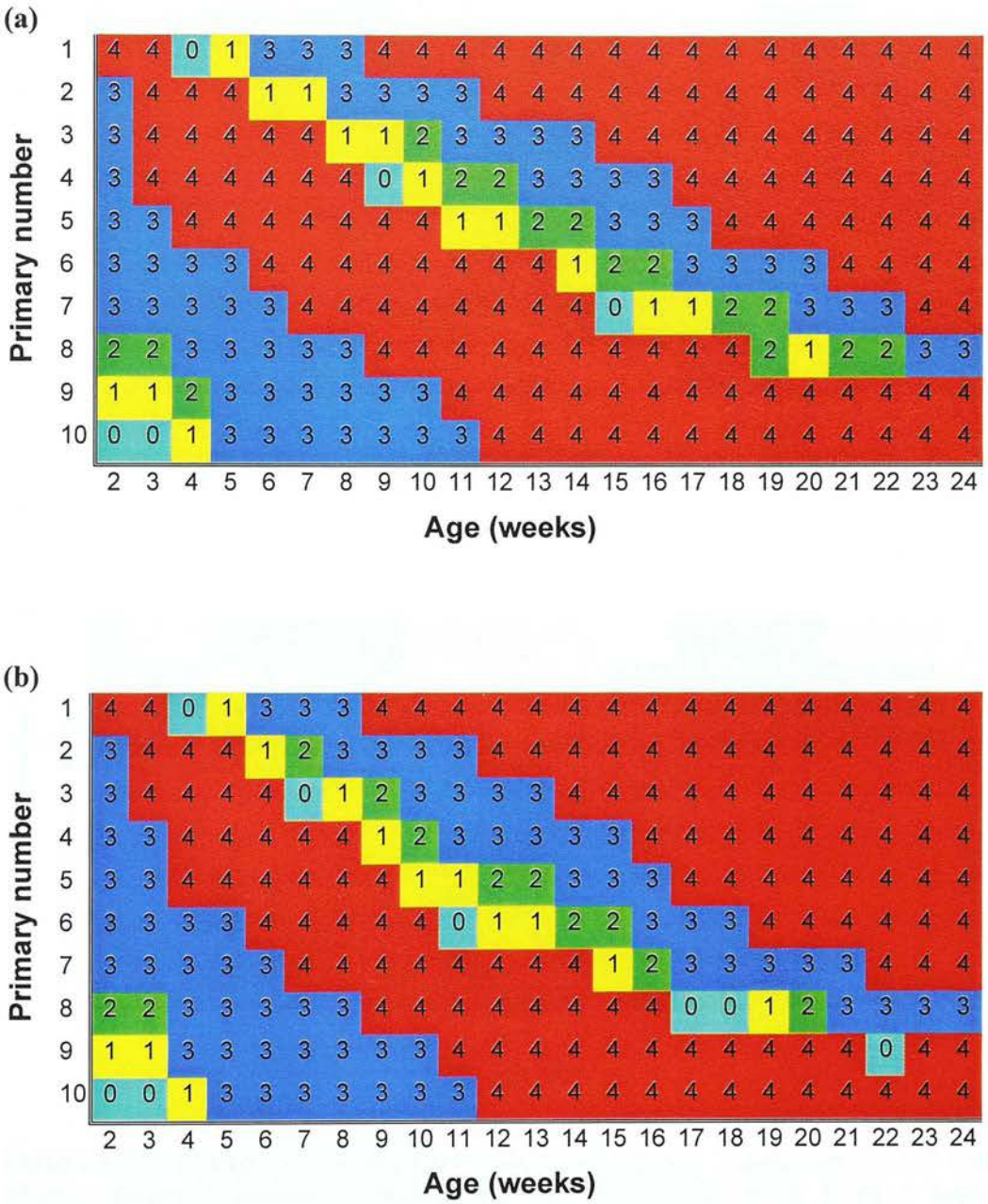

Figure A2.1 Pattern of feather growth and development, weekly from 2-24 weeks of age, of the 10 individual primary remiges in male (a) and female (b) BUT Big 5 turkeys. Numbers relate to the stage of development scores described in Table A2.1.

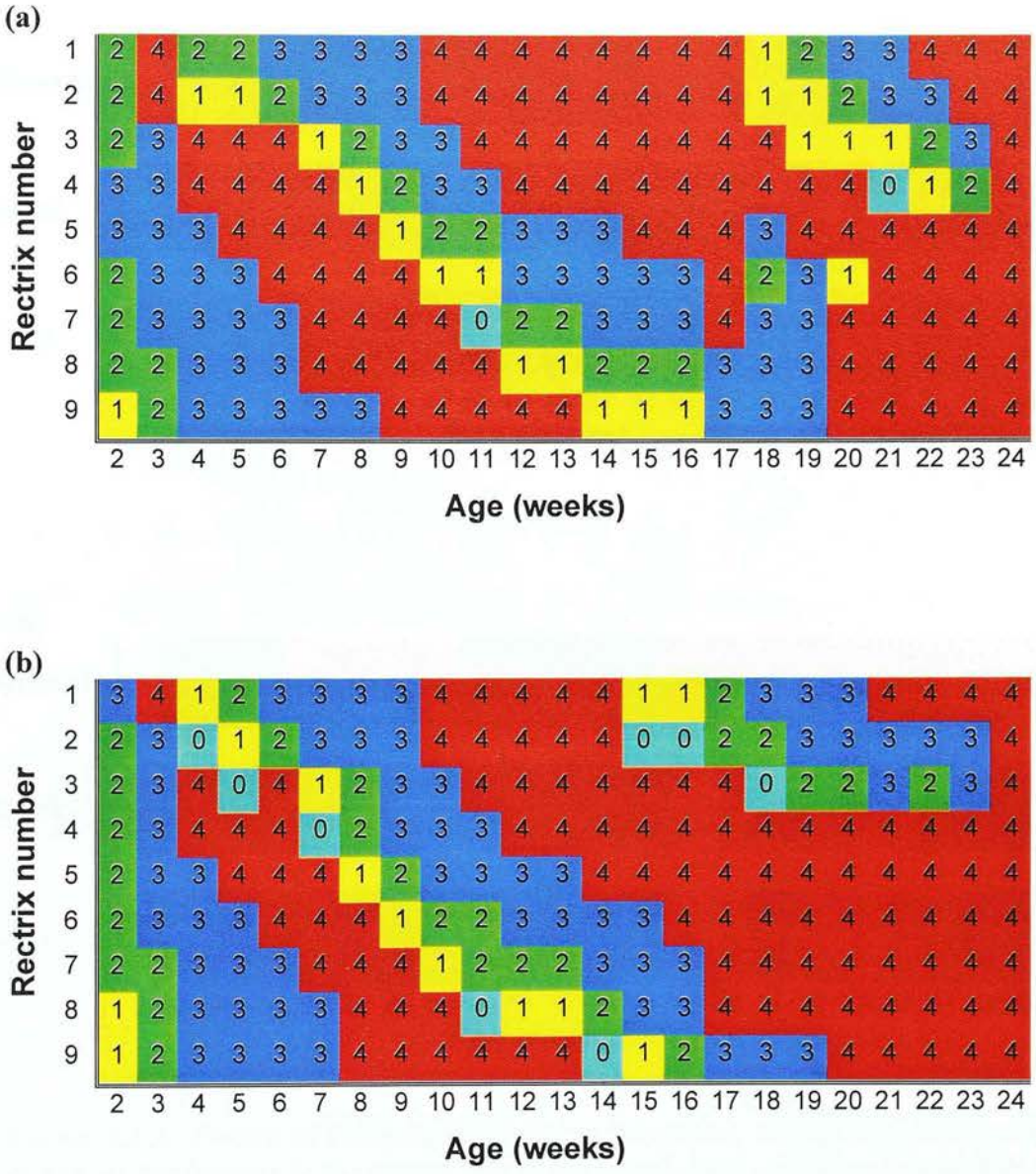

Figure A2.2 Pattern of feather growth and development, weekly from 2-24 weeks of age, of the 9 individual rectrices in male (a) and female (b) BUT Big 5 turkeys. Numbers relate to the stage of development scores described in Table A2.1.

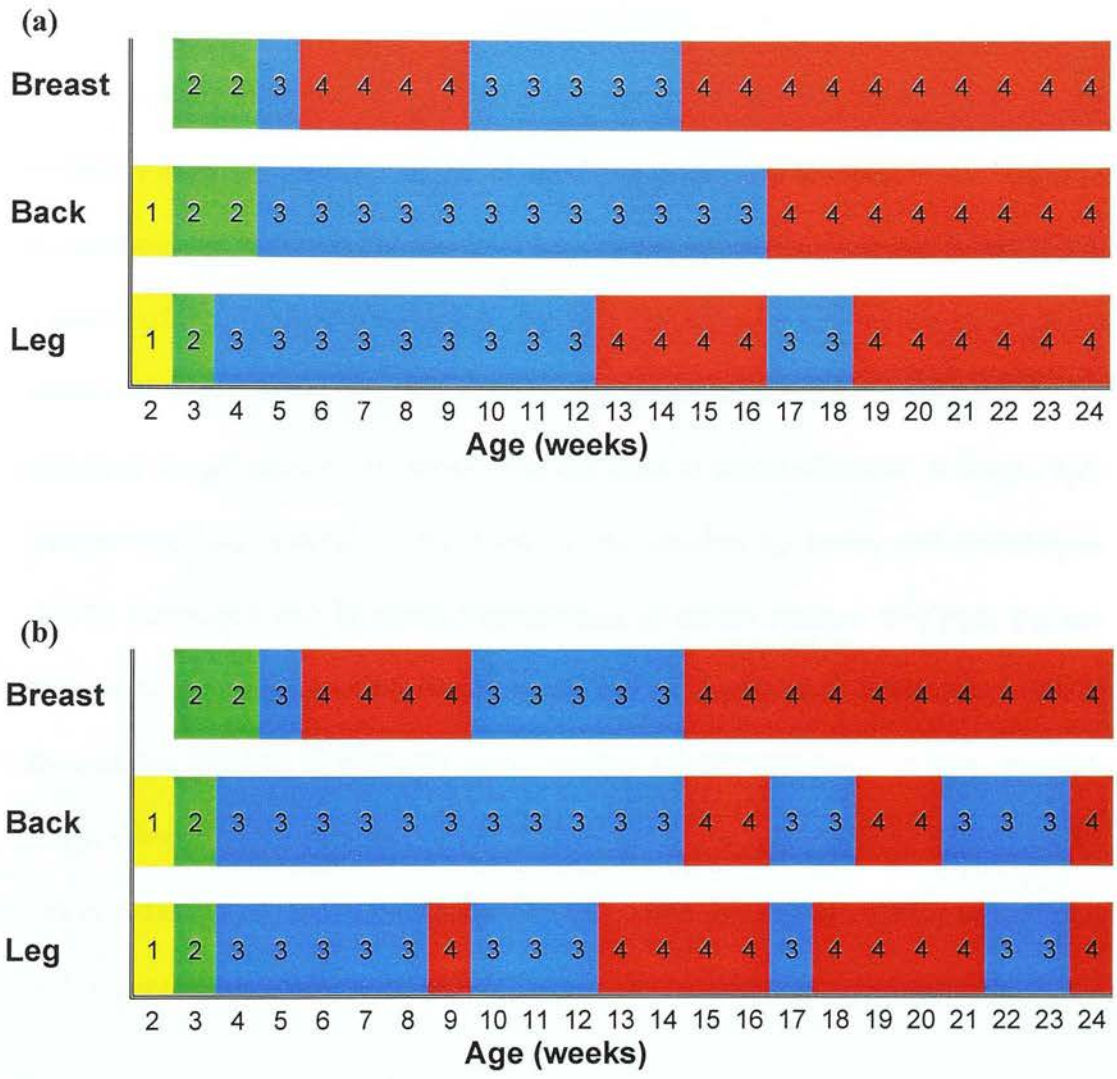

Figure A2.3 Pattern of feather growth and development, weekly from 2-24 weeks of age, of the feathers in the central line of the breast, back and leg tracts in male (a) and female (b) BUT Big 5 turkeys. Numbers relate to the stage of development scores described in Table A2.1.

A2.4 Discussion

The aim of the study was to provide a description of the progress of moult in the modern turkey. Successive stages of moulting were clearly seen in the primary remiges and the rectrices. The scores of development presented were the scores of the majority of the 10 birds examined at each age. However, in each of the 10 birds the pattern of development was found to be similar with only small variations in the timing of the progress of the moult. The direction of moult observed in the remiges and rectrices was similar to that found in the chicken by Lucas and Stettenheim (1972). Between 2 and 24 weeks 2 generations of mature remiges and 3 generations of mature rectrices were observed in the turkey. Lucas and Stettenheim (1972) showed the chicken to have the same number of generations of mature feathers. When comparing the progress of moult observed in the remiges and rectrices of the turkey with those found in the chicken by Lucas and Stettenheim (1972) the patterns and time of moult appear to be similar.

While the progress of moult was clear in the remiges and rectrices it was more difficult to follow in the body tracts. Several stages of feather development could be observed in each tract but development scores were allocated according to the stage of development of the majority of feathers in the centre of each tract. Feather growth has been shown to commence in the centre of each tract spreading to other follicles around it (Voitkevich, 1966). This method of scoring did not provide such an accurate pattern of moult for the back and thigh tracts as was obtained for the remiges and rectrices. The feathers in the body tracts were smaller making an

accurate assessment of the stage of development more difficult. The smaller feathers also appeared to mature more rapidly and examination at weekly intervals could have resulted in some of the developmental stages being missed. These difficulties could explain the apparent differences between the males and females in the number of generations of mature feathers observed in the back and thigh tracts. Similar difficulties were found when examining the breast tract but this tract was smaller than the other body tracts and showed less variation in the stage of development of the feathers within the tract. The pattern of development obtained for the breast tract in the turkey was similar to that shown for the chicken by Lucas and Stettenheim (1972) with the two generations of feathers maturing at the same ages in both species. The first generation of breast feathers was recorded as mature at 6 weeks of age and this was the age used for recording feather growth in the experiments presented in this thesis. Moulting patterns of the traditional Nebraska Spot turkey were not examined but the results of the comparative study presented earlier indicate that moulting patterns of the modern and traditional turkeys might be similar (see Chapter 3).

During the rearing period the remiges and rectrices undergo a continuous cycle of feather development and renewal. The description of the progress of moult in the modern turkey shows how the generations of feathers mature between 2 and 24 weeks of age.

APPENDIX 3:

Feather Cover in Selected and Rejected Turkeys

A3.1 Introduction

The modern turkey has been selected for breast muscle yield and part of the selection procedure has involved a visual assessment of the conformation of the breast. Selectors have been looking for a large broad muscle and appropriate muscle shape as well as increased body weight. Poor breast feathering may be a direct result of the large breast muscle, increased pressure and capillary damage as discussed in Chapter 6. Alternatively it is possible that selection for muscle conformation has been accompanied by selection for poor breast feathering. Poor breast feathering may make the breast size and conformation easier to assess and the selectors may have been inadvertently selecting for poor breast feathering.

The aim of this field study was to observe turkeys at selection and make a qualitative score of the breast feathering in birds that were selected and those that were rejected as breeding birds.

A3.2 Methods

The study was carried out on a BUT farm in Cheshire at the time of a 14 week selection of breeding birds. Male Big 5 turkeys were given a score from 0 to 5 for breast feather cover according to the criteria in Table A3.1 and a measure was made,

using a ruler, of the width of the bare area of skin between the feather tracts half way along the keel bone. The scoring was carried out immediately before the birds were passed to the selectors and then a record was made as to whether the bird was subsequently selected or rejected.

Table A3.1 Scoring criteria for breast feathering at selection.

Score	Description of scoring criteria
0	No feather growth on the cranial half of the sternal tracts. Cranial skin of breast completely exposed.
1	Cranial skin of breast mainly exposed with only short pin feathers present on the cranial half of sternal tracts.
2	Cranial skin of breast mainly exposed with sparse covering of more developed feathers on cranial half of sternal tracts.
3	Cranial skin of sternal tract with largely complete feather cover, though skin can still be see through the feathers.
4	Feathers on cranial half of sternal tract appear to be the same length and shape as the feathers on the caudal half of the sternal tract. Only the apteria is not covered completely. No exposed area of skin is present on the sternal tracts.
5	Full breast feathering. Complete covering of feathers. No skin is exposed. As seen in the Nebraska Spot.

Analysis of variance was used to test for differences in the feather scores and exposed skin measures between selected and rejected turkeys.

A3.3 Results

The feather scoring showed that selected turkeys had a significantly ($P<0.001$) lower mean feather score than the birds that were rejected at the 14 week selection (Figure A3.1). The mean measurement of the exposed area of skin between the sternal feather tracts was 9.9cm for selected and 9.1cm for rejected turkeys (SED 0.46). This difference was not found to be significant.

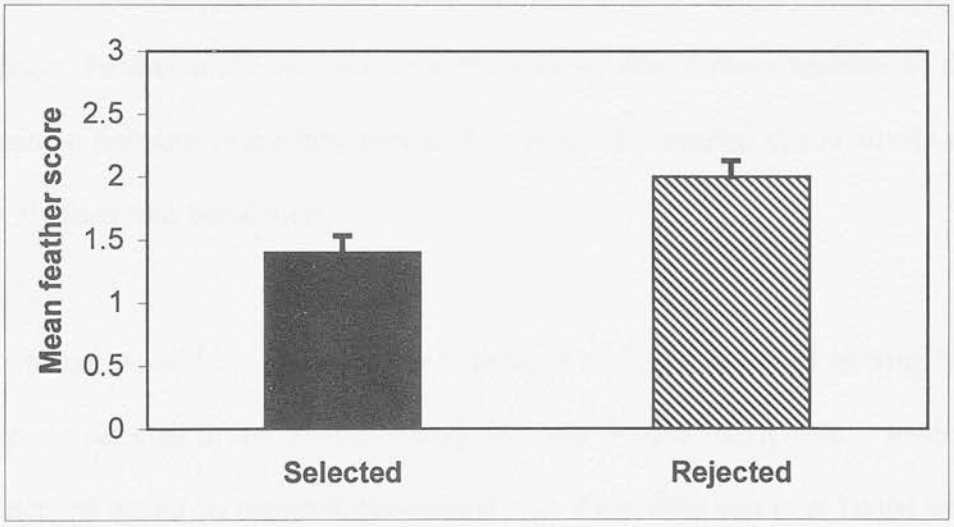

Figure A3.1 Mean (± SED) feather score (0-5) of male Big 5 BUT turkeys at 14 week selection.

A3.4 Discussion

Differences were found in the breast feather scoring of the selected and rejected turkeys. However it was not possible to say whether the poor feather score of the selected birds was a result of selection of birds with poor feathering, or if the poor

feathering was a consequence of the long term selection for breast muscle size and good conformation. In order to distinguish true selection for muscle conformation it would be necessary to compare the breast muscle yield in selected and rejected turkeys as well as feather score. This was not possible because the birds scored were being selected as commercial breeding turkeys.

It was interesting that the difference in feather score between the two groups was small with feathering being poor in both groups. This was reflected in the similar measurements of the width of the exposed area of skin in the selected and rejected turkeys. Feather score may not be extreme in modern turkeys because of the high selection pressure over a long period of time that has resulted in a relatively uniform poorly feathered population.

It was not possible to disregard the hypothesis that poor breast feathering has been actively selected in the modern turkey. A more detailed study across several flock selections would be required. However it does seem clear that poor breast feathering could in some way be linked to the selection process.

APPENDIX 4: Published Papers

The following papers relating to experiments described in this thesis have been published.

Wylie, L. M. and Hocking, P. M. (1997). Effect of dietary zinc on breast feathering in growing turkeys. British Poultry Science, 38: S42-S43.

Wylie, L. M. and Hocking, P. M. (1998). Comparative study of feathering in modern and traditional turkeys. British Poultry Science, 39: S20-S21.

Wylie, L. M. and Hocking, P. M. (1998). Effect of temperature and restricted feeding on feathering in turkeys. In: *Proceedings of the 10th European Poultry Conference, World's Poultry Science Association Israel Branch*, Jerusalem, Israel 21-26 June 1998.

Wylie, L. M. and Hocking, P. M. (1999). Effect of lowering dietary protein concentration on feathering in modern and traditional turkeys. In: *Proceedings of the World's Poultry Science Association United Kingdom Branch Spring Meeting*, Scarborough, 24-25 March 1999.

Wylie, L. M. and Hocking, P. M. (1999). Investigating feather growth as a means of preventing breast lesions in turkeys. In: *Proceedings of the 22nd Technical Turkey Conference,* Cheshire, England, 14-16 April 1999.

Effect of dietary zinc on breast feathering in growing turkeys

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Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) cause depressed welfare and are an important source of economic loss in market turkeys through downgrading and condemnation of carcasses. Such lesions are a common problem in modern turkeys and feathers may offer some protection to the breast from possible instigating causes of these conditions. Observations by Hocking (1995) suggest that feather growth in modern compared with traditional strains is poor and feathers may be absent over the breast region.

A zinc deficiency is known to result in brittle feathers and poor feather growth in chickens (Rahman *et al.* 1961) and quail (Fox and Harrison 1964). Poor feather growth on the breast of turkeys may result from a zinc deficiency. This study compares feather growth in birds with increased zinc concentrations in the diet.

Three turkey diets were prepared and titanium dioxide (TiO_2) added as an indigestible marker. Diet 1 contained 100 mg/kg zinc, diet 2 twice this amount (200 mg/kg) and diet 3 three times (300 mg/kg). Diet samples were taken for analysis of their zinc contents. The diets were allocated to pens using a randomised block design. 120 day-old poults (BUT Big5 male line) were randomly allocated among twelve pens. Birds were weighed each week for eight weeks and the food intake recorded. At four weeks of age, four birds per pen were selected randomly and the lengths and

widths of the sternal feather tracts were measured. The length of one feather from the top and bottom of each of the two tracts was measured (4 feathers per bird). These measurements were repeated on the same birds from each pen at eight weeks of age. Droppings and blood samples from one bird per pen were collected at 4 and 8 weeks for analysis of their zinc contents.

	Diet 1	Diet 2	Diet 3	SED
Zinc concentration	100 mg/kg	200 mg/kg	300 mg/kg	
Mean sternal tract length 4 weeks	12.0	11.6	11.8	0.481
Mean sternal tract length 8 weeks	15.9	15.8	15.6	0.481
Mean sternal tract width 4 weeks	1.4	1.4	1.4	0.206
Mean sternal tract width 8 weeks	3.2	3.1	3.0	0.206
Mean cranial feather length 4 weeks	2.2	2.1	2.1	0.311
Mean cranial feather length 8 weeks	2.7	2.6	2.9	0.311
Mean cranial width length 4 weeks	2.5	2.5	2.5	0.162
Mean cranial width length 8 weeks	4.3	4.3	4.4	0.162

The size of the sternal tract and lengths of the feathers did not differ with increasing concentration of zinc in the diet (Table). No differences in plasma zinc were found. Feather cover on the breast of turkeys was not improved by increasing the zinc concentration in the diet from 100 mg/kg to 300 mg/kg.

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Comparative study of feathering in modern and traditional turkeys

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Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) cause depressed welfare and are an important source of economic loss in market turkeys through downgrading and condemnation of carcasses. Such lesions are a common problem in modern turkeys and feathers may offer some protection to the breast from possible instigating causes of these conditions. Observations by Hocking (1995) suggest that feather growth in modern compared with traditional strains is poor and feathers may be absent over the breast region. This study quantifies and compares feather growth in a modern commercial turkey (B.U.T. Big5 Male Line) with that in an unrelated traditional line (Nebraska Spot) and investigates the lack of breast feathers in the large modern bird.

Forty-eight day-old poults of each strain, 24 males and 24 females, were allocated to 12 pens using a randomised block design. Birds were fed on a standard turkey starter diet until 4 weeks of age and then on a standard turkey grower diet. Birds were weighed each week and the food intake recorded. After 2, 4, 6, 8, 10, 15, 20 and 25 weeks, 3 birds of each strain and sex (one bird from each pen), selected at random at the start of the trial, were removed from the experiment and killed. The length and width of the feather tracts on the breast and back were measured. The lengths of four individual back feathers and one feather from the cranial and caudal region of each breast tract (4 breast feathers), were recorded. The two longest feathers on the tail and on each wing were measured. Feather follicle density on the breast tract was measured by counting the individual follicles within a fixed area ($4cm^2$). The birds were weighed before and after plucking to determine the weight of the feathers. After plucking, the left breast muscle was removed and weighed. The data were transformed to natural logarithms before analysis of a linear model with effects for age, strain, sex and their interactions.

Modern B.U.T. turkeys are significantly larger than the traditional Nebraska Spot, with B.U.T. birds weighing 2.5 times the body weight of the Nebraska Spot. There is, however, no significant difference in feather weight between the two lines (Table). Feather lengths from the wing, tail, back (data not given) and the caudal region of the breast are not significantly different (Table). The feathers from the cranial region of the breast tract are of different lengths in the two lines, with the modern B.U.T. turkey having significantly shorter feathers in this area than the traditional Nebraska Spot (Table). The density of feather follicles on the breast tract is significantly higher in the traditional turkey.

The results suggest that feather weight has not increased with the increased body weight of the modern turkey. The lower follicle density on the breast tracts of the modern turkey may be caused by the breast skin stretching to cover the much larger area of muscle without an increase in the total number of feather follicles. The growth of breast feathers, from the cranial region of the breast tract, appears to be impaired in the modern turkey.

Table. Feathering in Modern and Traditional turkeys (means 2-25 weeks)

	Modern	Traditional	S.E.D.	Significance
Body Weight ln (g)	8.2 (*3641*)	7.3 (*1480*)	0.03	***
Feather Weight ln (g)	4.7 (*110*)	4.6 (*100*)	0.05	NS
Feather / Body Weight %	3.0	6.8	-	-
Follicle Number ln (n)	2.4 (*11*)	3.1 (*22*)	0.03	***
Muscle Weight ln (g)	5.9 (*365*)	4.8 (*122*)	0.04	***
Back Tract Length ln (mm)	5.6 (*276*)	5.4 (*217*)	0.01	***
Breast Tract Length ln (mm)	5.0 (*146*)	4.9 (*129*)	0.01	***
Breast Tract Width ln (mm)	3.3 (*27*)	3.2 (*25*)	0.03	**
Cranial Breast Feather Length ln (mm)	3.3 (*27*)	3.6 (*37*)	0.05	***
Caudal Breast Feather Length ln (mm)	3.8 (*45*)	3.7 (*41*)	0.03	NS

This work was funded by MAFF and B.U.T.

Hocking, P.M. (1995) Defective growth of breast feathers in modern turkeys. *British Poultry Science* **36**:845.

Effect of Temperature and Restricted Feeding on Feathering in Turkeys

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Observations by Hocking (1995) suggest that feather growth in modern compared with traditional strains of turkey is poor and feathers may be absent over the breast region. Rapidly growing modern turkeys produce large quantities of body heat that they must lose in order to maintain constant body temperature. Reduced feathering may be an adaptive response to practical ambient temperatures.

Feather growth was compared in birds kept at high (26oC) and low (15oC) ambient temperatures with feeding either restricted, to reduce growth rate, or ad libitum. At 2 weeks of age 100 male turkeys (B.U.T. Big 5 Male Line) were randomly allocated to individual cages kept at 15oC or 26oC and provided with food ad libitum, or restricted to a degree which maintained body weight at 50% of the ad libitum fed birds. Feather and feather tract measurements were recorded at 6 weeks of age.

Restricting the growth of the birds to 50% of ad libitum decreased the length of feathers by 25% but the growth of the breast feathers was unaffected. The results suggest that the growth of breast feathers in ad libitum fed male turkeys is impaired.

Reference

Hocking, P. M. (1995) Defective growth of breast feathers in modern turkeys. British Poultry Science 36: 845.

Effect of lowering dietary protein concentration on feathering in modern and traditional turkeys

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Observations by Hocking (1995) suggest that feather growth in modern compared with traditional strains of turkey is poor and feathers may be absent over the breast region. The protein requirement for feather growth is high and comparable to that for breast muscle, suggesting that competition for protein could be the cause of defective feather growth in the rapidly growing modern turkey. The aim of this experiment was to investigate the effect of feeding a low protein diet to a modern commercial turkey (B.U.T. Big5 Male Line) and an unrelated traditional turkey (Nebraska Spot). Feather and muscle growth were compared in birds fed on diets with decreasing protein concentration to test the hypothesis that body growth, and in particular breast muscle growth, are maintained at the expense of feather growth when protein concentrations in the diet are deficient.

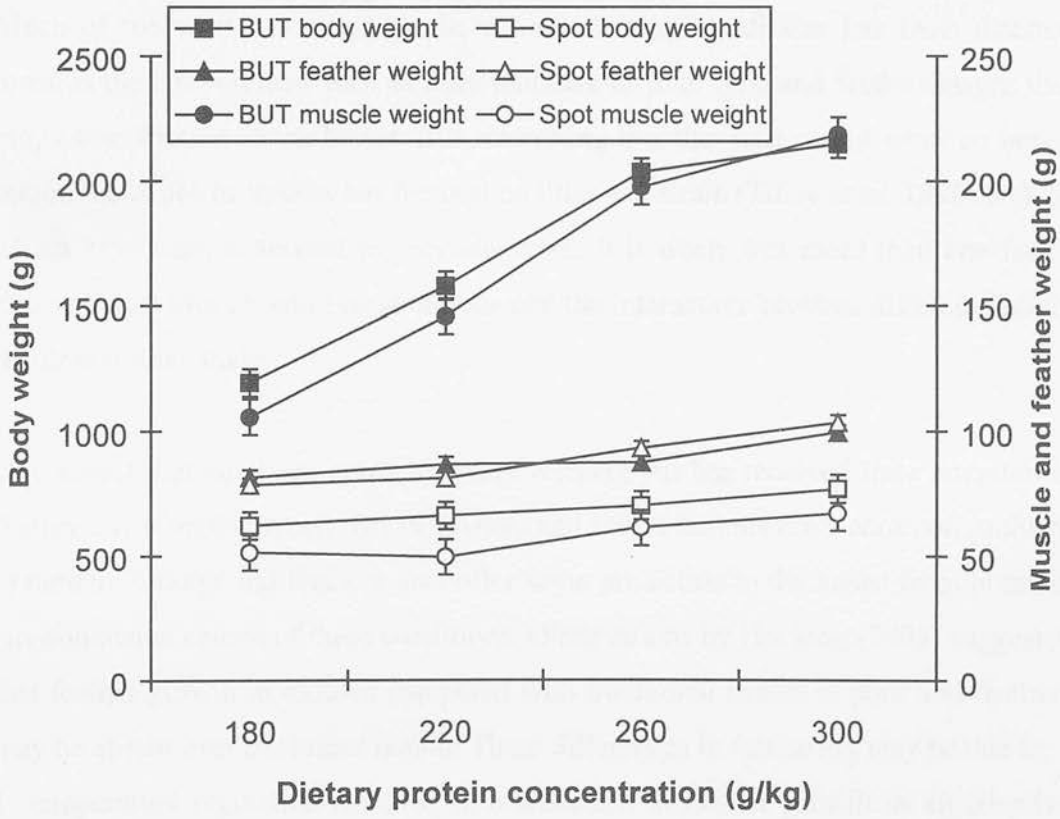
Sixty-four day-old poults of each strain, 32 males and 32 females, were allocated to 32 pens using a randomised block design. Birds were fed on a standard turkey starter diet until one week of age. Four experimental diets were formulated, using the same ingredients, to obtain crude protein concentrations of 180, 220, 260 and 300g/kg. The metabolisable energy concentration (ME) of each diet was formulated at 12.0 MJ/kg. Each diet was randomly allocated to two pens of each strain and sex and birds had *ad libitum* access to the diet between one and 6 weeks of age. Food intake per pen was recorded weekly. At 6 weeks of age four birds were removed from each pen and killed. The lengths of four individual back feathers and one feather from the cranial and caudal region of each breast tract (4 breast feathers) were recorded. The two longest feathers on the tail and on each wing were measured. The birds were weighed before and after plucking to determine the weight of the feathers. After plucking, the left breast muscle was removed and weighed. The data were compared by analysis of variance with effects for sex, strain, protein concentration and their interactions.

A significant ($P<0.001$) interaction between strain and dietary protein concentration was observed for body weight and breast muscle weight. Lowering the dietary protein concentration from 300 g/kg to 180 g/kg reduced the body weight of the modern turkey by 44% compared with a reduction of 20% in the traditional turkey (Figure). The same decrease in dietary protein concentration resulted in the breast muscle weight of the modern turkey being reduced by 52% compared with 24% in the traditional bird (Figure). A significant ($P<0.05$) interaction between strain and protein concentration showed that feather weight was reduced by 19% in the modern turkey and by 25% in the traditional turkey (Figure). Feather lengths from the back, tail, wing and caudal region of the breast were significantly ($P<0.001$) shorter when protein concentration was reduced,

but there was no interaction with strain. The feathers from the cranial region of the breast also showed a significant ($P<0.001$) strain by dietary protein concentration interaction. In the traditional turkey the cranial breast feathers decreased in length from 26mm to 19mm with reduced dietary protein, however, in the modern turkey these feathers were increased in length from 14mm to 25mm (SED 1.0).

The results suggest that, in the modern turkey, protein is preferentially partitioned to feather growth over muscle growth. When crude protein concentrations in the diet of the modern turkey are deficient, feather growth is maintained at the expense of body growth, and in particular breast muscle growth. Improved growth of breast feathers from the cranial region of the breast tract was achieved by lowering the dietary protein concentration.

Figure. Effect of strain and dietary protein concentration on body weight, breast muscle weight and feather weight.



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Investigating feather growth as a means of preventing breast lesions in turkeys

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Introduction

Sternal Bursitis (breast blisters) and Focal Ulcerative Dermatitis (breast buttons) cause depressed welfare and are an important source of economic loss in market turkeys through downgrading and condemnation of carcasses. Although much work has been carried out to examine the causes of breast blisters and breast buttons in both chickens and turkeys little progress has been made in reducing the incidence of these conditions.

Much of the work investigating the causes of these conditions has been directed towards the environment such as litter moisture or litter type and feeder designs that may cause friction to the breast. It is interesting that the most recent work on breast button incidence in turkeys has focused on litter and strain (Tilley *et al.* 1996) both of which have been examined in previous work. It is likely that more than one factor causes breast blisters and breast buttons and the interaction between different factors requires further study.

One aspect that has been noted by many workers but has received little attention is feather cover on the breast. Breast blisters and breast buttons are a common problem in modern turkeys and feathers may offer some protection to the breast from possible environmental causes of these conditions. Observations by Hocking (1995) suggested that feather growth in modern compared with traditional strains is poor and feathers may be absent over the breast region. These differences in feathering may be due to:

1. temperature regulation resulting in a reduction in feather growth as an adaptive response by the birds to the increased heat production resulting from their fast growth rates;
2. competition between muscle and feather growth for essential nutrients such as amino acids or minerals;

3. selection for increased breast muscle in the modern turkey resulting in poor feather cover as the skin is stretched to cover a larger area without an increase in the number of feathers.

By investigating the causes of poor breast feathering of turkeys it may be possible to improve feathering in the modern bird and to reduce the incidence of breast blisters and breast buttons.

Feather growth comparison

A comparative study of feathering in modern and traditional turkeys was carried out from hatch to 25 weeks of age. The aim of the study was to quantify and compare feather growth in a modern commercial turkey strain (B.U.T. Big5 Male Line) with that in an unrelated traditional line (Nebraska Spot), with special reference to the lack of breast feathers in the large modern bird.

Males and females of each turkey strain were killed at 2, 4, 6, 8, 10, 15, 20 and 25 weeks of age and measurements taken of the breast and back feather tracts and the breast, back, wing and tail feathers. The carcass was weighed before and after plucking to determine the weight of the feathers, and the density of feather follicles on the breast tracts were measured.

Although there was a large difference in body weight between the two turkey strains, with modern turkeys up to 2.5 times the body weight of the traditional turkeys, there was no significant difference in the weight of the feathers (Table 1). When feather weight was expressed as a percentage of the body weight the modern birds had 1.8% feathers compared with the traditional birds which had 4.2% feathers. Feather lengths from the wing, tail, back and the caudal region of the breast were not significantly different between lines. Another interesting result is that the feathers from the cranial (upper) region of the breast tract were significantly longer in the smaller traditional birds. It was also found that the density of feather follicles on the breast tract was significantly higher in the traditional turkey.

The results suggest that feather weight has not increased with the increased body weight of the modern turkey. The lower follicle density on the breast tracts of the modern turkey may be caused by the breast skin stretching to cover the much larger area of muscle without an increase in the total number of feather follicles. The growth of breast feathers from the cranial region of the breast tract appears to be impaired in the modern turkey.

Table 1. Feathering in Modern and Traditional turkeys (means 2-25 weeks)

	Modern	**Traditional**	**Significance**
Body Weight (g)	3641	1480	***
Feather Weight (g)	110	100	NS
Feather / Body Weight %	3.0	6.8	-
Follicle Number (n)	11	22	***
Muscle Weight (g)	365	122	***
Back Tract Length (mm)	276	217	***
Breast Tract Length (mm)	146	129	***
Breast Tract Width (mm)	27	25	**
Cranial Breast Feather Length (mm)	27	37	***
Caudal Breast Feather Length (mm)	45	41	NS

Temperature and restricted feeding

Rapidly growing modern turkeys produce large quantities of body heat that they must lose in order to maintain constant body temperature. An experiment was carried out to test the hypothesis that poor breast feathering is an adaptive response to practical ambient temperatures. Feather growth was compared in turkeys (B.U.T. Big 5 Male Line) kept at high (26°C) or low (15°C) ambient temperatures and fed *ad libitum* or restricted to a degree that maintained body weight at 50% of the *ad libitum* fed birds. Feather lengths, follicle density, feather weight and body weights were recorded at 6 weeks of age.

Temperature did not affect feather growth - birds kept at 15°C or 26°C showed no significant difference in feather lengths, feather follicle density or feather weight. Food restriction maintained body weight at 50% of the *ad libitum* fed birds and decreased the lengths of feathers from the wing, tail, back and caudal breast by 10% to 25% ($p<0.001$). However, food restriction increased the length of the cranial breast feathers by 20% ($p<0.001$). Feather follicle density was also significantly increased.

The results show that the two temperatures had no effect on feathering in 6-week old turkeys, however, restricting the growth of the birds to 50% of *ad libitum* decreased the lengths of all feathers except for the cranial breast feathers, which were increased in length. The results suggest that the growth of cranial breast feathers in *ad libitum* fed male turkeys is impaired.

Nutrition

Low protein

The modern turkey has been selected for increased breast muscle yield and rapid growth rates and it is likely that protein requirements for muscle growth are high. The protein requirement for feather growth is also high and competition for dietary protein could be the cause of defective feather growth in the rapidly growing modern turkey. An experiment was carried out to investigate the effect of feeding a low protein diet to a modern commercial turkey (B.U.T. Big 5 Male Line) and an unrelated traditional line (Nebraska Spot). Feather and muscle growth were compared in birds fed on diets with decreasing protein concentration to test the hypothesis that body growth, and in particular breast muscle growth, are maintained at the expense of feather growth when protein concentrations in the diet are deficient.

Modern and traditional turkeys were fed ad *libitum* on one of 4 experimental diets containing crude protein concentrations of 180, 220, 260 and 300g/kg. Feather lengths, follicle density, feather weight and body weights were recorded at 6 weeks of age.

Lowering the dietary protein concentration from 300 g/kg to 180 g/kg reduced the body weight of the modern turkey by 44% compared with a reduction of 20% in the traditional turkey (Figure 1). Feather weight was reduced by 19% in the modern turkey and by 25% in the traditional turkey (Figure 1). In the traditional turkey the cranial breast feathers decreased in length from 26mm to 19mm with reduced dietary protein, whereas in the modern turkey these feathers were increased from 14mm to 25mm (SED 1.0).

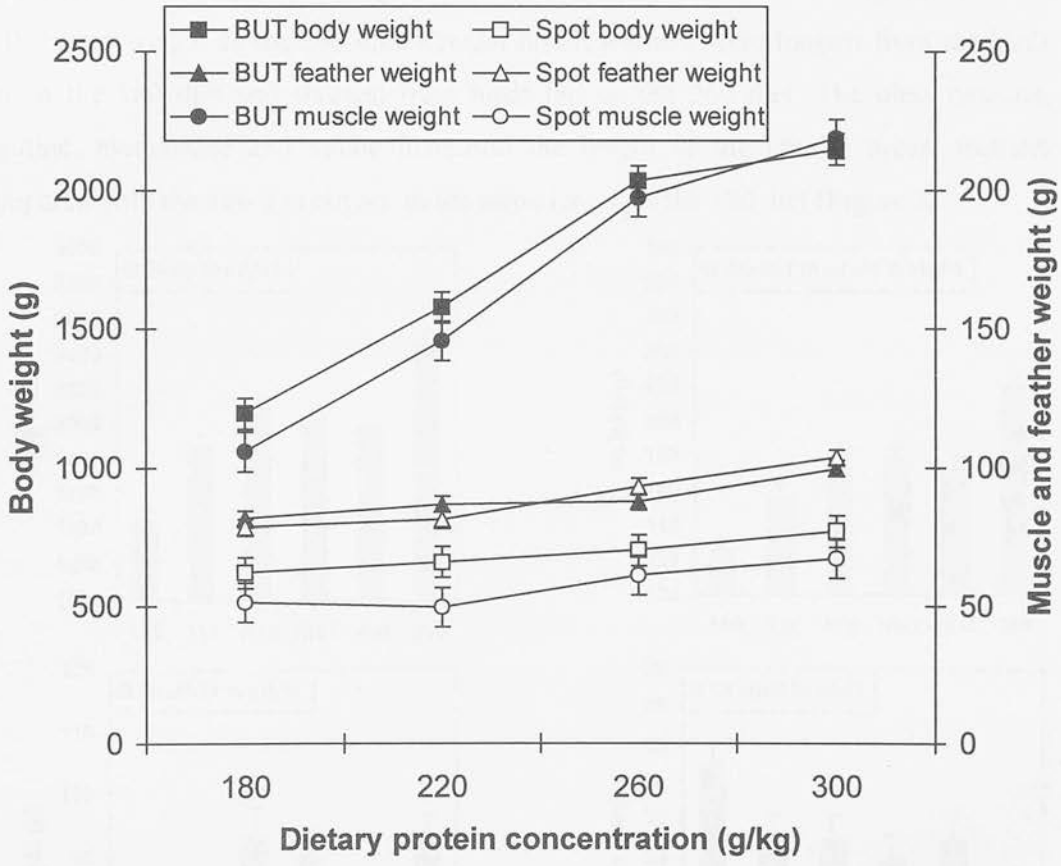

Figure 1. Effect of strain and dietary protein concentration on body weight, breast muscle weight and feather weight.

Amino acids

An experiment was carried out to investigate the effect on feather and body growth of increasing the dietary concentration of amino acids that are important for feather growth. The diet from the low protein experiment with a crude protein concentration of 180g/kg

was used as a basal diet. Individual amino acids (arginine, methionine, valine and tyrosine) were added to the basal diet to increase their concentration to the level found in the diet with a crude protein concentration of 260g/kg.

The diets supplemented with amino acids resulted in birds having a higher body weight and breast muscle weight than birds fed on the 180 diet but the body and muscle weights did not increase to those of the birds fed on the 260 diet (Figure 2). Increasing tyrosine resulted in a reduced feather weight compared with the 180 diet while valine had no effect on feather weight. Both arginine and methionine increased the weight of feathers to the same weight as the 260 diet. Cranial breast feathers were longest from the birds fed on the 180 diet and shortest from birds fed on the 260 diet. The diets tyrosine, arginine, methionine and valine increased the length of the cranial breast feathers compared with the 260 diet but not to the same length as the 180 diet (Figure 2).

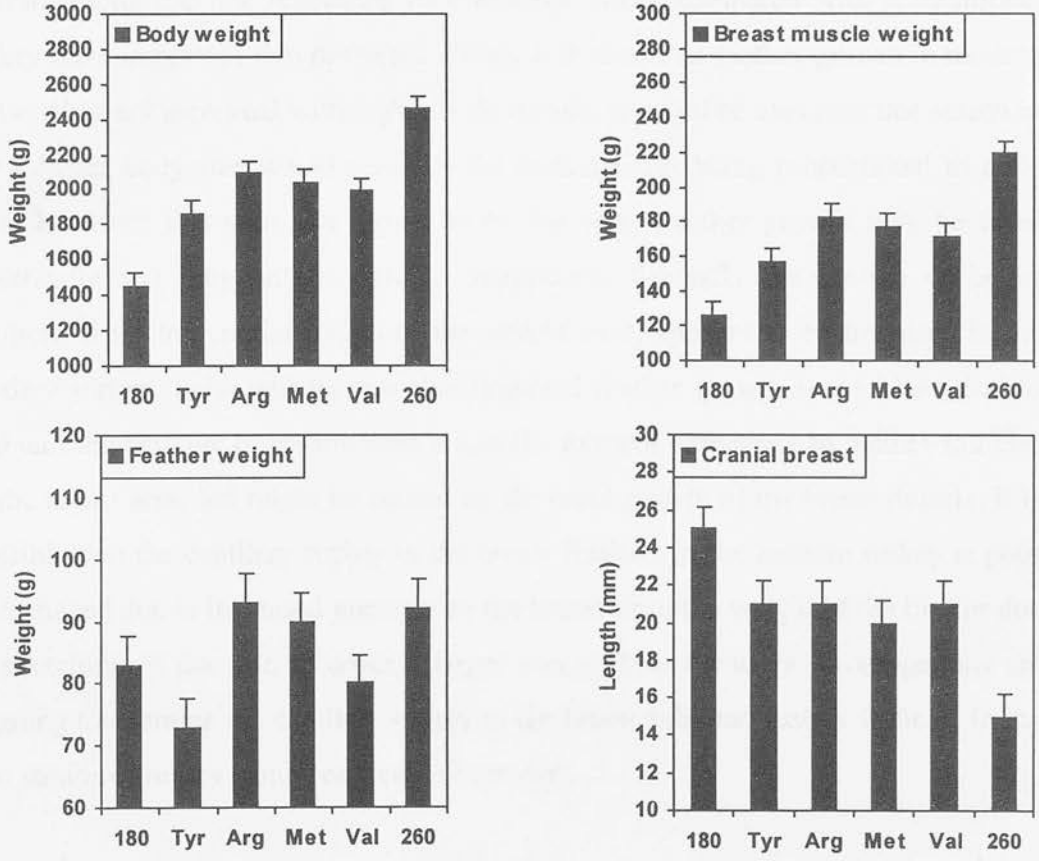

Figure 2. Effect of diet on mean (± SED) body, breast muscle and feather weights and cranial breast feather length.

The results of the two nutrition experiments suggest that, in the modern turkey, protein was preferentially partitioned to feather growth and that the amino acids arginine and methionine were used for increased feather growth in preference to muscle growth. When crude protein concentrations in the diet of the modern turkey are deficient, feather growth is maintained at the expense of body growth, and in particular breast muscle growth. Improved growth of breast feathers from the cranial region of the breast tract was achieved by lowering the dietary protein concentration. The impaired growth of cranial breast feathers would appear to be caused by the rapid breast muscle growth of the modern turkey and not because of a deficiency of specific amino acids.

Discussion

Investigations into the feathering of a modern turkey compared with a traditional turkey have suggested two problems. Firstly it is clear that feather growth in modern turkeys has not increased with higher body weight. It might be expected that selection for a larger body size would result in the feather cover being proportional to body size, however, this does not appear to be the case. Feather growth may be fixed genetically and may not be easy to manipulate. Secondly the growth of breast feathers, from the cranial region of the sternal tract, appears to be impaired in the modern turkey. It is unlikely that this impaired feather growth is an adaptation to ambient temperature or results from a specific nutrient deficiency to feather follicles in the breast area, but might be caused by the rapid growth of the breast muscle. It is possible that the capillary supply to the breast feathers in the modern turkey is poor or damaged due to increased pressure on the breast from the weight of the bird or due to stretching of the skin to cover a larger area of breast muscle. Investigations are ongoing to examine the capillary supply to the breast skin and feather follicles in the two strains of turkey using confocal microscopy.

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