SKIN BREAKING STRENGTH
IN BROILER CHICKENS

by

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DEDICATION

To , my beloved wife

A woman of valour who can find
For her price is far above rubies?

Proverbs 31:20

For you, who gave me the benefit of your love, your courage, and your support and for Ron and Itai -- thank you for teaching me the most important lesson of all:

Whatever you can do,
or dream you can, begin it.
Boldness has genius, power,
and magic in it.

-Goethe-

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When I first came to Blacksburg, I entered the office of Dr. Teekell, Dean of the Graduate School, and he asked me in which department I was to be a student. When I told him the Poultry Science Department, he smiled. "Then you're not just joining a department," he said, "you're becoming part of a family." In the three years since my arrival, I have indeed felt like a part of that family.

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INTRODUCTION

The skin serves as a barrier between higher organisms and the external environment, affording protection against mechanical damage and providing insulation for purposes of heat conservation. The skin is also the largest sensory organ of the body and contains receptors sensitive to temperature, pressure, and pain. Skin tissue is continually replaced and displays a remarkable regenerative ability.

The strength of the skin is of importance in poultry meat processing. Fragile skin results in an increased incidence of downgrades due to skin tears. Several approaches have been used in attempts to obtain maximal feather removal with a minimal occurrence of broken skin. Feather pulling force and feather release have been quantitated mechanically. The incidence of torn skin in relation to scalding temperatures and the design of mechanical pickers has been studied. The effects of various neuromimetic drugs, anesthetics, and tranquilizing drugs on feather release have been tested and, more recently, the effects of crude papain have been investigated.

Nutritional and other environmental effects on skin strength of poultry have been virtually ignored experimentally. The purpose of the research reported in this dissertation, therefore, was to examine factors of potential importance in contributing to skin strength.

Initially, a procedure to measure skin strength was developed and evaluated. This method was then used to examine the effects of calorie-protein ratios of the diet on skin breaking strength, and to investigate the relationships among skin composition, skin thickness, and skin breaking strength. Finally, the effect of supplemental ascorbic acid and heat stress on skin breaking strength was evaluated.

REVIEW OF LITERATURE

Skin composition and strength. According to Lucas and Stettenheim (1972b), chicken skin is composed of the epidermis, which contains a cornified layer of dead cells at the surface, the dermis, and the subcutis or hypodermis. The epidermis and dermis are largely composed of fairly densely arranged collagen fibers while the hypodermis, which is separated from the dermis by a thin layer of connective tissue called the elastic lamina, contains comparatively loosely arranged collagen fibers. The hypodermis, or subcutis, is separated from underlying muscle fibers by an elastic tissue called the deep fascia.

Although skin structure is well established, factors influencing skin fragility have not been extensively investigated. Numerous skin properties likely contribute to skin strength. Chicken skin, for example, contains substantial quantities of elastin. Elastin is composed of helically coiled polypeptide chains cross-linked by the amino acids desmosine and isodesmosine, which are synthesized from lysine in a reaction requiring copper. Copper deficiency results in defective cross-linking in both collagen and elastin, which in turn reduces tissue elasticity and eventually causes rupture of the fibrous proteins (Stryer, 1975; Underwood, 1977; Lehninger, 1978; Hunsaker et al., 1984).

Collagen is a principal component of all fibrous connective tissues, but collagen differs chemically and morphologically among various tissues. Collagen fibers in tendon, for example, are arranged in parallel bundles and, while very strong, have little capacity for stretching. In skin, collagen fibers form an interlocking network and, as a result, are comparatively elastic in nature.

Skin collagen is a high molecular weight protein composed of cross-stranded fibrils. The repeating subunits are strands of triple helix tropocollagen molecules arranged head to tail in a parallel but staggered manner (McGilvery, 1979). The strands are cross-linked by dehydrolysino-leucine. The three polypeptide chain coils within the tropocollagen triple helix are maintained by hydrogen bonding (Muench, 1982).

About one-third of the amino acids found in the collagen molecule are glycine. Collagen also contains alanine, proline, hydroxyproline, and hydroxylysine, the latter two amino acids being found in few other tissues. Hydroxyproline is synthesized from proline through hydroxylation mediated by a specific enzyme requiring ascorbic acid as a reducing agent (Blank and Peterkofsky, 1975). Other cofactors including iron (Kao et al., 1975), copper (Stryer, 1975), thiamine (Alvarez and Gilbreath, 1982), and oxygen (Uitto and Prockop, 1974) are also

required for collagen biosynthesis.

Substantial fat is stored within adipocytes in the hypodermis of the skin (Lucas and Stettenheim, 1972b). Fatty acids contained in skin tissues can be transported via plasma lipoprotein fractions to the skin, or can result from de novo synthesis within skin tissue (Yeh and Leveille, 1973; Nir and Lynn, 1978). The total lipid content of the skin of growing chicks varies between 20 and 40% (Yeh and Leveille, 1973; Bartov et al., 1974; Smith et al., 1977; Grey et al., 1983), is positively correlated with total carcass lipid, and is influenced by calorie-protein ratios of the diet (Bartov et al., 1974). The fat content of the skin differs between sexes and Bartov et al. (1974) suggested that increased dietary protein intake would minimize skin fat deposition.

In one of the few experiments in which differences in the composition of chicken skin were investigated, Smith et al. (1977) examined the influence of diet on collagen formation. No influence of protein-carbohydrate ratios, vitamin C, or iron on insoluble collagen concentrations or on collagen cross-linking was observed. Either supplemental ascorbic acid or increased dietary protein significantly increased total collagen concentrations, but no increase in total collagen occurred when diets containing both supplemental ascorbic acid and additional protein were fed.

A limited amount of data suggests that skin composition is influenced by sexual dimorphism. Smith et al. (1977) found a greater amount of soluble collagen in skin from females than in skin from males, but total collagen was greater in the skin of males. These authors suggested that higher levels of fat and corresponding lower levels of collagen in the skin of females could make their skin more susceptible to tearing during processing. In this regard, skin from females has been shown to contain more fat and less protein and collagen than skin from males in broiler chickens (Grey et al., 1983), goslings (Nitsan et al., 1981), and rats (Zika and Klein, 1971). Moreover, Edwards et al. (1973) observed more skin tears in female than in male broilers during processing.

Age differences in skin composition and skin strength may also occur. Edwards et al. (1973) reported that the incidence of torn skin was higher in broilers at nine weeks of age than at younger ages. Suderman et al. (1980), however, reported that skin protein remained fairly constant at different ages while other investigators (Nitsan et al., 1981; Grey et al., 1983) observed a general decline in skin protein concentrations with increasing age.

Ascorbic acid and heat stress. Although ascorbic acid, or vitamin C, is not considered to be nutritionally indispensable for domestic fowl, the physiological

utilization of this nutrient increases under stressful conditions (Hill and Garren, 1958; Thornton and Moreng, 1958; Nestor et al., 1972; Chatterjee, 1978; Bondi, 1982; Chaney, 1982; Pardue and Thaxton, 1984). As a result, it has been suggested that ascorbic acid may be an essential nutrient for domestic fowl subjected to stress (Pardue et al., 1983; Pardue and Thaxton, 1984). Data to support this contention, however, have been contradictory.

A number of investigators have examined the ability of supplemental ascorbic acid to alleviate the detrimental effects of heat stress on reproduction in laying hens. Supplemental ascorbic acid was reported by some workers to increase egg production of laying hens subjected to heat stress (Perek and Kendler, 1962; 1963; Hunt and Aitken, 1962; Peebles and Brake, 1983), while no beneficial effects were observed by others (Thornton and Moreng, 1959; Harms and Waldroup, 1961; Hunt and Aitken, 1962; Naber et al., 1963; Nestor et al., 1972). Some laboratories reported that additional ascorbic acid improved interior or exterior shell quality (Thornton and Moreng, 1958; 1959; El-Boushy et al., 1968; Nockels et al., 1968; Lyle and Moreng, 1968; Herrick and Nockels, 1969), while others reported that ascorbic acid was without effect on egg quality (Heywang and Kemmerer, 1955; Harms and Waldroup, 1961; Hunt and Aitken, 1962; Arscott et al., 1962; Perek and Kendler, 1962; 1963; Naber

et al., 1963; Heywang et al., 1964; Dorr and Nockels, 1972; Nestor et al., 1972).

In contrast to effects on egg production, surprisingly few experiments have been conducted to examine the ability of ascorbic acid to improve the growth rate of stressed chicks. Growth responses to ascorbic acid were reported by March and Biely (1953). Pardue et al. (1983) reported that ascorbic acid added to the diet improved body weights of chicks exposed to heat stress for a period of two days. Schmeling and Nockels (1978) observed a growth response to supplemental ascorbic acid in chicks treated with ACTH and suggested that ascorbic acid reduced tissue catabolism by interfering with the synthesis or secretion of corticoids.

If ascorbic acid is capable of protecting chicken against stress under some conditions, a number of physiological properties of this vitamin could explain the effects. Pardue and Thaxton (1984) provided evidence that supplemental ascorbic acid ameliorated immunosuppressive effects of exogenous cortisol and postulated that this nutrient is an anti-immunosuppressive agent in the chicken. There is also evidence that ascorbic acid reduces the increase in body temperature associated with heat stress (Thornton, 1962; Grimes and Moreng, 1965; Ahmad et al., 1967; Lyle and Moreng, 1968). These latter observations suggest that ascorbic acid may be involved in mechanisms

controlling heat expenditure. Richards (1970, 1971) proposed that vasomotor activity in the extremities, resulting in increased blood flow, is a principal mechanism by which regulation of internal body temperature is achieved throughout a wide range of moderate ambient temperatures.

Classical concepts of stress, originally developed from observations with mammalian species (Selye, 1950; Mulrow, 1973; Gale, 1973), appear to be generally applicable to the chicken (Frankel, 1970; Siegel, 1971). According to these concepts, "stress" activates the hypothalamus-pituitary-adrenal axis to release ACTH into the adrenal cortex. ACTH stimulates intensive steroidogenesis and corticosterone secretion (Edens and Siegel, 1975), which helps the animal accommodate the stressor by providing energy through mobilization of long-term energy stores (Williams, 1985). The initial intensive steroidogenesis, however, is followed by a rapid depletion of adrenal cholesterol, which serves as a precursor of the steroid hormones (Wolford and Ringer, 1963). Adrenal ascorbic acid levels have also been reported to decrease in chickens exposed to acute stress (Freeman, 1970; Siegel, 1980). This depletion of adrenal ascorbic acid is associated with an immediate increase in the concentration of ascorbic acid in plasma and leucocytes (Ben-Nathan et al., 1976). When chickens are subjected to physiological stress over longer periods, however, plasma

ascorbic acid levels tend to decrease (Hill and Garren, 1958; Perek and Kendler, 1963; Nockels et al., 1973).

Although stress certainly influences adrenal function, the effects of stress on adrenal weights of chickens are not clear. Brown et al. (1958) and Bates et al. (1960) failed to observe adrenal hypertrophy in response to stress. Wolford and Ringer (1963) reported that the right adrenal glands of hens exposed to cold stress and withheld from feed and water for 15 hours were heavier than the right adrenals of control hens, but total adrenal weights did not differ. Schmeling and Nockels (1978), however, reported that feeding ascorbic acid to vitamin A deficient chicks reduced adrenal hypertrophy.

In summary, the role of ascorbic acid in stress reactions in the fowl is poorly understood. Both specific and nonspecific responses to various environmental stimuli occur (Siegel, 1985), and ascorbic acid may be involved in either or both types of responses.

CHAPTER I

SKIN BREAKING STRENGTH IN CHICKENS: COMPARISONS AMONG GENETIC COMBINATIONS

INTRODUCTION

Although skin strength is important in various areas of poultry production and processing, there is a paucity of literature concerning factors affecting variation in this trait. This chapter contains information concerning breaking strength of skin covering the breasts and thighs of chickens obtained from various mating combinations.

MATERIALS AND METHODS

The apparatus for determining skin strength was fashioned from an apparatus used by Alvarez and Gilbreath (1982) to evaluate the repair of skin wounds in rats. Skin samples were attached to coil-spring clamps; one clamp was stationary while the other was attached to a large plastic bottle. At the midpoint of each sample, a notch was made on each side of the skin to provide a uniform weak point of 2 mm which prevented tearing at the site where the skin was attached to the clamps. Pressure was provided by adding water to the bottle until the section broke. Breaking strength was expressed as g/skin sample required for tearing to occur (Figure 1). Reliability of the procedure was determined by a preliminary experiment using two samples (left and right side) of thigh and breast skin from each of 50 chickens. Correlations between sides were .90 and .91 for thigh and breast skin, respectively.

The chicks used in the experiment reported here were progeny of the S_{23} generation of high-weight (H) and low-weight (L) chickens selected for body weight at 56 days of age (Siegel and Cherry, 1981). Comparisons also involved F_1 reciprocal crosses between males and females of the two lines (HL and LH) and an F_2 generation obtained from an HL x HL mating from S_{22} generation parents. Parental lines and filial generations were produced by mass matings. Symbols

denoting mating combinations refer to the sire line first, e.g. HL refers to F_1 progeny from a cross of H sires and L dams.

All chicks were hatched on April 6, 1983, toe-clipped for identification, sexed, vaccinated against Marek's disease, and reared on litter floors under ad libitum feeding. At 70 days of age, ten females from each mating combination were weighed and sacrificed by cervical dislocation. Uniform sections (7 x 1 cm) of skin were removed from the prolateral region of both sides of the breast and from the lateral region of the left and right thighs (Lucas and Stettenheim, 1972a) and breaking strength was measured immediately. The lateral values for each site were averaged, transformed to natural logarithms, and subjected to analysis of variance with mating combinations and skin sites (breast and thigh) as main variables. When significance was obtained for mating combinations, means were separated by Duncan's multiple range test. Nonlinear contrasts (Scheffe, 1970) were used to assess heterosis and recombination effects.

RESULTS AND DISCUSSION

As expected, because of prior selection for high and low body weight, considerable differences were found among mating combinations for body weight (Table 1). Regardless of mating combination, breaking strength was significantly greater for breast than for thigh skin. Breaking strength of thigh skin was significantly less for females from the LL parental line than for the other mating combinations which did not differ from each other. Similar patterns were obtained for breast skin, with the LL chicks having weaker skins than the HH and LH chicks. Values for thigh skin from the HL and F_2 mating combinations were intermediate and did not significantly differ from the other populations. Percentage heterosis for thigh and breast skin breaking strength was 24.7 and 7.1, respectively; with the value for thigh skin being significant. Percentage recombination (3.3 and -6.1 for thigh and breast skin, respectively), was not significant.

Breaking strength values among mating combinations were positively associated with that of body weight. Spearman's rank correlations of body weight with breast and thigh breaking strength were .80 and .90, respectively, suggesting that the changes occurred as correlated responses to selection for body weight.

The procedure outlined in this chapter appeared to be

sufficiently sensitive to allow an assessment of breaking strength of chicken skin, and the data showed that skin strength is influenced by both additive and nonadditive genetic variation.

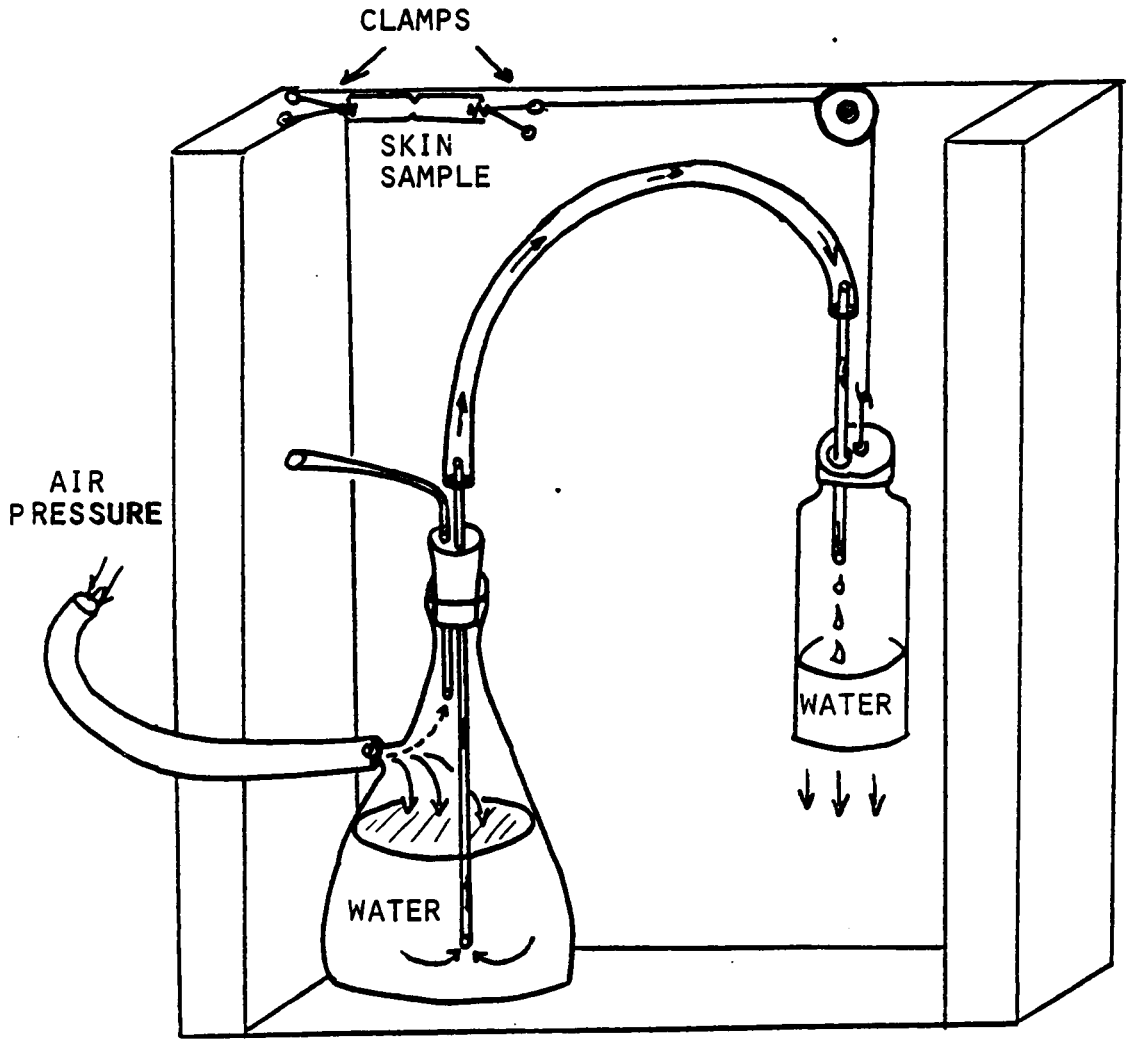

Figure 1. Apparatus for determining skin strength.

Table 1. Means and standard errors for body weight and breaking strength of skin from five different mating combinations of chickens

Mating Combination	Body Wt	Breaking Strength*	
		Thigh	Breast
	-g-	-g-	-g-
HH	1612 ± 77^{a}	823 ± 59^{Aa}	1021 ± 50^{Ba}
LH	1028 ± 97^{b}	830 ± 64^{Aa}	1009 ± 69^{Ba}
HL	832 ± 102^{b}	717 ± 81^{Aa}	923 ± 50^{Bab}
F_2	1016 ± 65^{b}	691 ± 68^{Aa}	857 ± 36^{Bab}
LL	311 ± 22^{c}	418 ± 30^{Ab}	783 ± 60^{Bb}

^{A,a}Different upper case letters designate significant site differences ($P \leq .05$). Different lower case letters designate significant population differences ($P \leq .05$). The site by mating combination interaction was not significant.

*Average of values from right and left side of each bird.

CHAPTER II

BREAKING STRENGTH AND COMPOSITION OF THE SKIN OF BROILER CHICKS: RESPONSE TO DIETARY CALORIE-PROTEIN RATIOS

INTRODUCTION

Because fragile skin increases the incidence of downgrades, skin strength is important in poultry processing. Factors contributing to skin strength, however, are largely unknown. Edwards et al. (1973) observed more skin tears in females than in males, and in older than in younger broilers. Smith et al. (1977) also observed a greater incidence of torn skin in females than in males, and postulated that higher levels of skin fat, accompanied by a reduction in total collagen concentrations, made the skin of females more susceptible to tearing.

In the experiment reported here, skin breaking strength was evaluated in relation to its chemical composition. Measurements were obtained at several ages and at different body sites in male and female broilers fed diets differing in the ratio of calories to protein (C:P).

MATERIALS AND METHODS

At 1 day of age, 40 male and 40 female commercial broiler chicks were wingbanded and assigned to treatment groups at random. The chicks were reared in electrically-heated battery brooders in groups of 10 chicks of the same sex. All chicks were initially fed Diet A (Table 2) which contained a C:P ratio of 140 kcal to 1% protein. At 14 days of age, 2 dietary regimes, designated as either wide or narrow depending on their relative C:P ratios, were each imposed on 2 groups of each sex. The narrow C:P regime consisted of C:P ratios of 140:1 (A), 160:1 (B), and 180:1 (C), fed 14-28, 28-42, and 42-56 days of age, respectively. The wide C:P regime consisted of diets containing C:P ratios of 160:1 (B), 180:1 (C), and 200:1 (D) fed during the same age periods. All diets were formulated using ingredient composition values provided by the National Research Council (1977). Feed and water were provided ad libitum and lighting was continuous.

At 28, 42, and 56 days of age, the birds were weighed individually to the nearest g, and 5 individuals from each sex-diet treatment were killed by cervical dislocation. They were then defeathered (dry picking) and uniform sections of skin were removed immediately from the lateral body apterium (breast) and from the areas delineated by the dorsopelvic (back) and femoral (thigh) feather tracts for

measuring breaking strength using the procedures described in Chapter I. Chemical analyses for fat (Folch-Jordi and Sloane-Stanley, 1965), protein (Nir et al., 1974), and water (Association of Official Analytical Chemists, 1975) were performed on all skin samples. Total skin collagen was estimated from hydroxyproline concentrations as described by Logan et al. (1950).

The data were analyzed by analysis of variance with age, sex, site and diet considered as fixed effects. The statistical model was:

$$Y_{ijklm} = \mu + A_i + S_j + D_k + L_l + (AS)_{ij} + (AD)_{ik} + (AL)_{il} + (SD)_{jk} + (SL)_{jl} + (DL)_{kl} + (ASD)_{ijk} + (ASL)_{ijl} + (SDL)_{jkl} + (ADL)_{ikl} + (ASDL)_{ijkl} + e_{ijklm}$$

where i = 1, 2, 3 ages, j = 1, 2 sexes, k = 1, 2 dietary regimes, l = 1, 2, 3 sites, and m = 1, 2 . . . n individuals. Prior to analysis, breaking strength values were transformed to natural logarithms because means and variances were correlated. Percentages were transformed to arc sine square roots for analyses. When age and site differences were significant, the means were separated by Duncan's multiple range test. When significant interactions among main variables occurred, the data were analyzed within pairs of variables. Product-moment correlation coefficients between breaking strength and skin composition were also

calculated within each site (Sokal and Rohlf, 1969). In addition, the data were subjected to multiple regression analysis using step-wise procedures with breaking strength considered as the dependent variable and age, diet, and site considered independent.

RESULTS

Main effects. Means and standard errors for skin breaking strength and skin composition traits of male broilers are presented in Table 3. Comparable data for females are reported in Table 4. Body weights of both males and females are contained in Table 5.

When main effects were examined (Table 6), the males were heavier and had stronger skin than females. Skin from females contained more fat and less protein and collagen then skin from males. No sexual dimorphism in skin moisture was observed.

Assuming that breaking strength values are indicative of skin strength, chicks fed diets with comparatively narrow C:P ratios were heavier and had stronger skin than those fed diets with wider C:P ratios. Feeding the wide C:P diets resulted in an increase in skin fat and decreases in the moisture, protein, and collagen contents of the skin.

Skin breaking strength increased between 28 and 56 days of age, with the value at 42 days of age being intermediate. Comparatively more fat and less moisture, protein, and collagen were contained in the skin at 56 days of age than at 28 days of age. Skin composition values at 42 days of age were intermediate to values obtained at 28 and 56 days of age.

Breast skin was stronger than skin from the thigh.

Skin from the back exhibited intermediate values for breaking strength. Back skin also contained more fat and less moisture, protein, and collagen than skin from the breast or thigh. Skin from the breast had more fat and less moisture, protein, and collagen than thigh skin.

Interactions among main variables. Significant sex by site interactions for skin breaking strength and for the percentages of protein, collagen, moisture, and fat in skin were obtained (Table 7). Although females had weaker skin than males regardless of site, the magnitude of the sexual dimorphism differed among sites. The ratio of female to male skin breaking strength was 94%, 89%, and 82% for breast, back, and thigh, respectively. Percentage fat in thigh skin was lower in males than in females, but no significant differences in skin fat due to sex were obtained in breast or back skin. No differences in moisture were obtained between sexes for breast or back skin, but thigh skin from the males contained more moisture than that from females. Skin from males was consistently higher in protein than skin from females, but the difference was most pronounced in thigh skin. Collagen concentrations of back and thigh skin were greater in males than in females, but the collagen content of breast skin did not differ significantly due to sex.

Age by diet interactions for breaking strength and for

fat, moisture, and protein concentrations of the skin are presented in Table 8. Broilers fed diets containing narrower C:P ratios had stronger skin than those fed the wide C:P diets at all ages examined, with the difference being significant at 56 days. Differences in skin composition due to diet, while pronounced at younger ages, disappeared by 56 days of age.

None of the significant age by site interactions (Table 9) were of the crossover type. Regardless of age, thigh skin exhibited the highest values for protein, collagen, and moisture; back skin exhibited the lowest values for moisture, protein, and collagen, the highest value for percentage fat, and was intermediate in breaking strength; breast skin while strongest exhibited intermediate values for fat, water, protein, and collagen.

Correlations and regressions. At all skin sites examined, correlations between breaking strength and the skin composition traits measured were low and not significant (Table 10). Step-wise regression analyses also failed to reveal any consistent cause-effect relationships between breaking strength and skin composition.

DISCUSSION

The tendency of males to have stronger skin than females (Table 6) was consistent with previous observations that the incidence of torn skin occurring during processing was greater in female than in male broilers (Edwards et al., 1973, Smith et al., 1977). Smith et al. (1977) proposed that the propensity for female skin to be weaker than that of males was related to increased concentrations of fat and/or decreased collagen concentrations. In the present study, skin from females tended to contain more fat and less protein and collagen than skin from males (Table 6). Similar observations were reported in broiler chicks by Grey et al. (1983) and in goslings by Nitsan et al. (1981). The significant sex by site interactions obtained in the present study, however, were not entirely consistent with the theory that increased fat and decreased protein or collagen contents of the skin are associated with weaker skin. Although breaking strength of skin from males was greater than that of females at all ages examined (Table 6), skin fat was significantly greater in females only for thigh skin. Numerically, percentage fat in breast skin was higher for males than for females. Protein and collagen concentrations of the skin, however, were consistently greater in males than in females irrespective of site.

If increases in skin fat result in weaker skin, feeding

diets with wide C:P ratios would be expected to reduce skin breaking strength; Bartov et al. (1974) reported that decreased dietary protein resulted in an increased deposition of fat in the skin of broilers. Feeding the wide C:P diets did result in weaker skin which was associated with a general increase in skin fat and decreases in the moisture, protein, and collagen concentrations of the skin (Table 6). It may be of importance, however, that differences in breaking strength due to diet were more pronounced at 56 days of age while differences in skin composition were more pronounced at younger ages (Table 8). No significant dietary differences in skin composition were observed at 56 days of age when the age by diet interaction was examined.

Although there were inconsistencies, differences in breaking strength and skin composition between males and females, and between chicks fed diets differing in C:P ratios, tended to support the conclusion that weak skin is associated with increased fat and decreased protein or collagen concentrations of the skin (Smith et al., 1977). Different patterns, however, emerged when site and age differences were examined.

Edwards et al. (1973) reported that the incidence of torn skin was higher in broilers at 9 weeks than at younger ages, inferring that the skin of chickens becomes weaker

with age. The present results were inconsistent with this inference. Breaking strength tended to increase with age (Table 6), although the patterns varied between diets (Table 8) and among sites (Table 9). For example, a decrease in breaking strength of the skin occurred between 42 and 56 days of age when the diets containing wide C:P ratios were fed, but breaking strength of skin increased during this period in response to the narrow C:P diets (Table 8). Moreover, thigh and back skin tended to become stronger with age while breast skin did not (Table 9).

Changes in skin composition with age indicated that skin strength was not solely associated with increased fat and decreased protein and collagen concentrations. Increases in breaking strength with age were accompanied by increases in skin fat and decreases in moisture and collagen concentrations. Skin protein content increased from 28 to 42 days of age but decreased from 42 to 56 days of age. Reports in the literature concerning age effects on skin protein are also ambiguous. Suderman et al. (1980) reported that skin protein remained fairly constant at different ages while other investigators (Nitsan et al., 1981, Grey et al., 1983) observed a general decline in protein concentrations in the skin with age. Nonetheless, changes in the protein content of the skin failed to parallel those in breaking strength. Neither were the significant age by diet (Table

8) nor age by site (Table 9) interactions indicative of a consistent relationship between skin composition and skin strength.

Similar to the age effects, differences in the composition and strength of skin at different sites did not indicate that breaking strength was associated with increased fat or decreased protein or collagen concentrations. Skin from the back contained more fat and less protein and collagen than skin from the other sites, but exhibited intermediate values for breaking strength (Table 6). Although breast skin contained more fat and less protein and collagen than that from the thigh, it was considerably stronger than thigh skin. Skin from the thigh, which was weakest among the sites examined, had the least fat and the most protein and collagen. These site differences were generally consistent at the different ages (Table 9) and between sexes (Table 7); the significant interactions were not of the crossover type.

Differences in skin composition and skin strength due to sex, diet, age, and skin site revealed no consistent relationship between the strength and chemical composition of the skin. Correlation and multiple regression analyses were also ineffective in relating skin strength to any of the chemical characteristics measured. Perhaps a more detailed chemical or histological examination of specific

skin layers would be beneficial in elucidating physical or chemical factors determining skin strength.

Table 2. Composition of diets

	Diet			
	A	B	C	D
	----------%			
Ingredient				
Yellow corn	65.38	62.23	72.53	78.89
Dehulled soybean meal	24.10	17.90	13.90	13.90
Meat and bone meal	7.50	7.60	7.90	4.10
Wheat bran	0.80	8.10	3.60	1.10
Ground limestone	0.55	0.55	0.50	0.50
Hydrolyzed fat	1.00	3.00	1.00	1.00
DL-methionine	0.17	0.12	0.07	0.01
Salt	0.25	0.25	0.25	0.25
Vitamin & trace mineral mix*	0.25	0.25	0.25	0.25
Calculated analysis				
Crude protein	21.4	19.4	17.3	16.0
Metab. energy, kcal/kg	3016	3080	3130	3267
C:P ratio	140	160	180	200

*Vitamin and trace mineral premix provided per kg of diet: vitamin A 8000 IU, vitamin D_3 3000 IU, vitamin E 10 IU, vitamin K 2 mg, Riboflavin 4 mg, d-pantothenic acid 10 mg, thiamine 1 mg, niacin 35 mg, choline chloride 350 mg, vitamin B_{12} 8 µg, folic acid 0.5 mg, ethoxyquin 250 mg, pyridoxine 5 mg, biotin 0.4 mg, manganese 80 mg, zinc 112 mg, iron 62 mg, copper 16 mg, iodine 0.1 mg, selenium 0.1 mg.

Table 3. Means and standard errors for skin breaking strength and skin composition of male broilers fed diets with comparatively wide (W) and narrow (N) ratios of calories to protein

Skin Site	Age	Diet	Breaking strength	Fat	Water	Protein	Collagen
	-d-		-%-	-%-	-%-	-%-	-% of prot.-
Breast	28	W	984 ± 46	37.7 ± 2.4	50.2 ± 2.4	12.1 ± 0.2	71.7 ± 1.1
		N	995 ± 30	22.7 ± 1.5	58.5 ± 1.4	12.7 ± 0.3	78.5 ± 3.3
	42	W	1038 ± 42	38.5 ± 0.6	48.7 ± 1.4	12.3 ± 0.6	70.6 ± 1.5
		N	1144 ± 51	33.9 ± 1.0	51.7 ± 1.4	12.9 ± 0.2	77.1 ± 1.5
	56	W	945 ± 14	44.1 ± 0.7	43.9 ± 1.0	12.1 ± 0.3	67.6 ± 2.2
		N	1049 ± 20	44.4 ± 2.6	44.9 ± 1.9	12.4 ± 0.3	74.4 ± 2.3
Back	28	W	736 ± 46	53.6 ± 1.6	38.2 ± 1.7	9.2 ± 0.3	68.7 ± 0.5
		N	847 ± 52	40.4 ± 1.2	49.1 ± 1.3	10.6 ± 0.3	70.3 ± 1.3
	42	W	850 ± 36	51.1 ± 2.2	37.6 ± 1.2	8.7 ± 0.2	57.6 ± 1.4
		N	869 ± 55	43.6 ± 1.4	44.1 ± 0.8	10.1 ± 0.3	64.6 ± 1.0
	56	W	789 ± 70	50.3 ± 1.7	39.0 ± 1.6	9.6 ± 0.2	62.1 ± 1.3
		N	952 ± 52	53.3 ± 1.2	36.6 ± 0.7	9.4 ± 0.5	68.5 ± 3.0
Thigh	28	W	701 ± 30	27.4 ± 2.1	57.2 ± 6.7	15.4 ± 0.4	75.4 ± 4.4
		N	737 ± 36	24.7 ± 1.2	58.9 ± 1.0	16.4 ± 0.3	83.1 ± 2.1
	42	W	695 ± 8	26.8 ± 2.5	56.1 ± 2.3	15.8 ± 0.3	78.6 ± 2.6
		N	560 ± 65	20.3 ± 0.4	60.9 ± 0.8	15.4 ± 0.3	88.2 ± 1.9
	56	W	738 ± 25	27.9 ± 1.0	55.2 ± 1.0	16.4 ± 0.2	81.3 ± 2.1
		N	821 ± 36	29.9 ± 2.1	54.1 ± 1.2	15.9 ± 0.3	86.4 ± 1.4

Table 4. Means and standard errors for skin breaking strength and skin composition of female broilers fed diets with comparatively wide (W) and narrow (N) ratios of calories to protein

Skin Site	Age	Diet	Breaking strength	Fat	Water	Protein	Collagen
	-d-		-%-	-%-	-%-	-%-	-% of prot.-
Breast	28	W	877 ± 24	34.9 ± 2.1	53.4 ± 2.1	11.1 ± 0.2	75.6 ± 2.6
		N	938 ± 39	27.2 ± 1.3	61.0 ± 1.2	11.8 ± 0.3	77.9 ± 1.4
	42	W	1081 ± 38	37.7 ± 1.1	48.9 ± 1.2	11.3 ± 0.1	74.8 ± 1.8
		N	1097 ± 42	34.8 ± 0.7	50.1 ± 1.2	12.4 ± 0.2	70.6 ± 2.1
	56	W	831 ± 33	42.6 ± 2.0	46.1 ± 2.4	10.1 ± 0.4	65.9 ± 2.2
		N	1003 ± 25	38.0 ± 2.3	51.8 ± 2.7	11.1 ± 0.3	61.3 ± 2.2
Back	28	W	673 ± 21	50.6 ± 2.0	39.8 ± 1.9	9.4 ± 0.2	65.9 ± 2.4
		N	743 ± 33	45.0 ± 1.8	44.9 ± 1.3	10.1 ± 0.3	70.1 ± 1.5
	42	W	746 ± 46	51.7 ± 2.3	37.6 ± 2.3	7.1 ± 0.1	57.9 ± 2.4
		N	770 ± 15	45.0 ± 0.8	43.3 ± 0.7	7.9 ± 0.1	61.0 ± 1.0
	56	W	753 ± 43	51.7 ± 2.6	34.5 ± 2.6	7.6 ± 0.3	59.9 ± 1.1
		N	800 ± 27	55.4 ± 1.2	36.4 ± 1.2	8.2 ± 0.4	56.4 ± 1.5
Thigh	28	W	591 ± 27	35.0 ± 1.5	51.3 ± 1.5	13.7 ± 0.2	82.8 ± 1.4
		N	536 ± 38	26.2 ± 1.2	60.4 ± 1.5	14.4 ± 0.1	79.9 ± 1.5
	42	W	473 ± 9	32.8 ± 1.5	53.2 ± 1.3	12.9 ± 0.3	78.8 ± 0.8
		N	551 ± 27	24.2 ± 0.9	59.1 ± 0.7	13.6 ± 0.3	77.2 ± 1.8
	56	W	576 ± 33	37.8 ± 1.8	48.1 ± 1.1	13.1 ± 0.6	70.2 ± 2.4
		N	761 ± 40	38.6 ± 1.6	48.7 ± 0.9	13.5 ± 0.1	70.5 ± 3.3

Table 5. Means and standard errors of body weights of male and female broilers summarized within age-diet groups

Age	Diet	Males	Females
		-g-	-g-
28	Wide	709 ± 42	665 ± 22
	Narrow	783 ± 16	617 ± 40
42	Wide	1302 ± 44	1049 ± 43
	Narrow	1321 ± 21	1202 ± 49
56	Wide	1515 ± 121	1621 ± 79
	Narrow	1884 ± 50	1689 ± 52

Table 6. Body weights, breaking strength, and chemical composition of skin by sex, diet, age, and site with main effects pooled

Main Effect	Body Weight	Breaking Strength	Fat	Moisture	Protein	Collagen
	-g-	-g-	-%-	-%-	-%-	-% of prot.-
Sex						
Male	1069[a]	860[a]	38[b]	49[a]	12.6[b]	74[a]
Female	989[b]	763[b]	40[a]	48[a]	11.1[a]	70[b]
Diet						
Wide	981[b]	779[b]	41[a]	47[b]	11.5[b]	70[b]
Narrow	1078[a]	843[a]	36[b]	51[a]	12.2[a]	73[a]
Age						
28	702[c]	779[b]	36[b]	52[a]	12.3[a]	75[a]
42	1196[b]	821[ab]	37[b]	49[b]	11.7[b]	71[b]
56	1677[a]	835[a]	43[a]	45[c]	11.6[b]	69[c]
Site						
Breast	---	996[b]	37[b]	51[b]	11.9[b]	72[b]
Back	---	794[a]	50[a]	40[c]	8.9[c]	64[c]
Thigh	---	644[c]	29[c]	55[a]	14.2[a]	79[a]

[a,b,c]Within main effects, means in a column having the same superscript were not significantly different ($P \geq .05$).

Table 7. Sex by site interactions for skin breaking strength and fat, moisture, protein, and collagen concentrations of the skin

Site	Sex	Breaking strength	Fat	Moisture	Protein	Collagen
		-g-	-%-	-%-	-%-	-%-
Breast	Male	1026_{a}	37.9_{a}	49.7_{a}	12.4_{a}	73.3_{a}
	Female	966_{b}	35.9_{a}	52.0_{a}	11.3_{b}	71.0_{a}
Back	Male	841_{a}	48.7_{a}	40.6_{a}	9.6_{a}	65.3_{a}
	Female	748_{b}	51.0_{a}	39.4_{a}	8.4_{b}	61.9_{b}
Thigh	Male	709_{a}	26.3_{b}	57.1_{a}	15.9_{a}	82.2_{a}
	Female	581_{b}	32.4_{a}	53.4_{b}	13.6_{b}	76.6_{b}

$_{a,b}$Within sites, means in the same column having the same superscript were not significantly different ($P \geq .05$).

Table 8. Age by diet interactions for skin breaking strength and for fat, moisture, and protein concentrations of the skin

Age	Diet	Breaking strength	Fat	Moisture	Protein
		-g-	-%-	-%-	-%-
28	Wide	759_a	39.9_a	48.2_a	11.8_a
	Narrow	799_a	32.0_b	55.4_b	12.7_b
42	Wide	809_a	39.8_a	47.1_a	11.3_a
	Narrow	832_a	33.8_b	51.5_b	12.1_b
56	Wide	772_a	43.4_a	44.5_a	11.5_a
	Narrow	899_b	43.3_a	45.4_a	11.7_a

$_{a,b}$Within ages, means having the same superscript were not significantly different ($P \geq .05$).

Table 9. Age by site interactions for skin breaking strength and for fat, moisture, protein, and collagen concentrations of the skin

Age	Site	Breaking strength	Fat	Moisture	Protein	Collagen
		-g-	-%-	-%-	-%-	-%-
28	Breast	946_{a}	32.1_{b}	55.8_{a}	11.9_{b}	75.9_{b}
	Back	749_{b}	47.4_{a}	42.7_{b}	9.8_{c}	68.8_{c}
	Thigh	641_{c}	28.3_{c}	56.9_{a}	15.0_{a}	80.3_{a}
42	Breast	1090_{a}	30.2_{b}	50.1_{b}	12.2_{b}	73.2_{b}
	Back	809_{b}	48.0_{a}	40.6_{c}	8.5_{c}	60.3_{c}
	Thigh	563_{c}	26.2_{c}	57.3_{a}	14.4_{a}	80.7_{a}
56	Breast	957_{a}	42.3_{b}	46.7_{b}	11.4_{b}	67.3_{b}
	Back	825_{b}	54.2_{a}	36.6_{c}	8.7_{c}	62.0_{c}
	Thigh	724_{c}	33.5_{c}	51.5_{a}	14.7_{a}	77.1_{a}

$_{a,b,c}$Within ages, means having the same superscript were not significantly different ($P \geq .05$).

Table 10. Correlation coefficients between breaking strength and skin composition traits at the three sites: breast, back, and thigh

Traits	Site		
	Breast	Back	Thigh
Fat	.07	.04	-.13
Water	-.09	-.10	.10
Protein	-.23	.09	-.00
Collagen	.10	-.07	.11

CHAPTER III

SKIN BREAKING STRENGTH IN BROILERS: RELATIONSHIP WITH SKIN THICKNESS

INTRODUCTION

Skin damage during defeathering can have economic implications in poultry processing, particularly when accomplished at lower scalding temperatures. Biological and environmental factors contributing to skin fragility, however, have not been extensively investigated. More skin tears occur in female than in male broilers during processing (Edwards et al., 1973; Smith et al., 1977), and these differences can be measured mechanically (Chapter II). Differences in skin strength due to age, calorie-protein (C:P) ratios of the diet, and genetic background have also been reported (Edwards et al., 1973; Chapters I and II).

Although it has been suggested that increased skin fat and an associated decrease in protein or collagen concentrations of skin contributes to skin damage during processing (Smith et al., 1977), I was unable to correlate skin breaking strength with either fat, protein, or collagen concentrations (Chapter II). In the present study, therefore, skin of broiler chicks fed diets with different C:P ratios was examined histologically. Total skin thickness and thickness of different skin layers determined from photomicrographs of representative skin sections were then compared to breaking strength values.

MATERIALS AND METHODS

Specific details concerning rearing of the chicks and the diets used were provided previously (Chapter II). Briefly, commercial broiler chicks were caged in groups of 10 in electrically heated battery brooders. Each group contained 5 males and 5 females. All chicks were fed a corn-soybean meal basal diet containing a C:P ratio of 140:1 until 14 days of age. Beginning at this age, two dietary treatments designated as either wide or narrow depending on their relative C:P ratios were each imposed on 4 groups of chicks. The narrow C:P treatment consisted of diets containing C:P ratios of 140:1, 160:1, and 180:1 fed from 14 to 28, 28 to 42, and 42 to 56 days of age, respectively. The wide C:P treatment consisted of C:P ratios of 160:1, 180:1, and 200:1 fed at corresponding age intervals. Both feed and water were provided ad libitum. Lighting was continuous.

At 56 days of age, 5 females and 5 males from each feeding treatment were selected randomly, killed by cervical dislocation, and defeathered by dry picking. Uniform sections of skin from the lateral body apterium (breast) and from areas delineated by the dorsopelvic (back), femoral (thigh), and sternal (side) feather tracts (Lucas and Stettenheim, 1972a) were removed from each bird (Figure 2). One section from each region was used for breaking strength

determinations (Chapter I) and another was examined histologically. For histological examination, fresh 10 x 20 mm segments of skin were placed on dry paper towels and then rapidly immersed in a 10% buffered neutral formalin solution for 24 hr. This procedure gave flat specimens and helped avoid tangential sections. After fixation, tissues were dehydrated, cleaned and embedded in paraffin. Perpendicular sections 5 μm in thickness were cut and stained by the Masson's trichrome technique (Luna, 1968). Photographs of these sections under light microscopy were then taken and used to determine total skin thickness, thickness of the hypodermis, and the combined thickness of the epidermis and dermis using a Zeiss Mop 3 image analyzer. Epidermal and dermal skin layers were measured together because of the difficulty in distinguishing between them with the relatively low power photomicrographs used.

The data were analyzed by analysis of variance according to the following statistical model:

$$Y_{ijkl} = \mu + S_i + D_j + L_k + (SD)_{ij} + (SL)_{ik} + (DL)_{jk} + (SDL)_{ijk} + e_{ijkl}$$

where i = 1, 2 sexes, j = 1, 2 dietary regimes, k = 1, 2, 3, 4 sites, and l = 1, 2 . . . 5 individuals. Before analysis, values for breaking strength and the combined thickness of the epidermis and dermis were transformed to natural logarithms because means and variances were correlated.

When significant interactions among main variables occurred, the data were analyzed within pairs of variables. When site differences were significant, the means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

General effects. Pooled means for breaking strength, total skin thickness, thickness of the hypodermis, and thickness of the dermis plus epidermis are presented in Table 11. With the exception of values for skin from the side, breaking strength values were presented previously (Chapter II). Briefly, males had stronger skin than females. Feeding diets with wide C:P ratios, in comparison to diets with comparatively narrow C:P ratios, reduced skin strength. Among sites, skin from the breast was strongest while that from the side was weakest. Thigh skin was stronger than breast skin.

Skin strength generally appeared to be related inversely to total skin thickness. Females had thicker skin than males and skin from chicks fed wide C:P diets was thicker in comparison to skin from chicks fed narrow C:P diets. Skin from the breast was thinner than that from the side, while thigh and back skin exhibited intermediate values for skin thickness. In each of these instances, increased skin thickness was associated with decreases in breaking strength. Differences between back and thigh skin, however, were an exception. Thigh skin was thinner and weaker than skin from the back.

Differences in skin thickness tended to be positively associated with thickness of the hypodermis, a region

composed largely of adipose tissue but also containing some muscle, fascia, and skin adenexae. The dermis plus epidermis tended to be relatively constant or to be slightly thinner in thicker skin. This relationship between skin and hypodermal thickness was particularly pronounced in breast and side skin (Figures 3 and 4).

Diet effects. The relationship between skin thickness and breaking strength in response to C:P ratios of the diet is illustrated in Figures 5 and 6 for females and males, respectively. Irrespective of sex or site, breaking strength values were consistently higher for chicks fed narrow C:P diets. Total skin thickness tended to be reduced in response to narrow C:P diets although differences for breast and back skin of males and for thigh skin of females were not significant. Thickness of the hypodermis followed patterns similar to those for total skin thickness. The dermis plus epidermis, however, was consistently thicker in skin from chicks fed narrow C:P diets in comparison to skin from chicks fed wide C:P diets. These results suggest that increased skin breaking strength due to feeding the narrow C:P diets is associated with a thinner hypodermis and a thicker epidermis plus dermis.

Sexual dimorphism. Skin breaking strength and thickness comparisons between males and females are presented in Figures 7 and 8. When wide C:P diets were fed,

males had stronger skin than females with the differences being significant for breast and thigh skin (Figure 7). Similar results were obtained when narrow C:P diets were fed, although the differences were significant only for back skin (Figure 8). Similar to effects observed due to diet, increased breaking strength in males tended to be associated with reduced skin thickness which was primarily due to a reduction in the thickness of the hypodermis. In contrast to differences due to diet, however, increased breaking strength of male skin was not consistently associated with an increased combined thickness of the dermis and epidermis.

Site differences. Regardless of diet or sex, strength of the skin among sites in decreasing order of strength were breast > back > thigh > side (Figures 9 and 10). Skin strength tended to be inversely related to skin thickness but there were exceptions. Back skin, for example, was stronger but thicker in comparison to skin from the thigh. Moreover, there was little indication that increased skin strength was associated with an increased thickness of the dermis plus epidermis. No significant differences among sites for dermis plus epidermis thickness were obtained for males (Figure 9). With females, the dermis plus epidermis was thinner in skin from the side than in that from other sites.

General discussion. The epidermis is a thin layer of

tissue composed of stratified squamous cells capable of keratinization (Lucas and Stettenheim, 1972b). Because this layer of skin is only 4 to 6 cells thick in feathered areas, it is unlikely that the thickness of the epidermis contributed substantially to differences in skin thickness obtained with the procedures used in this study. Differences in the thickness of the dermis and the hypodermis, however, may explain many of my observations. The dermis is a compact, protein-rich fibrous layer composed largely of collagen but also containing substantial quantities of elastin and glycoproteins (Spearman, 1971). The hypodermis, while thicker than the dermis, is less dense and contains far fewer cells. The cells of the hypodermis are loosely arranged and substantial fat is stored in adipocytes. These morphological differences may result in different effects on skin strength. It is conceivable that increased thickness of the dermis would tend to increase skin strength while an increased thickness of the hypodermis would be associated with decreases in skin strength.

Evidence that the dermis contributes to skin strength was most pronounced when dietary effects were examined (Figures 5 and 6). Increased protein intake should minimize fat deposition in skin (Bartov et al., 1974), and this effect was apparently manifested as a decrease in the thickness of the hypodermis. Feeding narrow C:P diets also

consistently increased the combined thickness of the dermis and epidermis. The increase in skin strength associated with feeding narrow C:P diets, therefore, may have resulted from either an increased combined thickness of the dermis and epidermis, a decreased thickness of the hypodermis, or both.

The increased thickness of the hypodermis in females (Figures 7 and 8) was likely due to sexual dimorphism for fat deposition. With the exception of skin from the side, differences in breaking strength between males and females did not appear to be related to increased thickness of the dermis. The stronger skin of males, therefore, appeared to be primarily associated with a thinner hypodermis. With the exception of comparisons between back and thigh skin, differences in breaking strength observed among sites were consistent with an inverse relationship between skin strength and hypodermis thickness (Figures 9 and 10). Back skin, however, was stronger and tended to have a thicker hypodermis than skin from the thigh. Although the differences were not significant, the combined thickness of the dermis and epidermis tended to be greater in thigh skin than in back skin.

Since the dermis contains larger concentrations of protein and collagen and the hypodermis contains larger quantities of lipid, differences in breaking strength

associated with differences in thickness of these skin layers should be reflected by the chemical composition of the skin. As mentioned previously, I was unable to correlate skin breaking strength with either protein, collagen, or fat concentrations (Chapter II). This failure may have been due to the expression of composition data on a concentration or percentage basis, which can be misleading when total skin thickness is variable and the dermis is proportionately much smaller than the hypodermis. The relationship between skin thickness and breaking strength may also be too simplistic to explain intricate cause-effect relationships. Skin integrity is undoubtedly a complex phenomenon related to numerous morphological and physiological properties. Nonetheless, the results clearly indicate that an increase in skin thickness does not improve skin strength.

Table 11. Means and standard errors for skin breaking strength, total skin thickness, and thickness of the hypodermis plus dermis with sex, diet, and site effects pooled

Main Effect	Breaking Strength	Total Skin	Hypo-dermis	Dermis & Epidermis
	-g-	-mm-	-mm-	-mm-
Sex				
Male	771[a]	1.57[b]	1.33[b]	0.23[a]
Female	608[b]	1.71[a]	1.48[a]	0.23[a]
Diet				
Narrow	849[a]	1.55[b]	1.27[b]	0.27[a]
Wide	718[b]	1.73[a]	1.54[a]	0.19[b]
Site				
Breast	831[a]	1.01[d]	0.74[d]	0.26[a]
Thigh	576[c]	1.20[c]	0.96[c]	0.24[a]
Back	753[b]	1.55[b]	1.32[b]	0.23[a]
Side	513[d]	3.12[a]	2.96[a]	0.17[b]

[a,b,c,d]Within main effects, means in the same column having the same superscripts were not significantly different ($P < .05$).

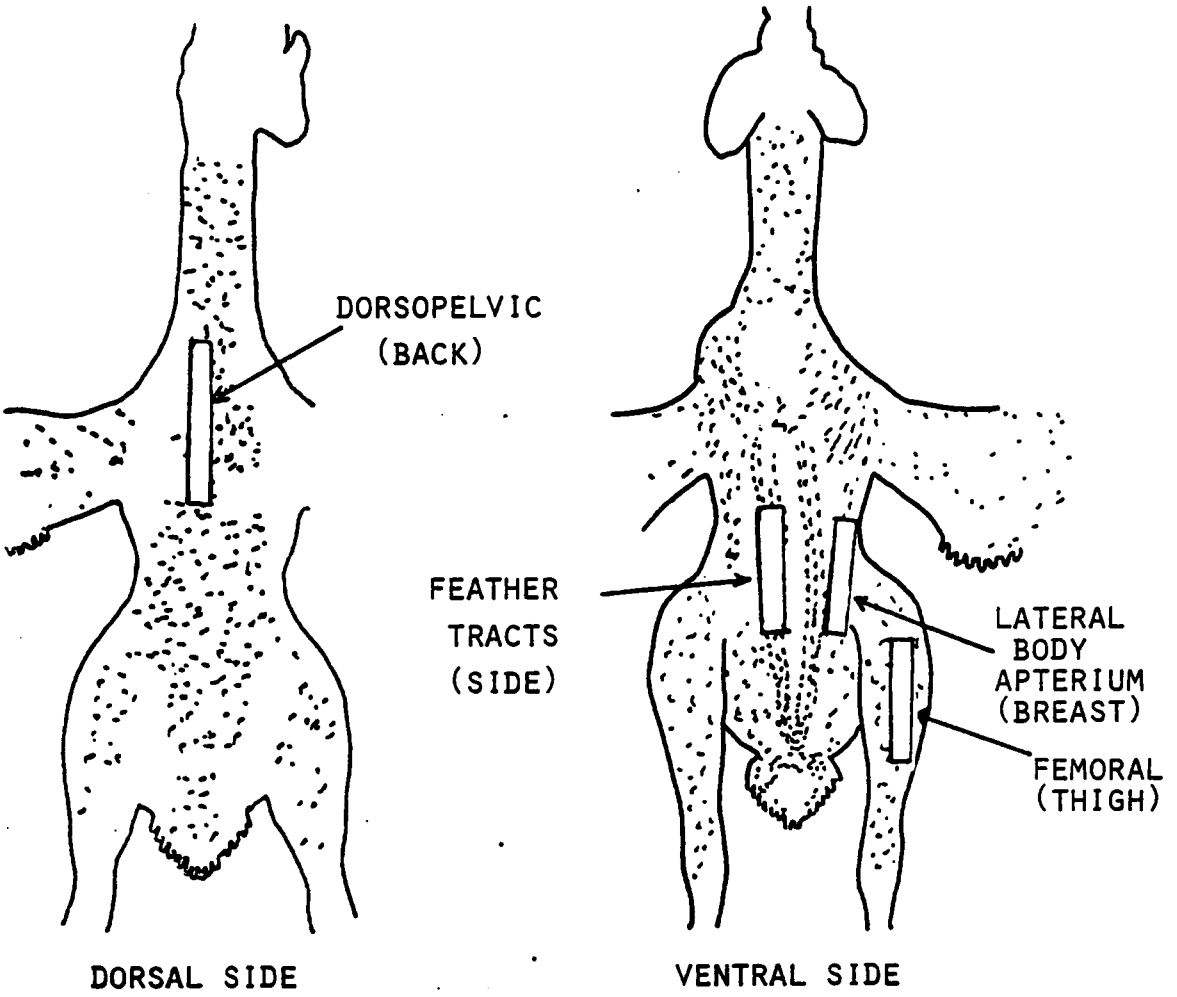

Figure 2. Sites at which skin samples were taken for breaking strength and chemical composition measurements.

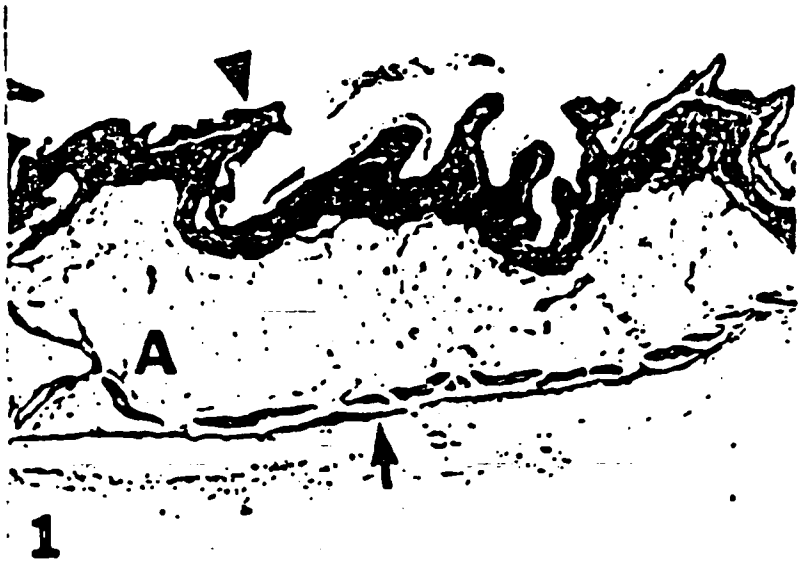

Figure 3. Section of skin from the breast of a female chick fed a wide C:P diet. The hypodermal layer (A) is bounded by dark staining epidermis and dermis (arrowhead) and deep fascia (arrow). Masson's trichrome stain x 22.

Figure 4. Section of skin from region of sternal feather tract (side) in a chick fed a wide C:P diet. Note the greater thickness of skin as compared to Figure 1 due to the thicker hypodermis (A). The latter is bounded by dark staining epidermis and dermis (arrowhead) and deep fascia (arrow). Masson's trichrome stain x 22.

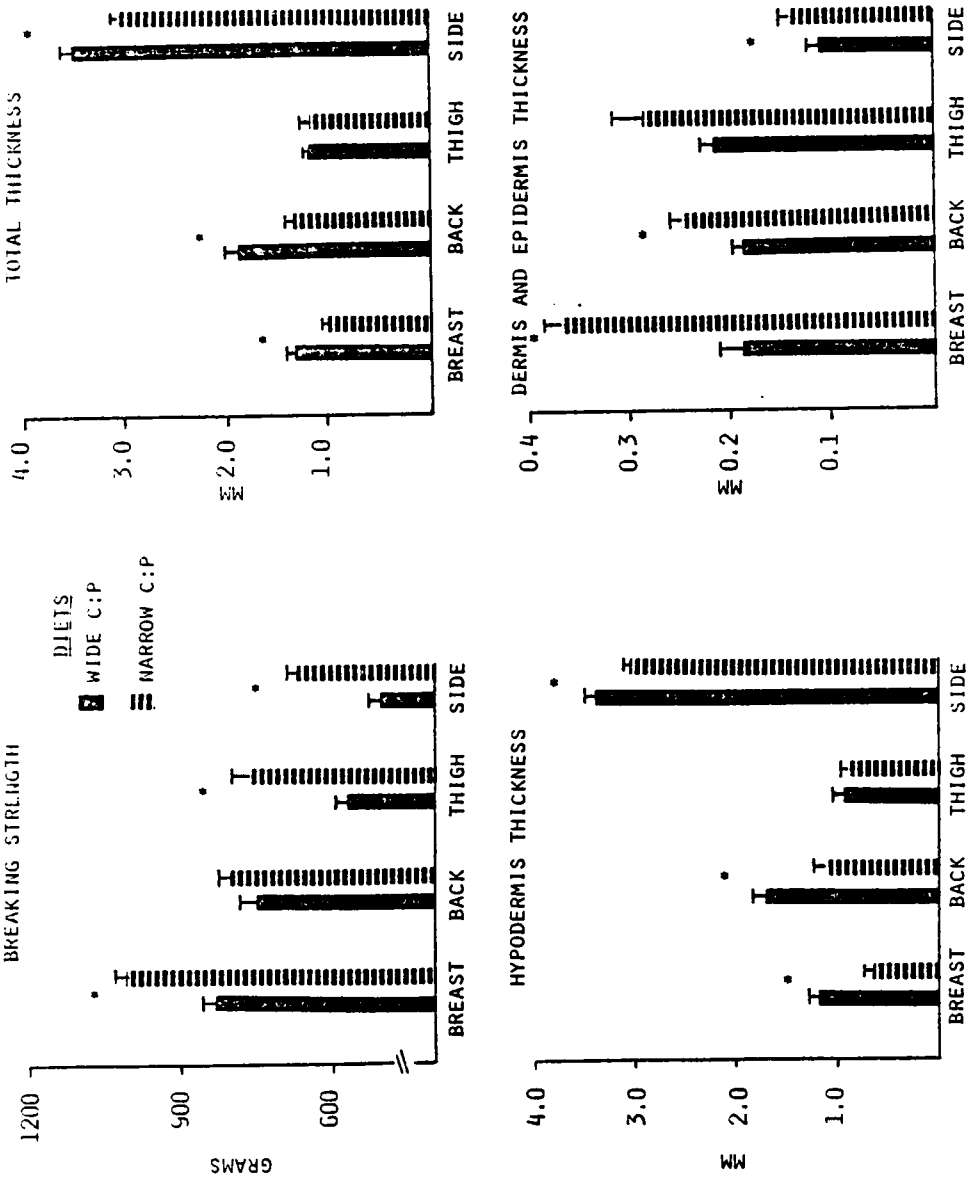

Figure 5. The relationship between skin breaking strength, total skin thickness, hypodermis thickness, and dermis plus epidermis thickness in female broilers fed diets containing comparatively wide and narrow C:P ratios. Values are means ± standard errors. Asterisks indicate a significant diet effect within site.

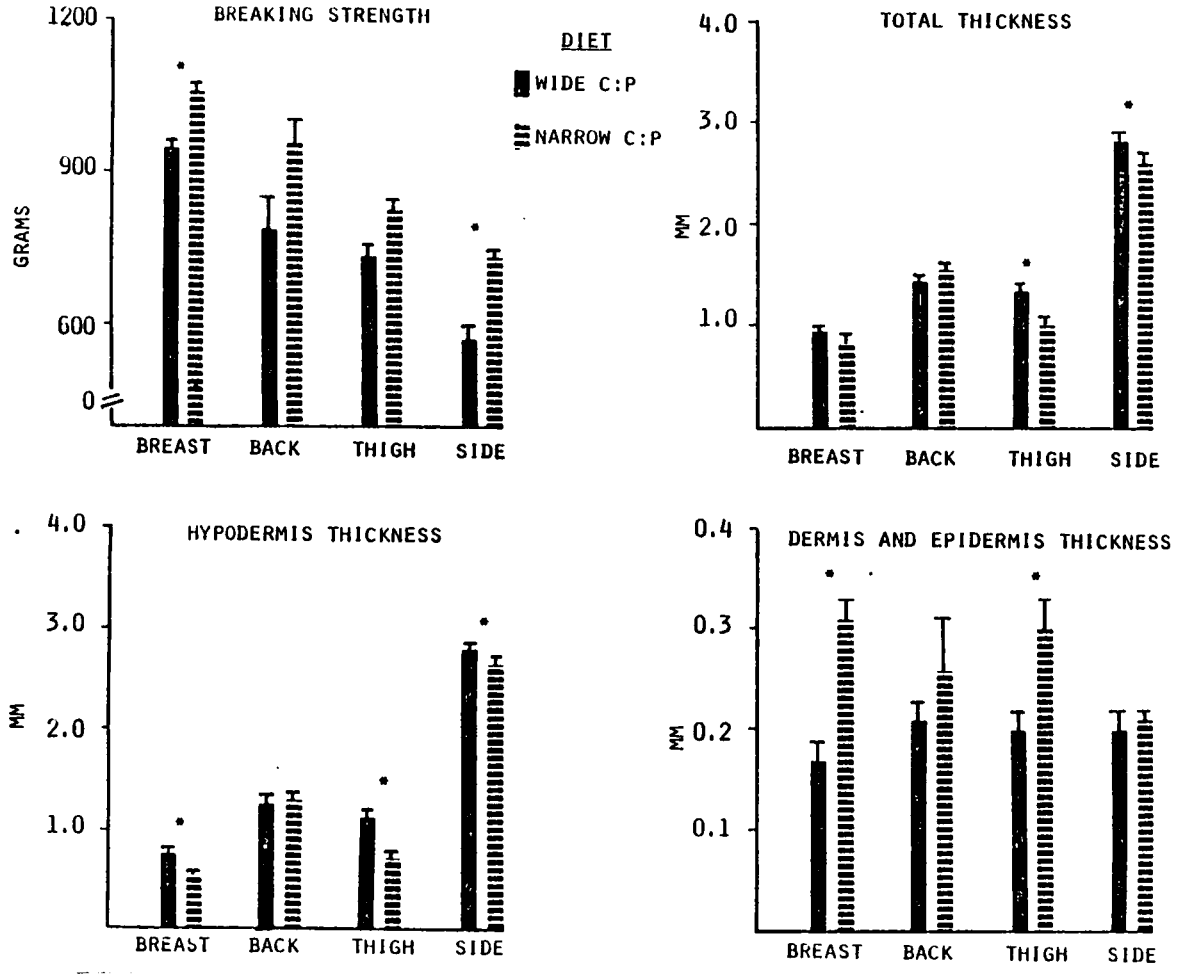

Figure 6. The relationship between skin breaking strength, total skin thickness, hypodermis thickness and dermis plus epidermis thickness in male broilers fed diets containing comparatively wide and narrow C:P ratios. Values are means ± standard errors. Asterisks indicate a significant diet effect within site.

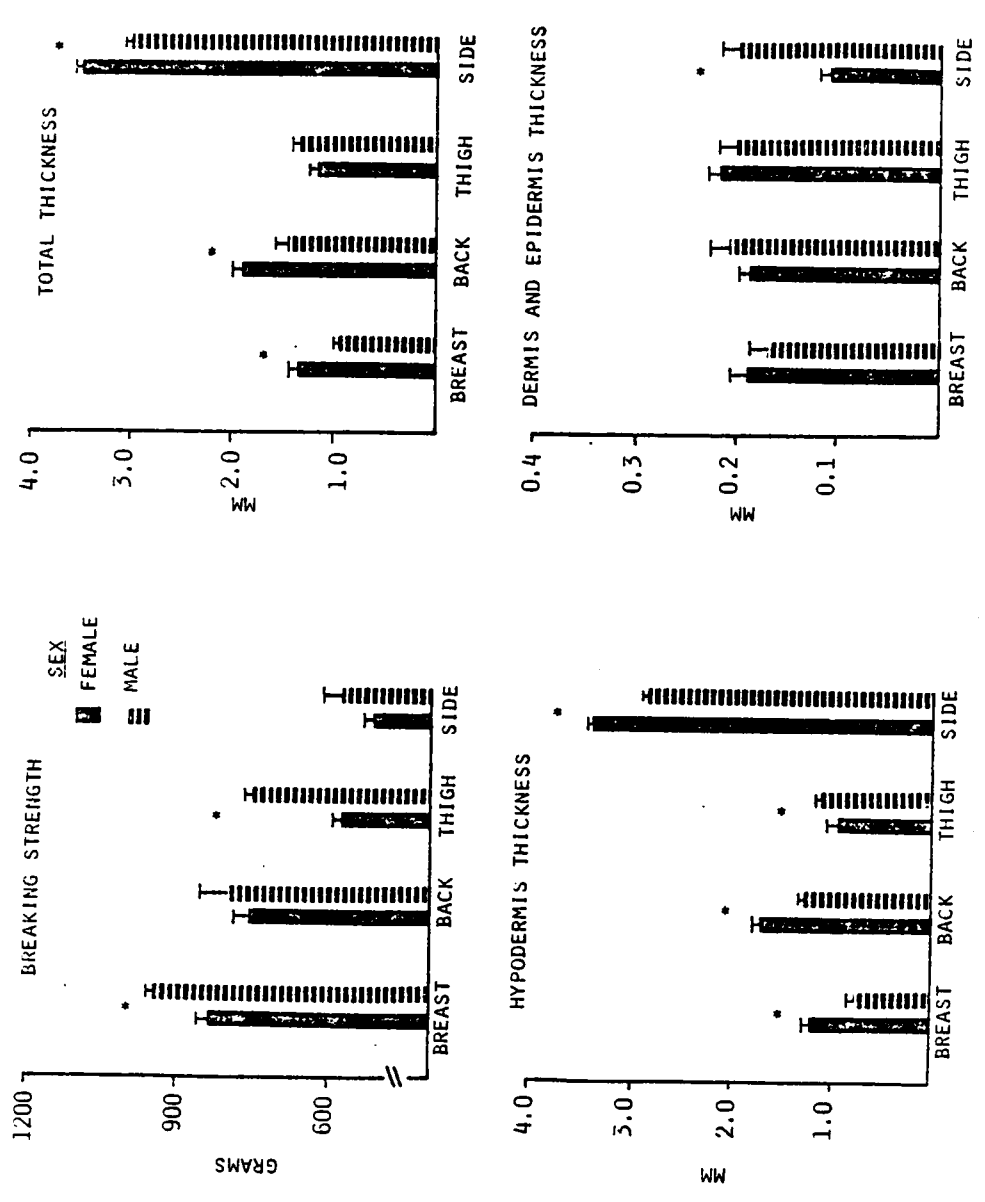

Figure 7. The relationship between skin breaking strength, total skin thickness, hypodermis thickness, and dermis plus epidermis thickness in male and female broilers fed diets containing wide C:P ratios. Values are means ± standard errors. Asterisks indicate a significant sex effect within site.

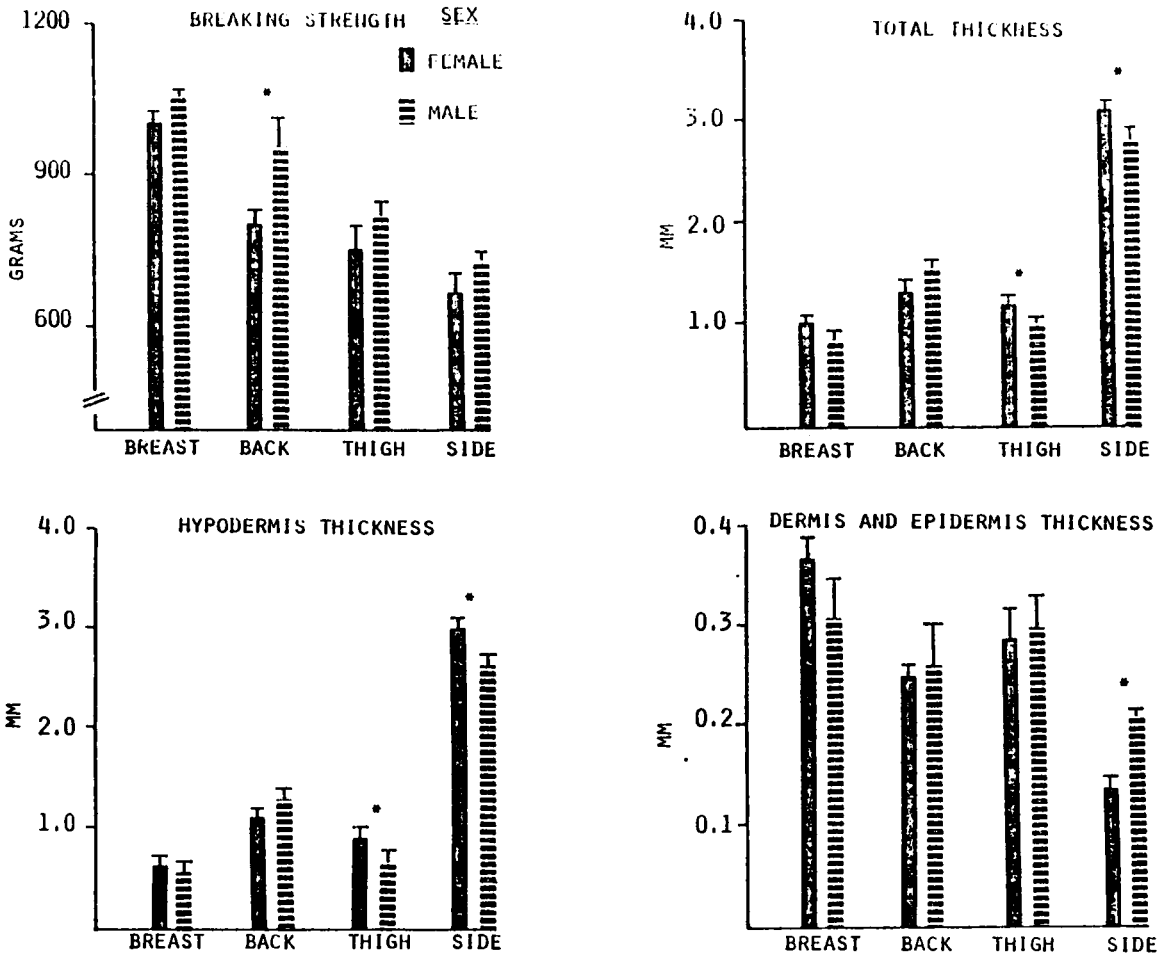

Figure 8. The relationship between skin breaking strength, total skin thickness, hypodermis thickness, and dermis plus epidermis thickness in male and female broilers fed diets containing narrow C:P ratios. Values are means ± standard errors. Asterisks indicate a significant sex effect within site.

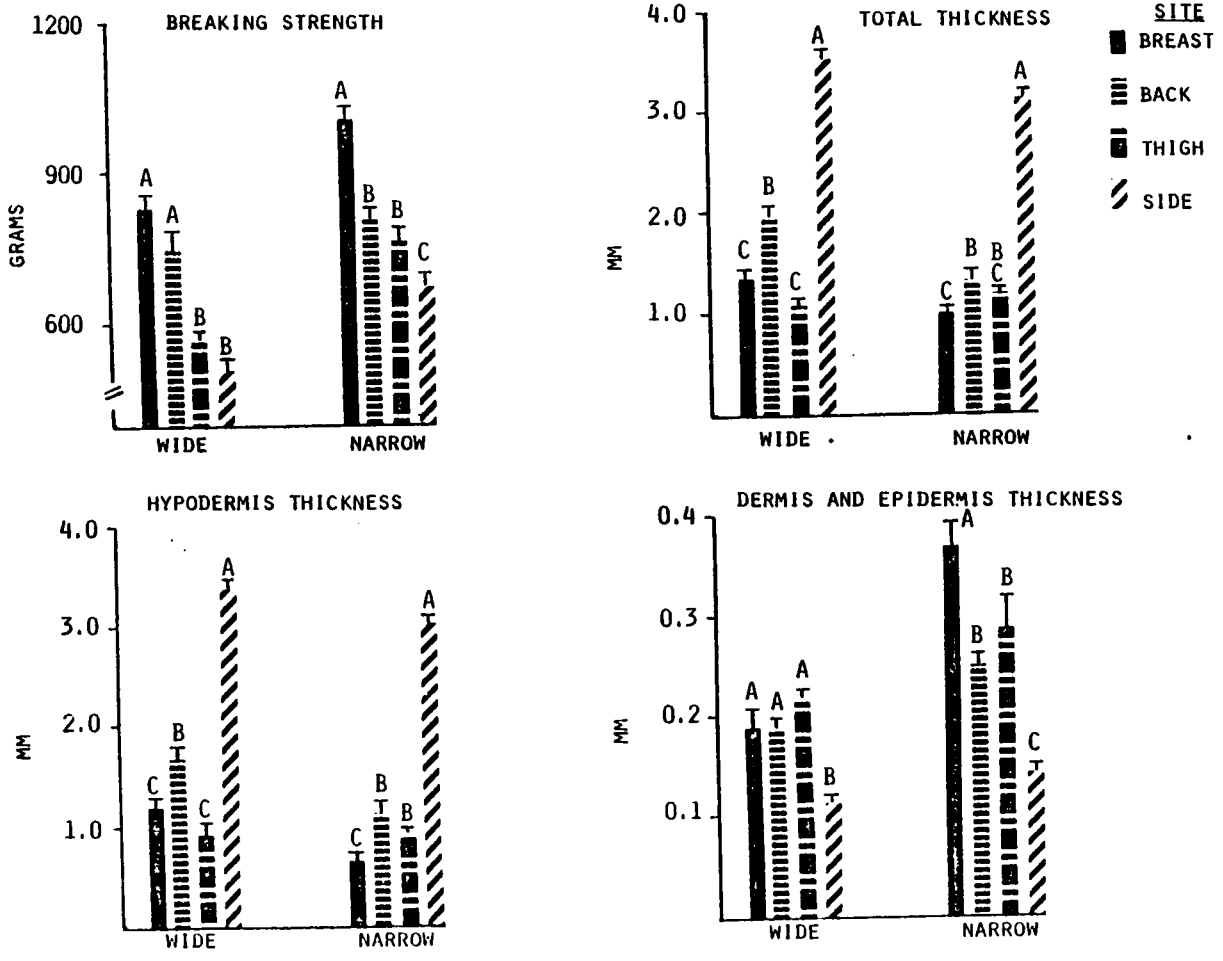

Figure 9. The relationship between skin breaking strength, total skin thickness, hypodermis thickness, and dermis plus epidermis thickness at different skin sites of female broilers. Values are means ± standard errors. Different letters indicate significant site effects within diet.

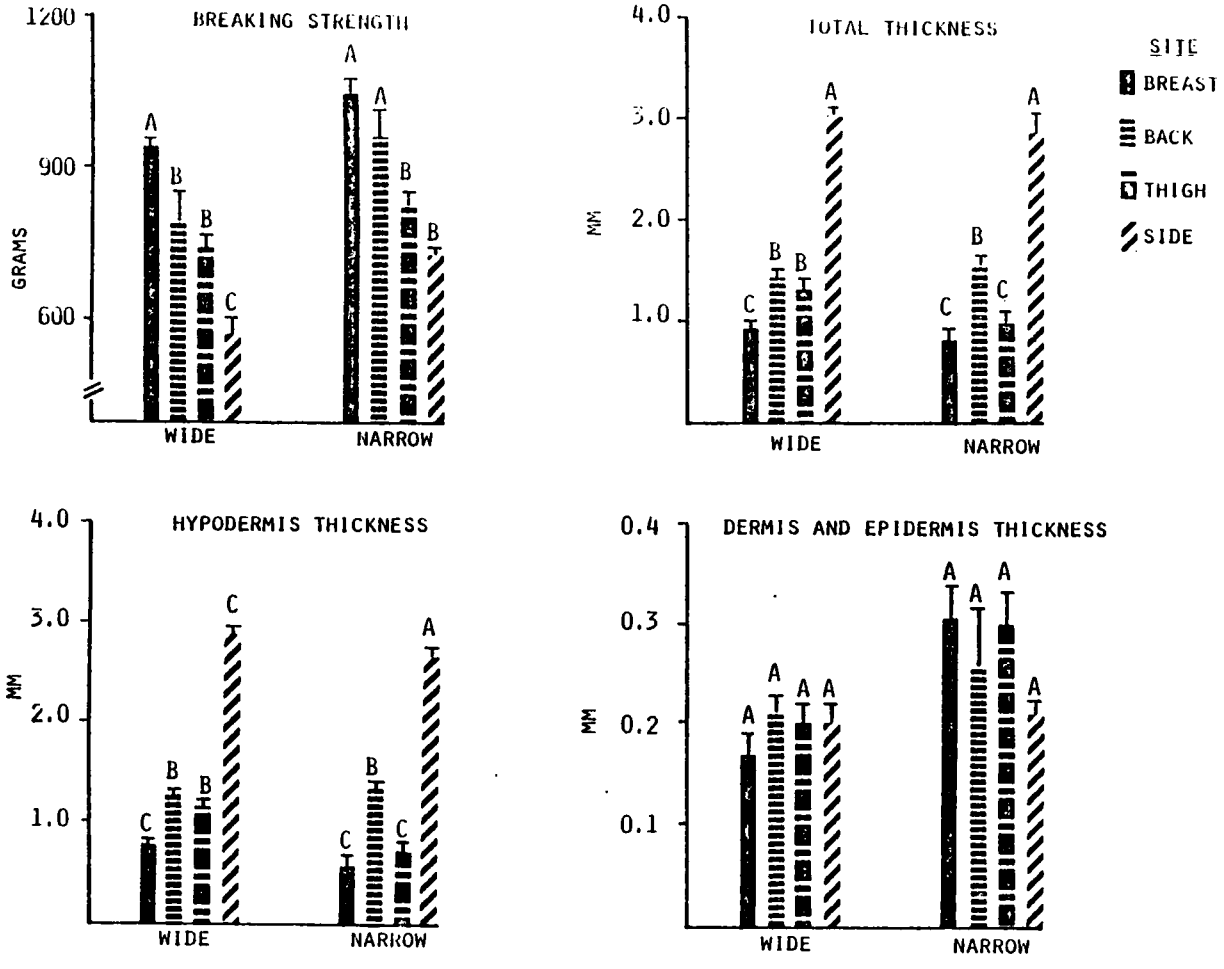

Figure 10. The relationship between skin breaking strength, total skin thickness, hypodermis thickness, and dermis plus epidermis thickness at different skin sites of male broilers. Values are means ± standard errors. Different letters indicate significant site effects within diet.

CHAPTER IV

BODY WEIGHT, BODY TEMPERATURE, AND SKIN STRENGTH OF BROILER CHICKS IN RESPONSE TO HEAT STRESS AND SUPPLEMENTAL ASCORBIC ACID

INTRODUCTION

Numerous morphological and physiological properties likely contribute to the integrity of the skin of chickens. Skin strength has been shown to vary due to sexual dimorphism (Edwards et al., 1973; Smith et al., 1977; Chapters II and III), to calorie-protein ratios of the diet (Chapters II and III), to age (Edwards et al., 1973; Chapter II), and genetic background (Chapter I). Skin strength tends to be inversely associated with total skin thickness (Chapter III), but attempts to correlate breaking strength with the chemical composition of the skin have been unsuccessful (Chapter II).

The principal objective of this study was to examine skin strength in relation to dietary ascorbic acid (vitamin C) and heat stress. Ascorbic acid serves as a cofactor for both prolyl and lysyl hydroxylase (Blank and Peterkovsky, 1975), enzymes which catalyze the hydroxylation of prolyl and lysyl residues during collagen biosynthesis. Although ascorbic acid is not considered to be an essential nutrient for the chicken, ascorbic acid synthesis may not proceed at a sufficient rate to maintain maximal skin collagen biosynthesis under heat stress conditions (Bondi, 1982).

MATERIALS AND METHODS

Two diets containing either 0 or 100 mg/kg of supplemental L-ascorbic acid were each fed ad libitum to 100 male and 100 female chicks beginning at 1 day of age. The chicks were reared in litter-covered floor pens with sexes intermingled. A continuous photoperiod was provided.

At 23 days of age, the chicks were randomly separated within diets into 6 treatment groups containing 30 males and 30 females. One group fed each diet was then subjected to either a constant ambient temperature of 23C, a constant ambient temperature of 32C or a daily temperature treatment (32/40C) consisting of 32C from 1600 to 1200 hr and 40C from 1200 to 1600 hr.

At 27 and 30 days of age, cloacal temperatures were measured to the nearest 0.1C at 1100 hr and again at 1400 hr. Temperatures were determined using a Fisher digital electronic thermometer and a YSI series 400 Thermistor probe (T2625).

At 34 days of age, the chicks were weighed individually to the nearest g and 5 males and 5 females from each diet-temperature treatment group were selected at random, killed by cervical dislocation, and defeathered by dry picking. Uniform sections of skin were then removed for breaking strength determinations using the procedures described in Chapter I. Skin samples were removed from the lateral body

apterium (breast) and from the areas delineated by the dorsopelvic (back) and femoral (thigh) feather tracts. The same samples used for breaking strength determinations were also analyzed chemically for percent lipid (Folch et al., 1965), percent protein (Nir et al., 1974), and percent moisture (Association of Official Analytical Chemists, 1975). Also, total skin collagen, expressed as a percentage of protein, was estimated from hydroxyproline concentrations as described by Logan et al. (1950).

Data were analyzed by analysis of variance within each sex and site with diet and ambient temperature considered as fixed effects. The statistical model was:

$$Y_{ij} = \mu + D_i + T_j + (DT)_{ij} + e_{ijk}$$

where i = 1, 2 diets, j = 1, 2, 3 temperatures, and k = 1, 2 . . . 5 individuals. Prior to analysis, body weight and breaking strength values were subjected to logarithmic transformations, and all percentages were transformed to arc sine square roots. When significance was obtained, the means were separated by Duncan's multiple range test. When significant interactions among main variables occurred, the data were analyzed within pairs of variables.

RESULTS

Body weight. Significant differences in body weight occurred in response to both ambient temperature and supplemental ascorbic acid (Table 12). Females subjected to the heat stress treatments (32C, 32/40C) were smaller than the controls maintained at 23C regardless of whether the diets contained supplemental ascorbic acid. No differences in body weight between chicks subjected to the 32C and 32/40C treatments were observed. Similar results were obtained with males except that body weight was similar at 23C and 32C when the diets contained supplemental ascorbic acid.

With the exception of the males subjected to the 32/40C ambient temperature treatment, supplemental ascorbic acid increased body weights of chicks exposed to the higher ambient temperature treatments (32C, 32/40C) in comparison with the controls maintained at 23C. Differences in body weight of the controls (23C) in response to supplemental ascorbic acid were not significant.

Body temperatures. Preliminary analyses indicated that body temperatures did not differ significantly between males and females, thus the data were pooled. At both 27 and 30 days of age, heat stress (32C, 32/40C) induced a significant increase in cloacal temperatures when measured at 1100 hr (Table 13). No differences between chicks subjected to the

32 and 32/40C treatments were observed. When body temperatures were measured at 1400 hr, a time when the chicks subjected to the 32/40C treatment were experiencing an ambient temperature of 40C, cloacal temperatures were higher in the chicks subjected to the 32/40C treatment than in those maintained at a constant temperature of 32C. With the exception of the 27-day-old chicks fed diets supplemented with ascorbic acid, cloacal temperature at 1400 hr were higher in the chicks maintained at 32C than in those maintained at 23C.

Increases in cloacal temperatures in response to heat stress were not alleviated by supplemental ascorbic acid. When temperatures of 30-day-old chicks were measured at 1100 hr, ascorbic acid supplementation resulted in a slight but significant increase in cloacal temperature irrespective of ambient temperature. A significant increase in cloacal temperatures in response to ascorbic acid supplementation was also observed in 27-day-old control (23C) chicks when temperatures were measured at 1400 hr. No other significant differences in body temperatures in response to supplemental ascorbic acid were obtained.

Skin breaking strength. Neither supplemental ascorbic acid nor heat stress had a consistent effect on skin strength (Table 14). In males, no significant differences were obtained for either breast skin or thigh skin. A

significant ambient temperature by ascorbic acid interaction for breaking strength of breast skin was observed, however. In the presence of supplemental ascorbic acid, breast skin from chicks subjected to the 32C treatment was weaker than the skin of the chicks maintained at 23C, while the chicks subjected to the 32/40C temperature treatment exhibited intermediate values for breast skin strength. When the diets were not supplemented with ascorbic acid, ambient temperatures did not affect breaking strength of the breast skin from males.

With females, breaking strength of back and thigh skin was significantly reduced by the 32C ambient temperature treatment. A similar decrease in breaking strength occurred for thigh skin in response to the 32/40C treatment, but breaking strength of back skin from the chicks subjected to the 32/40C treatment did not differ from control values. Ambient temperatures did not affect the strength of the breast skin of females when the diets were not supplemented with ascorbic acid. When ascorbic acid was added to the diet, however, breast skin from females maintained at 32C was stronger than skin from those maintained at 23C or from those subjected to the 32/40C ambient temperature treatment.

Skin composition. No significant differences in either lipid (Table 15) or moisture (Table 16) concentrations in the skin were observed in response to supplemental ascorbic

acid or heat stress. Both protein (Table 17) and collagen concentrations (Table 18), however, differed in response to these treatments.

Protein concentrations of the skin are presented in Table 17. Regardless of sex, no significant differences for breast skin were observed. The effects of heat stress on protein concentrations of back and thigh skin were inconsistent between sexes. In males, the protein content of back skin was lower in the chicks subjected to the 32/40C ambient temperature treatment than in those maintained at 23C or 32C. The protein content of back skin from females did not differ significantly due to ascorbic acid or heat stress. The protein content of thigh skin was increased in response to heat stress in females, with the differences between chicks under the 23C and 32/40C temperature treatments being significant. Heat stress did not influence the protein content of thigh skin from males.

Supplemental ascorbic acid significantly increased the protein content of back and thigh skin of males irrespective of ambient temperature, but ascorbic acid did not affect protein levels in skin from the breast of males. No differences in skin protein due to supplemental ascorbic acid occurred in females.

Heat stress tended to increase skin collagen concentrations (Table 18). In every instance in which

significant differences due to ambient temperature occurred, an increase in skin collagen in response to increased ambient temperature was observed.

Supplemental ascorbic acid tended to increase collagen concentrations in breast and back skin from males. An increase in skin collagen in response to ascorbic acid also occurred in the breast skin from females subjected to the 32/40C ambient temperature treatment. No significant responses to ascorbic acid were observed for back skin from females or for thigh skin from chicks of either sex.

DISCUSSION

The chicks maintained under the higher ambient temperatures were apparently unable to maintain homeothermy; increases in body temperature resulted from the higher ambient temperature treatments (Table 13). This heat stress consistently resulted in reduced body weights (Table 12). The chicks subjected to the 32/40C temperature treatment tended to have higher body temperatures than those maintained at 32C when measurements were taken during the period in which the chicks receiving the 32/40C treatment were exposed to an ambient temperature of 40C. Although these body temperature data suggest that the chicks receiving the 32/40C treatment were stressed more than those maintained at 32C, no consistent differences in body weight between chicks from these two groups occurred. Nevertheless, both body weight and body temperature data indicated that the chicks exposed to the higher ambient temperatures experienced heat stress.

Heat stress did not appear to impair collagen biosynthesis. To the contrary, increases in skin collagen tended to accompany heat stress (Table 18). An increase in the collagen content of skin from males also tended to result in response to supplemental ascorbic acid. Smith et al. (1977) also observed increased skin collagen concentrations in response to supplemental ascorbic acid.

Neither lipid (Table 15) nor moisture (Table 16) content of the skin differed significantly in this experiment, but ascorbic acid tended to increase the protein content of skin from males (Table 17). It has been suggested that reduced skin fat and/or an associated increase in protein or collagen concentrations results in stronger skin (Smith et al., 1977). This clearly did not occur. There appeared to be absolutely no relationship between differences in breaking strength and differences in skin composition due to heat stress or to supplemental ascorbic acid. These results were consistent with previous observations that differences in breaking strength of the skin due to sexual dimorphism, calorie-protein ratios of the diet, or to body site from which skin samples were taken could not be explained by differences in the chemical composition of the skin (Chapter II).

Although supplemental ascorbic acid did not improve skin breaking strength, it did ameliorate the depressive effect of heat stress on body weight (Table 12). This response was not entirely unexpected. Although ascorbic acid is not considered an essential nutrient for domestic fowl, its biosynthesis may be insufficient under stressful conditions (Hill and Garren, 1958; Thornton and Moreng, 1958; Nestor et al., 1972; Pardue and Thaxton, 1984). Depletion of ascorbic acid from the adrenals and plasma of

stressed birds has been observed by several laboratories (Satterfield et al., 1940; Hill and Garren, 1958; Perek et al., 1959; Perek and Elat, 1960; Nockels et al., 1973).

The ability of ascorbic acid to alleviate the effects of stress on egg production and egg quality has been examined in numerous experiments. The results have been inconsistent. Supplemental ascorbic acid improved egg production in some experiments (Perek and Kendler, 1962, 1963; Hunt and Aitken, 1962; Peebles and Brake, 1983) while no benefits were observed in others (Thornton and Moreng, 1959; Harms and Waldroup, 1961; Hunt and Aitken, 1962; Naber et al., 1963; Nestor et al., 1972). Supplemental ascorbic acid was also reported to be beneficial in improving interior or exterior egg qualities in some studies (Thornton and Moreng, 1958, 1959; El-Boushy et al., 1968; Nockels et al., 1968; Herrick and Nockels, 1969; Lyle and Moreng, 1968), but ineffective in others (Heywang and Kemmerer, 1955; Harms and Waldroup, 1961; Hunt and Aitken, 1962; Arscott et al., 1962; Perek and Kendler, 1962, 1963; Naber et al., 1963; Heywang et al., 1964; Dorr and Nockels, 1971; Nestor et al., 1972).

In contrast to effects on egg production, surprisingly few experiments have been conducted to determine the effectiveness of supplemental ascorbic acid in alleviating growth depression due to stress. Pardue et al. (1983)

reported a beneficial effect of ascorbic acid on body weight when broilers were exposed to heat stress over a period of two days. Schmeling and Nockels (1978) reported a growth response to supplemental ascorbic acid and attributed the results to an inhibition of corticosterone synthesis. Growth responses to ascorbic acid supplementation were also observed by March and Biely (1953). Supplemental ascorbic acid, therefore, may be beneficial in counteracting reduced growth in chicks due to stress, but the mechanism needs clarification. Contrary to previous reports (Ahmad et al., 1967; Lyle and Moreng, 1968), ascorbic acid did not reduce body temperature of heat-stressed birds in this study (Table 13). This failure suggests that ascorbic acid does not prevent heat stress; rather, it apparently minimizes the stress insult.

Table 12. Means and standard errors for body weight of 34-day old male and female broiler chicks in response to heat stress and ascorbic acid supplementation

Sex	Ascorbic Acid	Ambient temperature 23	32	32/40	$\bar{x}$
	-mg/kg-	----------g----------			
Male	0	1105 ± 36^{aA}	986 ± 36^{bB}	1003 ± 23^{aB}	1031
	100	1115 ± 28^{aA}	1103 ± 23^{aA}	1031 ± 21^{aB}	1083
	$\bar{x}$	1110	1044	1017	
Female	0	1021 ± 25^{aA}	858 ± 38^{bB}	878 ± 20^{bB}	919
	100	1045 ± 25^{aA}	953 ± 17^{aB}	947 ± 24^{aB}	982
	$\bar{x}$	1033	906	912	

^{ab,AB}Within sexes, means in a column having the same lower case superscript were not significantly different and means in a row having the same upper case superscript were not significantly different ($P > .05$).

Table 13. Means and standard errors for cloacal temperature of broiler chicks in response to heat stress and supplemental ascorbic acid

Age	Time	Ascorbic Acid	Ambient temperature 23	32	32/40	$\bar{x}$
-d-	-hr-	-mg/kg-	----------C----------			
27	1100	0	41.7 ± 0.2	42.1 ± 0.1	42.3 ± 0.1	41.9^{a}
		100	41.5 ± 0.1	42.0 ± 0.1	41.8 ± 0.5	41.7^{a}
		$\bar{x}$	41.6^{B}	42.0^{A}	42.0^{A}	
27	1400	0	41.5 ± 0.1^{aC}	42.1 ± 0.1aB	42.7 ± 0.2^{bA}	42.4
		100	41.7 ± 0.1^{aB}	41.9 ± 0.1aB	43.5 ± 0.4^{aA}	42.3
		$\bar{x}$	41.6	42.2	43.1	
30	1100	0	41.7 ± 0.1	41.7 ± 0.2	42.0 ± 0.2	41.9^{b}
		100	41.9 ± 0.1	42.0 ± 0.1	42.2 ± 0.2	42.0^{a}
		$\bar{x}$	41.8^{B}	42.0^{A}	42.1^{A}	
30	1400	0	41.4 ± 0.1	41.7 ± 0.1	43.1 ± 0.1	42.1^{a}
		100	41.6 ± 0.2	42.4 ± 0.1	43.0 ± 0.2	42.3^{a}
		$\bar{x}$	41.5^{C}	42.0^{B}	43.0^{A}	

^{ab,ABC}Within age-time subgroups, means in a row having different upper case superscripts were significantly different while those in a column having the same lower case superscripts were significantly different ($P \leq .05$). When ascorbic acid by ambient temperature interactions were not significant, significance was denoted by main effects only.

Table 14. Means and standard errors for skin breaking strength of broiler chicks in response to heat stress and ascorbic acid supplementation

Sex	Site	Ascorbic Acid	Ambient temperature 23	32	32/40	$\bar{x}$
		-mg/kg-	-----------g-----------			
Male	Breast	0	733 ± 23^{a}	764 ± 42^{a}	814 ± 33^{a}	770
		100	814 ± 35^{aA}	696 ± 25^{aB}	725 ± 34a^{AB}	745^{a}
		$\bar{x}$	774	730	770	
	Back	0	598 ± 23	630 ± 43	532 ± 25	587^{a}
		100	620 ± 24	587 ± 26	650 ± 43	619^{a}
		$\bar{x}$	609^{A}	608^{A}	591^{A}	
	Thigh	0	618 ± 32	605 ± 47	557 ± 27	593^{a}
		100	620 ± 23	513 ± 30	581 ± 46	571^{a}
		$\bar{x}$	619^{A}	559^{A}	569^{A}	
Female	Breast	0	637 ± 35^{aA}	724 ± 36^{aA}	660 ± 32^{aA}	674
		100	598 ± 12^{aB}	761 ± 28^{aA}	557 ± 27^{bB}	639
		$\bar{x}$	618	742	608	
	Back	0	554 ± 26	482 ± 16	572 ± 32	536^{a}
		100	568 ± 19	541 ± 24	543 ± 19	551^{a}
		$\bar{x}$	561^{A}	512^{B}	558^{A}	
	Thigh	0	509 ± 23	431 ± 24	424 ± 39	455^{a}
		100	528 ± 24	488 ± 24	448 ± 19	488^{a}
		$\bar{x}$	518^{A}	460^{B}	436^{B}	

^{ab,ABC}Within sex-site subgroups, means in a row having different upper case superscripts were significantly different while those in a column having different lower case superscripts were significantly different ($P \leq .05$). When ascorbic acid by ambient temperature interactions were not significant, significance was denoted by main effects only.

Table 15. Means and standard errors for lipid content of the skin of broiler chicks in response to heat stress and supplemental ascorbic acid

Sex	Site	Ascorbic Acid	Ambient Temperature 23	32	32/40	$\bar{x}$
		mg/kg	----------%----------			
Male	Breast	0	39.3 ± 2.1	39.1 ± 1.4	39.9 ± 1.6	39.4^{a}
		100	37.6 ± 1.0	43.6 ± 1.4	42.9 ± 1.7	41.4^{a}
		$\bar{x}$	38.4^{A}	41.4^{A}	41.4^{A}	
	Back	0	54.2 ± 2.1	54.4 ± 1.4	55.0 ± 1.7	54.5^{a}
		100	56.0 ± 1.6	56.0 ± 1.6	56.2 ± 1.7	54.8^{a}
		$\bar{x}$	53.2^{A}	55.2^{A}	55.6^{A}	
	Thigh	0	29.6 ± 1.5	29.5 ± 2.1	29.6 ± 1.4	29.6^{a}
		100	28.7 ± 0.8	31.6 ± 1.6	32.1 ± 1.4	30.8^{a}
		$\bar{x}$	29.2^{A}	30.6^{A}	30.8^{A}	
Female	Breast	0	41.2 ± 0.8	44.1 ± 2.1	40.5 ± 1.6	41.9^{a}
		100	40.3 ± 1.1	43.5 ± 1.3	41.1 ± 1.9	41.6^{a}
		$\bar{x}$	40.8^{A}	43.8^{A}	40.8^{A}	
	Back	0	55.3 ± 1.5	58.9 ± 1.8	56.3 ± 1.9	56.8^{a}
		100	57.3 ± 1.5	56.9 ± 1.8	57.4 ± 1.4	57.2^{a}
		$\bar{x}$	56.3^{A}	57.9^{A}	56.8^{A}	
	Thigh	0	34.7 ± 0.8	33.6 ± 1.4	30.7 ± 0.8	33.0^{a}
		100	32.9 ± 1.1	34.5 ± 1.9	35.6 ± 1.2	34.3^{a}
		$\bar{x}$	33.8^{A}	34.0^{A}	33.2^{A}	

^{ab,ABC}Within sex-site subgroups, means in a row having different upper case superscripts were significantly different while those in a column having different lower case superscripts were significantly different ($P \leq .05$). Ascorbic acid by ambient temperature interactions were not significant.

Table 16. Means and standard errors for moisture content of the skin of broiler chickens in response to heat stress and ascorbic acid supplementation

Sex	Site	Ascorbic Acid	Ambient Temperature 23	32	32/40	$\bar{x}$
		mg/kg	----------%----------			
Male	Breast	0	49.6 ± 1.8	48.6 ± 1.3	49.6 ± 1.6	49.3[a]
		100	50.2 ± 1.2	45.3 ± 1.1	46.0 ± 1.2	47.2[a]
		$\bar{x}$	49.9[A]	47.0[A]	47.8[A]	
	Back	0	37.1 ± 2.3	36.6 ± 1.2	37.5 ± 1.4	37.1[a]
		100	38.7 ± 0.9	35.8 ± 1.3	35.6 ± 1.5	36.7[a]
		$\bar{x}$	37.9[A]	36.2[A]	36.6[A]	
	Thigh	0	56.1 ± 1.4	55.2 ± 1.7	56.2 ± 1.3	55.8[a]
		100	56.5 ± 0.8	53.6 ± 1.0	53.6 ± 1.0	54.6[a]
		$\bar{x}$	56.3[A]	54.4[A]	54.9[A]	
Female	Breast	0	48.1 ± 0.8	46.5 ± 1.4	48.3 ± 1.4	47.6[a]
		100	48.7 ± 1.2	45.5 ± 0.9	48.5 ± 1.7	47.6[a]
		$\bar{x}$	48.5[A]	46.0[A]	48.4[A]	
	Back	0	37.4 ± 1.3	33.7 ± 1.6	36.7 ± 1.4	35.9[a]
		100	35.3 ± 1.5	35.9 ± 1.5	35.6 ± 1.2	35.6[a]
		$\bar{x}$	36.4[A]	34.8[A]	36.2[A]	
	Thigh	0	52.3 ± 0.8	54.2 ± 1.7	55.0 ± 0.9	53.8[a]
		100	54.1 ± 1.1	52.4 ± 1.7	53.5 ± 1.3	53.2[a]
		$\bar{x}$	53.2[A]	53.3[A]	54.2[A]	

[ab,ABC]Within sex-site subgroups, means in a row having different upper case superscripts were significantly different while those in a column having different lower case superscripts were significantly different ($P \leq .05$). Ascorbic acid by ambient temperature interactions were not significant.

Table 17. Means and standard errors for protein content of the skin of broiler chickens in response to heat stress and ascorbic acid supplementation

Sex	Site	Ascorbic Acid	Ambient Temperature 23	32	32/40	$\bar{x}$
		mg/kg	----------%----------			
Male	Breast	0	12.0 ± 0.4	12.2 ± 0.4	10.9 ± 0.4	11.7^{a}
		100	12.2 ± 0.2	11.4 ± 0.4	11.5 ± 0.4	11.7^{a}
		$\bar{x}$	12.1^{A}	11.8^{A}	11.2^{A}	
	Back	0	8.4 ± 0.3	8.7 ± 0.2	7.7 ± 0.3	8.3^{b}
		100	9.2 ± 0.1	8.8 ± 0.2	8.3 ± 0.2	8.8^{a}
		$\bar{x}$	8.8^{A}	8.8^{A}	8.0^{B}	
	Thigh	0	14.1 ± 0.4	15.0 ± 0.3	13.9 ± 0.5	14.3^{b}
		100	14.9 ± 0.3	15.3 ± 0.3	14.7 ± 0.3	15.0^{a}
		$\bar{x}$	14.5^{A}	15.2^{A}	14.3^{A}	
Female	Breast	0	11.0 ± 0.4	11.4 ± 0.4	11.0 ± 0.2	11.1^{a}
		100	11.1 ± 0.3	11.6 ± 0.3	10.8 ± 0.6	11.2^{a}
		$\bar{x}$	11.0^{A}	11.5^{A}	10.9^{A}	
	Back	0	7.1 ± 0.1	7.5 ± 0.3	7.4 ± 0.2	7.3^{a}
		100	7.6 ± 0.2	7.6 ± 0.1	7.2 ± 0.3	7.5^{a}
		$\bar{x}$	7.4^{A}	7.6^{A}	7.3^{A}	
	Thigh	0	13.0 ± 0.3	13.5 ± 0.4	14.0 ± 0.2	13.5^{a}
		100	13.0 ± 0.2	13.4 ± 0.3	13.9 ± 0.4	13.4^{a}
		$\bar{x}$	13.0^{B}	13.4^{AB}	14.0^{A}	

^{ab,ABC}Within sex-site subgroups, means in a row having different upper case superscripts were significantly different while those in a column having different lower case superscripts were significantly different ($P \leq .05$). Ascorbic acid by ambient temperature interactions were not significant.

Table 18. Means and standard errors for collagen content of the skin of broiler chickens in response to heat stress and ascorbic acid supplementation

Sex	Site	Ascorbic Acid	Ambient Temperature 23	32	32/40	$\bar{x}$
		mg/kg	---------% of protein----------			
Male	Breast	0	62.1 ± 3.1^{bB}	70.8 ± 2.1^{bA}	66.5 ± 3.0^{aAb}	66.8
		100	72.7 ± 3.0^{aA}	77.0 ± 1.7^{aA}	71.9 ± 2.5^{aA}	73.9
		$\bar{x}$	67.4	73.9	69.2	
	Back	0	52.2 ± 4.9	57.9 ± 2.1	63.3 ± 3.2	57.8^{b}
		100	65.0 ± 2.7	61.1 ± 1.8	68.2 ± 2.3	64.8^{a}
		$\bar{x}$	58.6^{B}	59.5^{B}	65.8^{A}	
	Thigh	0	68.2 ± 2.3	82.0 ± 2.5	78.2 ± 1.9	76.1^{a}
		100	74.3 ± 2.8	83.7 ± 2.8	79.6 ± 2.8	79.2^{a}
		$\bar{x}$	71.2^{B}	82.8^{A}	78.9^{A}	
Female	Breast	0	66.3 ± 2.6^{aA}	63.6 ± 1.2^{aA}	63.0 ± 2.4^{bA}	64.3
		100	59.2 ± 2.9^{aB}	69.2 ± 2.9^{aA}	70.4 ± 1.6^{aA}	66.3
		$\bar{x}$	62.8	66.4	66.7	
	Back	0	56.2 ± 2.1^{aA}	59.2 ± 1.6^{aA}	53.5 ± 8.5^{aA}	56.3
		100	48.7 ± 3.0^{aC}	57.5 ± 2.3^{aB}	60.3 ± 1.6^{aA}	55.5
		$\bar{x}$	52.4	58.4	56.9	
	Thigh	0	72.3 ± 1.5	73.4 ± 2.1	76.2 ± 2.4	74.0^{a}
		100	67.6 ± 2.2	76.5 ± 2.8	76.3 ± 2.5	73.5^{a}
		$\bar{x}$	70.0^{B}	75.0^{A}	76.2^{A}	

^{ab,ABC}Within sex-site subgroups, means in a row having different upper case superscripts were significantly different while those in a column having different lower case superscripts were significantly different ($P \leq .05$). When ascorbic acid by ambient temperature interactions were not significant, significance was denoted by main effects only.

CHAPTER V

SUPPLEMENTAL ASCORBIC ACID AND HEAT STRESS IN BROILER CHICKS

INTRODUCTION

Chickens resemble mammals in that numerous morphological and physiological changes accompany stress. In chickens, acute stress is associated with rapid increases in blood ACTH (Edens and Siegel, 1975), enhanced steroidogenesis (Siegel, 1985; Freeman, 1985), increased utilization of adrenal cholesterol (Wolford and Ringer, 1963), and increased secretion of catecholamines by the adrenal medulla (Edens and Siegel, 1973, 1975). Increased catecholamine secretion results in decreases in skin temperature due to peripheral vasodilation (Richards, 1970; Misson, 1982), and increases in heart rate, blood pressure, and blood glucose concentrations (Freeman, 1970).

Although ascorbic acid is not considered an essential dietary constituent for the chicken, this nutrient is apparently involved in stress responses. Ascorbic acid secretion from the adrenal gland is stimulated by stressors (Freeman, 1970), sometimes resulting in increased concentrations of ascorbic acid in the peripheral circulatory system (Briggs and Toepel, 1958). Low plasma levels of ascorbic acid are associated with reduced immunological function in the fowl (Pardue and Thaxton, 1984) as well as mammals (Pauling, 1976; Prinz et al., 1980). Exogenous corticosteroids reduce plasma ascorbic acid levels and dietary ascorbic acid ameliorates this

depletion (Pardue and Thaxton, 1984). Moreover, supplemental ascorbic acid may increase body weights of chicks subjected to heat stress (Pardue et al., 1983; Chapter IV) and it has been suggested that ascorbic acid may be an essential nutrient for the domestic chicken under stressful conditions (Bondi, 1982).

The purpose of the experiment reported here was to examine further the ability of dietary ascorbic acid to ameliorate the effects of heat stress on commercial broiler chicks. The potential of supplemental ascorbic acid in increasing skin strength was also investigated. Previous results (Chapter IV) indicated that supplemental ascorbic acid increased skin collagen concentrations but no significant increases in skin breaking strength were detected.

MATERIALS AND METHODS

Sixty male and 60 female commercial broiler chicks were assigned randomly into groups of 10 chicks of the same sex and caged in electrically-heated battery brooders. Corn-soybean meal based starting diets containing either 0, 200, or 400 mg of ascorbic acid/kg of diet were each fed to two groups of males and two groups of females beginning at one day of age. Both feed and water were provided ad libitum. Lighting was continuous.

At 21 days of age, the chicks were transferred to litter-floor pens in groups of 20. Each group contained 10 males and 10 females fed the same diet. Beginning at 33 days of age, half the chicks fed each diet were exposed to a constant ambient temperature of 23C while the remainder was subjected to a constant ambient temperature of 34C. These two temperature treatments were maintained until the experiment was terminated when the chicks were 49 days of age.

The chicks were weighed individually to the nearest g at 40 days of age and surface (foot pad) and core (cloacal) body temperatures were measured to the nearest 0.1C. Surface body temperatures were measured from the right foot pad using a Fisher Digital Electronic Thermometer and YSI series 400 Thermistor probe (T2605). Core temperatures were measured using similar techniques except a different probe

(T2625) was used.

At 49 days of age, blood samples were collected from the brachial vein for the determination of plasma ascorbic acid (Zannoni, 1974), and for heterophil and lymphocyte counting (Gross and Siegel, 1983). Each leucocyte count was for 60 total cells including both heterophils and lymphocytes. Heterophil-lymphocyte (H/L) ratios were then determined by dividing the number of heterophils by the number of lymphocytes.

Immediately after blood was collected, the chicks were weighed and killed by cervical dislocation. Within five minutes after death, both adrenal glands were removed, pooled, weighed to the nearest 0.1 mg, and stored in cold 5% TCA solutions for the subsequent determination of ascorbic acid concentrations (Zannoni, 1973). Uniform sections of skin were then removed from the back at the area delineated by the dorsopelvic feather tract for skin breaking strength determinations (Chapter I).

The data were analyzed within sexes by analyses of variance with diet and ambient temperature considered fixed effects. The statistical model was:

$$Y_{ijk} = \mu + D_i + T_j + (DT)_{ij} + e_{ijk}$$

where i = 1, 2, 3 diets, j = 1, 2 ambient temperatures, and k = 1, 2 . . . 5 individuals. Prior to analyses, body,

adrenal, spleen, and bursa of Fabricius weights, and breaking strength values were transformed to natural logarithms because means and variances were correlated. Adrenal, spleen, and bursa of Fabricius weights expressed on a body weight basis (g/100 g), adrenal ascorbic acid concentration, surface-core (S/C) ratios, and H/L ratios were transformed to arc sine square roots. When differences due to diet were significant, the means were separated by Duncan's multiple range test.

RESULTS

Body weight. Prior to the initiation of the ambient temperature treatments at 23 days of age, supplemental ascorbic acid did not significantly affect body weights (Table 19). Subsequent to the initiation of the temperature treatments, the chicks maintained at 23C were significantly heavier than those maintained at 34C, with the exception of the females at 40 days of age (Table 20). Irrespective of ambient temperature, supplemental ascorbic acid did not significantly affect body weight.

Body temperature. Core body temperatures of the chicks subjected to the 34C ambient temperature treatment were higher than those of chicks maintained at 23C (Table 21). Supplemental ascorbic acid did not significantly affect core body temperatures regardless of ambient temperature. A significant ascorbic acid by ambient temperature interaction occurred, however, for surface body temperature. Supplemental ascorbic acid increased surface temperatures of chicks maintained at 23C but not at 34C. Similar to core temperatures, surface temperatures were higher in response to the higher ambient temperature treatment (34C). S/C temperature ratios followed patterns similar to those for surface temperatures.

Skin breaking strength. Neither ambient temperature nor supplemental ascorbic acid significantly affected skin

strength (Table 22).

Heterophil and lymphocyte number. No significant differences in heterophil number, lymphocyte number or H/L ratios in response to ambient temperature or supplemental ascorbic acid were observed in female chicks (Table 23). Neither were there significant differences in these traits in males due to ambient temperature. Supplemental ascorbic acid, however, significantly influenced heterophil and lymphocyte number in males. Feeding diets supplemented with 200 mg ascorbic acid/kg of diet resulted in a significant decrease in the number of lymphocytes, a significant increase in heterophil number, and a significantly higher H/L ratio in comparison to chicks receiving diets without supplemental ascorbic acid. These responses in males were consistent across ambient temperature treatments; no significant ambient temperature by ascorbic acid interactions were observed.

Spleen, adrenal and bursa of Fabricius weights. Means and standard errors for the weights of the spleen, bursa, and adrenal glands are presented in Table 24; weights expressed as mg/100 g of body weight are contained in Table 25. Regardless of how expressed, supplemental ascorbic acid had no effect on spleen weight. The chicks subjected to the 34C treatment had smaller spleens than chicks maintained at 23C. These differences were not entirely a function of body

weight. When corrected for body weight, spleens from male chicks were still significantly smaller in response to the 34C ambient temperature treatment.

Increased ambient temperature resulted in reduced bursa weights, although the differences were not significant when weights were expressed on a body weight basis. Supplemental ascorbic acid did not significantly affect bursa weight.

Increased ambient temperature did not consistently affect adrenal weights. Differences in adrenal weights in response to ambient temperature were not significant in females. With male chicks, a reduction in adrenal weights in response to the 34C ambient temperature treatment was observed when the diets contained 400 mg/kg of supplemental ascorbic acid but not when the diets containing 0 and 200 mg/kg of ascorbic acid were fed. Moreover, no significant differences in adrenal weights of males due to ambient temperature were obtained when weights were expressed on a body weight basis.

Regardless of ambient temperature, supplemental ascorbic acid reduced adrenal weights in female chicks (Tables 24 and 25). In males, a significant reduction in adrenal weights occurred only in chicks maintained at 23C and only in response to 200 mg/kg ascorbic acid. No differences in adrenal weights expressed on a body weight basis in response to supplemental ascorbic acid were

obtained for males.

Plasma and adrenal ascorbic acid concentrations. The concentration of ascorbic acid in plasma was not significantly affected by ambient temperature (Table 26). The feeding of diets supplemented with 400 mg/kg of ascorbic acid to females resulted in a significant decrease in plasma ascorbic acid. Similar reductions, however, were not observed in males or when the diets of females contained 200 mg/kg of ascorbic acid.

Neither dietary ascorbic acid levels nor ambient temperature significantly affected adrenal ascorbic acid concentrations (µg/100 mg). The total amount of ascorbic acid contained in the adrenals, however, differed significantly in response to these treatments. Adrenal ascorbic acid was lower in both male and female chicks maintained at 34C than in those maintained at 23C. Supplemental ascorbic acid resulted in a significant reduction in adrenal ascorbic acid in females. Adrenal ascorbic acid was also reduced in males in response to dietary ascorbic acid, but the differences were not significant.

DISCUSSION

Contrary to previous observations (Pardue et al., 1983; Chapter IV), supplemental ascorbic acid failed to ameliorate the depressive effect of high ambient temperature on body weights of broiler chicks (Table 20). The reason for this failure is not known, although several major differences existed among experiments in which the effects of ascorbic acid were examined in heat-stressed chicks. The beneficial effect of ascorbic acid supplementation reported by Pardue et al. (1983) was for broilers exposed to heat stress for a period of two days. In the present experiment, the broilers were subjected to heat stress for a period of 16 consecutive days. It may be that supplemental ascorbic acid is beneficial under mildly or moderately stressful conditions but not under more severe or prolonged heat stress. In this regard, responses to supplemental ascorbic acid were more pronounced in broiler chicks maintained at a constant temperature of 32C than in broilers subjected to a cyclic temperature treatment in which temperatures reached 40C daily (Chapter IV). Moreover, many of the reported physiological responses to supplemental ascorbic acid have been observed after comparatively brief exposure to stressful stimuli (Wolford and Ringer, 1963; Edens and Siegel, 1975; Ben-Nathan et al., 1977; Gross and Siegel, 1983; Freeman, 1985).

Husbandry practices have also differed in experiments involving ascorbic acid and heat stress. Previous responses to supplemental ascorbic acid reported in this dissertation (Chapter IV) were with broiler chicks reared in litter-floor pens from one day of age. In the present experiment, the birds were reared in wire-floored battery brooders before being transferred to floor pens at 21 days of age. A high incidence of leg abnormalities occurred in these chicks, resulting in wide variation in body weights (note the standard errors for body weight in Table 21). These problems could have masked growth responses to ascorbic acid in the present study.

Despite the failure of supplemental ascorbic acid to reduce the detrimental effects of heat stress on body weight, several observations appeared to be of interest. Dietary ascorbic acid, for instance, tended to reduce adrenal weight (Tables 24 and 25). It might be postulated that this reduction was associated with diminished adrenal corticosterone synthesis. Nockels and her co-workers (Nockels et al., 1973; Schmeling and Nockels, 1978) reported that supplemental ascorbic acid depressed corticosterone synthesis and reduced adrenal hypertrophy in vitamin A-deficient chicks. In vitro studies (Sulimovici and Boyd, 1968; Shimizu, 1970) indicated that high levels of ascorbic acid inhibited corticosterone synthesis while lower levels

stimulated synthesis. Kitabchi (1967) reported that mitochondrial steroid 11-β-hydroxylation and microsomal 21-hydroxylation in beef adrenals were inhibited by supplemental ascorbic acid. There was no evidence, however, that the chicks in the present study exhibited the classical adrenal hypertrophy in response to stress. Adrenal weights were unaffected by heat stress regardless of ascorbic acid levels in the diets (Tables 24 and 25). Bates et al. (1940) and Brown et al. (1958) also failed to observe increased adrenal weights in response to heat stress. Wolford and Ringer (1963) reported that right adrenal glands were heavier in cold-stressed hens maintained without feed and water for 15 hr than the right adrenals of control birds, but left and total adrenal weights did not differ significantly from control values.

Adrenal ascorbic acid concentrations (μg/100 mg) were not significantly affected by heat stress (Table 26). Significant differences in the total ascorbic acid content of the adrenal glands due to ambient temperature appeared to reflect differences in total adrenal weight (Table 24). Although a depletion of ascorbic acid from the adrenal glands and plasma of birds has been reported to accompany stress (Satterfield et al., 1940; Hill and Garren, 1958; Perek et al., 1959; Perek and Elat, 1960; Nockels et al., 1973), avian species are less sensitive to ACTH

administration than are mammals (Greenman et al., 1967). Moreover, Freeman (1970) reported that ascorbic acid depletion from the adrenals of the fowl in response to ACTH stimulation, while very rapid, was only transient. In the present experiment, therefore, the time between the initial stimulation and sampling may have been too great to detect transient fluctuations in adrenal ascorbic acid levels.

General adaptation reactions to stress include involution of lymphoid tissue (Craig, 1981). The decreased bursa and spleen weights (Table 24), therefore, indicated that the birds exposed to the 34C ambient temperature treatment were under stress. Similar reactions by chickens to heat stress were previously reported (Pardue and Thaxton, 1982; Siegel, 1985). Supplemental ascorbic acid did not ameliorate these responses (Tables 24 and 25).

Although spleen and bursa weights (Tables 24 and 25), as well as body weights (Table 20), in response to ambient temperature were indicative of physiological stress, heterophil number, lymphocyte number and H/L ratios were not (Table 23). Ben-Nathan (1976) reported that the number of lymphocytes declined markedly after exposure to a temperature of 42C for one hr. Gross and Siegel (1983) observed a decrease in the number of lymphocytes, an increase in heterophils, and an increase in H/L ratios in response to fasting for two days or to corticosterone

administration in the feed for one day. An increase in H/L ratios was also observed 24 hr after inoculation with Escherichia coli, after 24 hr of social stress, and in response to collecting blood twice within a period of 24 hr. Because the H/L ratio measures a physiological change, Gross and Siegel (1983) concluded that H/L ratios should be a reliable indicator of long-term changes in environment. The failure to detect differences in heterophils, lymphocytes, or H/L ratios in the present experiment, however, suggests that H/L ratios may not be sensitive indicators of prolonged exposure to heat stress.

Consistent with previous observations (Chapter IV), ascorbic acid did not ameliorate increases in core body temperatures in response to heat stress (Table 21). Ascorbic acid also failed to reduce surface temperatures of heat-stressed birds. To the contrary, supplemental ascorbic acid tended to increase surface body temperatures of chicks maintained at 23C. The implications of this observation are not clear, but heat loss through naked skin sites is extremely important in maintaining internal homeothermy at ambient temperatures up to about 30C (Richards, 1970). From 26C to 35C, the thermal circulation index (indication of peripheral blood flow) at unfeathered sites can change 10- to 15-fold. Moreover, hens fed diets supplemented with ascorbic acid may be more sensitive in controlling their

internal body temperature than those fed diets without supplemental ascorbic acid (Thornton, 1962; Grimes and Moreng, 1965). It is conceivable that ascorbic acid can act to increase heat expenditure at unfeathered skin sites. Such responses would not be evident at very high ambient temperatures when evaporative cooling becomes the predominant homeothermic mechanism.

Previously, it was observed that both heat stress and supplemental ascorbic acid tended to increase collagen concentrations in the skin (Chapter IV). Although it has been suggested that skin collagen affects skin fragility (Smith et al., 1977), increased collagen concentrations did not appear to increase skin strength (Chapter IV). The results of the present experiment were consistent with those previous observations. Neither supplemental ascorbic acid nor heat stress significantly influenced skin strength (Table 22). Supplemental ascorbic acid, therefore, would appear to be of no benefit in reducing the incidence of torn skin in commercial broiler processing.

Table 19. Means and standard errors of body weights of male and female broiler chicks at 33 days of age in response to ascorbic acid supplementation

Ascorbic Acid	Females	Males
mg/kg	----------g----------	
0	927 ± 11[a]	981 ± 16[a]
200	914 ± 11[a]	990 ± 13[a]
400	895 ± 12[a]	1012 ± 12[a]

[a]Means in a column having the same superscript were not significantly different ($P \geq .05$).

Table 20. Means and standard errors for body weights of male and female broilers at 40 and 49 days of age in response to heat stress and ascorbic acid supplementation

Sex	Age	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
	-d-	-C-	----------g----------			
Female	40	23	1259 ± 53	1358 ± 27	1192 ± 51	1268^{A}
		34	1232 ± 61	1183 ± 27	1254 ± 35	1223^{A}
		$\bar{x}$	1243^{a}	1270^{a}	1223^{a}	
	49	23	1719 ± 70	1903 ± 92	1737 ± 86	1786^{A}
		34	1619 ± 60	1550 ± 31	1556 ± 23	1575^{B}
		$\bar{x}$	1669^{a}	1726^{a}	1646^{a}	
Males	40	23	1423 ± 57	1436 ± 45	1432 ± 65	1430^{A}
		34	1348 ± 78	1217 ± 76	1400 ± 57	1321^{B}
		$\bar{x}$	1386^{a}	1326^{a}	1416^{a}	
	49	23	2095 ± 63	1993 ± 66	2042 ± 89	2043^{A}
		34	1735 ± 106	1734 ± 68	1835 ± 67	1768^{B}
		$\bar{x}$	1915^{a}	1864^{a}	1938^{a}	

^{AB,a}Within age-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). Ambient temperature by ascorbic acid interactions were not significant.

Table 21. Means and standard errors for core (cloacal) and surface (foot pad), and the surface/core ratio of male and female broilers in response to heat stress and supplemental ascorbic acid

Trait	Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
		-C-	----------g----------			
Core Temp.	Female	23	41.8 ± 0.1	41.7 ± 0.1	41.8 ± 0.1	41.8^{B}
		34	42.1 ± 0.1	42.2 ± 0.1	42.0 ± 0.1	42.1^{A}
		$\bar{x}$	42.0^{a}	42.0^{a}	41.9^{a}	
	Male	23	41.7 ± 0.2	41.7 ± 0.1	41.8 ± 0.1	41.7^{B}
		34	42.0 ± 0.1	42.2 ± 0.1	42.2 ± 0.1	42.1^{A}
		$\bar{x}$	41.8^{a}	42.0^{a}	42.0^{a}	
Surface Temp.	Female	23	31.7 ± 1.0^{bB}	35.2 ± 0.2^{aB}	34.4 ± 1.0^{aB}	33.8
		34	39.2 ± 0.2^{aA}	38.8 ± 0.4^{aA}	38.7 ± 0.4^{aA}	38.9
		$\bar{x}$	35.4	37.0	36.6	
	Male	23	31.5 ± 0.5^{cB}	35.7 ± 0.4^{aB}	33.7 ± 0.8^{bB}	33.6
		34	38.7 ± 0.3^{aA}	39.6 ± 0.3^{aA}	39.0 ± 0.2^{aA}	39.1
		$\bar{x}$	35.1	37.6	36.4	
Surf./ Core Ratio	Female	23	$0.76 \pm .02^{bB}$	$0.84 \pm .01^{aB}$	$0.82 \pm .02^{aB}$	0.81
		34	$0.93 \pm .01^{aA}$	$0.92 \pm .01^{aA}$	$0.92 \pm .01^{aA}$	0.92
		$\bar{x}$	0.84	0.88	0.87	
	Male	23	$0.75 \pm .01^{cB}$	$0.86 \pm .01^{aB}$	$0.81 \pm .02^{bB}$	0.81
		34	$0.92 \pm .01^{aA}$	$0.94 \pm .01^{aA}$	$0.92 \pm .01^{aA}$	0.93
		$\bar{x}$	0.84	0.90	0.86	

^{ab,ABC}Within age-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). Ambient temperature by ascorbic acid interactions were not significant.

Table 22. Means and standard errors for skin breaking strength of male and female broiler chicks in response to heat stress and supplemental ascorbic acid

Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
	-C-	----------g----------			
Females	23	731 ± 41	958 ± 97	922 ± 92	870^{A}
	34	885 ± 124	941 ± 106	1030 ± 94	952^{A}
	$\bar{x}$	808^{a}	950^{a}	976^{a}	
Males	23	1024 ± 85	1271 ± 57	943 ± 93	1079^{A}
	34	1114 ± 122	1148 ± 98	1190 ± 54	1150^{A}
	$\bar{x}$	1072^{a}	1210^{a}	1066^{a}	

^{A,a}Means in a row having the same lower case superscript and those in a column having the same upper case superscript were not significantly different ($P \geq .05$).

Table 23. Means and standard errors for heterophil number, lymphocyte number, and heterophil/lymphocyte ratio in male and female broiler chicks in response to heat stress and supplemental ascorbic acid

Trait	Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
		-C-				
Hetero-	Female	23	18 ± 2	20 ± 3	13 ± 2	17^{A}
phils		34	17 ± 2	18 ± 2	20 ± 2	18^{A}
(cell count)		$\bar{x}$	18^{a}	19^{a}	16^{a}	
	Male	23	16 ± 2	20 ± 3	16 ± 2	17^{A}
		34	11 ± 2	21 ± 2	16 ± 2	16^{A}
		$\bar{x}$	14^{b}	20^{a}	16^{ab}	
Lymph-	Female	23	42 ± 2	40 ± 3	47 ± 2	43^{A}
ocytes		34	43 ± 2	42 ± 2	40 ± 2	42^{A}
(cell count)		$\bar{x}$	42^{a}	41^{a}	44^{a}	
	Male	23	44 ± 2	40 ± 3	44 ± 2	43^{A}
		34	50 ± 2	39 ± 2	44 ± 2	44^{A}
		$\bar{x}$	47^{a}	40^{b}	44^{ab}	
H/L	Female	23	.46 ± .08	.56 ± .05	.29 ± .06	44^{A}
Ratio		34	.42 ± .05	.44 ± .07	.51 ± .09	46^{A}
		$\bar{x}$	$.44^{a}$	$.50^{a}$	$.40^{a}$	
	Male	23	.37 ± .07	.52 ± .10	.38 ± .07	$.42^{A}$
		34	.22 ± .06	.54 ± .07	.38 ± .07	$.38^{A}$
		$\bar{x}$	$.30^{b}$	$.53^{a}$	$.38^{ab}$	

^{a,b,A}Within age-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). Ambient temperature by ascorbic acid interactions were not significant.

Table 24. Means and standard errors for spleen, bursa of Fabricius, and adrenal weights of male and female broiler chicks in response to heat stress and supplemental ascorbic acid

Trait	Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
		-C-	----------g----------			
Spleen	Female	23	3.06 ± .14	3.62 ± .28	3.38 ± .35	3.35^{A}
		34	2.89 ± .19	2.50 ± .34	2.84 ± .37	2.74^{B}
		$\bar{x}$	2.98^{a}	3.06^{a}	3.11^{a}	
	Male	23	3.94 ± .26	4.51 ± .76	3.36 ± .36	3.94^{A}
		34	3.02 ± .46	2.47 ± .39	2.23 ± .30	2.57^{B}
		$\bar{x}$	3.48^{a}	3.49^{a}	2.79^{a}	
Bursa	Female	23	1.05 ± .14	1.17 ± .15	1.23 ± .16	1.15^{A}
		34	0.92 ± .15	0.64 ± .08	0.91 ± .16	0.82^{B}
		$\bar{x}$	0.98^{a}	0.90^{a}	1.07^{a}	
	Male	23	0.97 ± .16	1.13 ± .22	1.60 ± .16	1.23^{A}
		34	0.88 ± .12	0.79 ± .12	0.85 ± .10	0.84^{B}
		$\bar{x}$	0.92^{a}	0.96^{a}	1.22^{a}	
Adrenal	Female	23	.118 ±.017	.099 ±.012	.109 ±.009	$.109^{A}$
		34	.129 ±.013	.095 ±.007	.073 ±.005	$.099^{A}$
		$\bar{x}$	$.124^{a}$	$.097^{b}$	$.091^{b}$	
	Male	23	$.163 \pm .015^{aA}$	$.084 \pm .023^{bA}$	$.144 \pm .009^{abA}$	.130
		34	$.123 \pm .022^{aA}$	$.106 \pm .013^{aA}$	$.079 \pm .012^{aB}$	.103
		$\bar{x}$	.143	.095	.112	

^{ab,AB}Within trait-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). With the exception of male adrenal weights, ascorbic acid by ambient temperature interactions were not significant.

Table 25. Means and standard errors for spleen, bursa of Fabricius, and adrenal weights of male and female broiler chicks in response to heat stress and supplemental ascorbic acid

Trait	Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
		-C-	----------mg/100 g body wt.---------			
Spleen	Female	23	179 ± 8	194 ± 24	193 ± 14	189^{A}
		34	180 ± 16	160 ± 21	182 ± 21	174^{A}
		$\bar{x}$	180^{a}	177^{a}	188^{a}	
	Male	23	188 ± 14	228 ± 35	165 ± 19	194^{A}
		34	185 ± 45	141 ± 19	120 ± 14	148^{B}
		$\bar{x}$	186^{a}	184^{a}	142^{a}	
Bursa	Female	23	61 ± 8	61 ± 6	72 ± 10	65^{A}
		34	58 ± 11	42 ± 5	58 ± 8	53^{A}
		$\bar{x}$	60^{a}	52^{a}	65^{a}	
	Male	23	46 ± 8	56 ± 8	82 ± 11	61^{A}
		34	52 ± 8	45 ± 5	47 ± 6	48^{A}
		$\bar{x}$	49^{a}	50^{a}	64^{a}	
Adrenal	Female	23	6.9 ± 1.0	5.2 ± 0.5	6.4 ± 0.6	6.2^{A}
		34	8.1 ± 1.0	6.2 ± 0.6	4.8 ± 0.5	6.4^{A}
		$\bar{x}$	7.5^{a}	5.7^{b}	5.6^{b}	
	Male	23	7.8 ± 0.8	4.8 ± 0.6	7.2 ± 0.8	6.6^{A}
		34	7.4 ± 1.8	6.1 ± 0.6	4.3 ± 0.6	5.9^{A}
		$\bar{x}$	7.6^{a}	5.4^{a}	5.8^{a}	

^{ab,AB}Within trait-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). Ambient temperature by ascorbic acid interactions were not significant.

Table 26. Means and standard errors for plasma and adrenal ascorbic acid concentrations in male and female broiler chicks in response to heat stress and supplemental ascorbic acid

Trait	Sex	Ambient Temp.	Supplemental Ascorbate (mg/kg) 0	200	400	$\bar{x}$
		-C-				
Plasma ascorbic acid (µg/ml)	Female	23	12.8 ± 1.0	22.2 ± 2.0	11.2 ± 2.6	15.4^{A}
		34	21.0 ± 3.6	16.0 ± 4.1	10.5 ± 3.1	15.8^{A}
		$\bar{x}$	16.9^{a}	19.1^{a}	10.8^{b}	
	Male	23	16.7 ± 2.2	17.8 ± 4.3	14.5 ± 1.8	16.3^{A}
		34	8.5 ± 2.0	13.9 ± 3.0	15.3 ± 500	12.6^{A}
		$\bar{x}$	12.6^{a}	15.8^{a}	14.9^{a}	
Adrenal ascorbic acid (µg)	Female	23	544 ± 76	416 ± 50	470 ± 68	477^{A}
		34	513 ± 50	365 ± 35	290 ± 46	389^{B}
		$\bar{x}$	525^{a}	390^{b}	380^{b}	
	Male	23	622 ± 87	376 ± 82	483 ± 80	494^{A}
		34	428 ± 61	359 ± 57	340 ± 45	376^{A}
		$\bar{x}$	525^{a}	368^{a}	412^{a}	
Adrenal ascorbic acid (µg/100 mg)	Female	23	461 ± 6	418 ± 8	435 ± 52	438^{A}
		34	398 ± 11	385 ± 26	387 ± 48	390^{A}
		$\bar{x}$	430^{a}	402^{a}	411^{a}	
	Male	23	398 ± 30	386 ± 28	330 ± 46	365^{A}
		34	396 ± 48	333 ± 15	438 ± 36	382^{A}
		$\bar{x}$	377^{a}	360^{a}	384^{a}	

^{ab,AB}Within trait-sex subgroups, means in a row having the same lower case superscripts and those in a column having the same upper case letters were not significantly different ($P \geq .05$). Ambient temperature by ascorbic acid interactions were not significant.

SYNTHESIS

The overall objective of the research reported in this dissertation was to examine factors contributing to skin strength in meat-type chickens. An apparatus, adapted from one used to evaluate the repair of skin wounds in rats (Alvarez and Gilbreath, 1982) was initially developed to measure skin breaking strength. Reliability of the procedure was determined by a preliminary experiment in which breaking strength values obtained from samples of thigh and breast skin from the right and left side of about 50 birds were compared. Correlations between sides were .90 and .91 for thigh and breast skin, respectively.

Skin breaking strength was then measured in lines of chickens divergently selected for high (H) and low (L) juvenile body weight, their F_1 reciprocal crosses (HL and LH) and an F_2 generation derived from HL and LH matings. In both lines, skin covering the breast had higher breaking strength than that covering the thigh. Skin of chicks from the L line was weakest, that from HH and LH matings was strongest, and that from HL and F_2 matings was intermediate. Percentage heterosis for breaking strength was significantly positive while percentage recombination was not significant. It was concluded that the procedure was sufficiently sensitive to allow the assessment of breaking strength of chicken skin.

Edwards et al. (1973) observed more skin tears in females than in males, and in older than in younger broilers. Smith et al. (1977) also observed a greater incidence of torn skin in females than in males and postulated that higher levels of skin fat, accompanied by a reduction in skin protein or collagen, made the skin of females more susceptible to tearing. In the present experiment, therefore, skin breaking strength was related to its chemical composition. Skin breaking strength, and protein, fat, moisture, and total collagen concentrations of skin from the breast, thigh, and back of male and female commercial broilers were measured in response to diets containing relatively wide or narrow C:P ratios. Comparisons were made at 28, 42, and 56 days of age.

Chicks fed diets containing relatively wide C:P ratios had weaker skin than those fed diets with narrower C:P ratios, with differences being greater at 56 days than at younger ages. Regardless of diet, males had stronger skin than females. Although the magnitude of the differences varied with age, breast skin was stronger than thigh skin with skin from the back being intermediate in strength. It was concluded that the observed differences in breaking strength were not consistently associated with fat, protein, moisture, or collagen concentrations of the skin.

Because skin breaking strength was not associated with

fat, protein, or collagen concentrations, skin from broiler chicks fed diets with different C:P ratios was examined histologically. Total skin thickness and thickness of different skin layers, determined from photomicrographs of representative skin sections, were then compared to breaking strength values. Males had stronger and thinner skin than females, with the differences in thickness due primarily to differences in the hypodermis. Regardless of sex, feeding diets containing wider C:P ratios resulted in weaker and thicker skin. Increased skin thickness due to these diets was associated with an increase in hypodermis thickness and a decrease in the combined thickness of the dermis and epidermis. Among sites, skin from the breast was strongest and that from the side was weakest, while skin from the back and thigh exhibited intermediate values for breaking strength. With the exception of differences between the back and thigh, increases in breaking strength observed between sites were associated with a reduction in total skin thickness and in the thickness of the hypodermis. It was concluded that increased hypodermis thickness and/or decreased combined thickness of the dermis and epidermis reduced skin strength.

Two experiments were also conducted in which skin strength was examined in response to heat stress and supplemental ascorbic acid. The rationale was that ascorbic

acid biosynthesis may not proceed at sufficiently rapid rates to maintain maximal skin collagen concentrations under heat stress conditions (Bondi, 1982). In the initial experiment, diets containing either 0 or 100 mg/kg of L-ascorbic acid were fed to commercial broiler chicks from 0 to 23 days of age. From 23 to 34 days of age, chicks fed each diet were subjected to a constant temperature of 23C, a constant temperature of 32C, or a treatment consisting of 32C from 1600 to 1200 hr and 40C from 1200 to 1600 hr. Traits measured included body weight, body temperature, skin breaking strength, and protein, lipid, moisture, and collagen concentrations in skin from the breast, back, and thigh.

The chicks subjected to the higher ambient temperature treatments (32C, 32/40C) exhibited higher body temperatures and lower body weights than those maintained at 23C. Supplemental ascorbic acid partially alleviated the reduction in body weight due to heat stress but did not reduce body temperatures. Both heat stress and supplemental ascorbic acid tended to increase collagen concentrations in the skin. The protein content of skin from males tended to be increased in response to supplemental ascorbate, while effects of heat stress on skin protein were inconsistent. Neither heat stress nor ascorbic acid significantly affected the moisture and lipid content of skin. Observed

differences in skin composition were not associated with differences in skin breaking strength. Differences in breaking strength were inconsistent in response to heat stress and ascorbic acid.

Although supplemental ascorbic acid did not appear to improve skin strength, the observation that supplemental ascorbic acid increased body weights of heat-stressed chicks appeared important, because economic losses due to heat stress are a major problem in broiler production. As a result, a final experiment was conducted to investigate further the relationships between heat stress and ascorbic acid nutriture. Supplemental ascorbic acid levels of 0, 200, and 400 mg/kg were used. The chicks were exposed to constant ambient temperatures of 23C and 34C from 33 to 49 days of age. Traits measured included body weight, skin breaking strength, surface and core body temperatures, plasma and adrenal ascorbic acid concentrations, heterophil and lymphocyte number, and weights of the spleen, bursa of Fabricius, and adrenal glands.

The results of the final experiment were consistent with previous observations that neither supplemental ascorbic acid nor heat stress significantly influenced skin strength. Contrary to previous results, however, supplemental ascorbic acid failed to ameliorate the depressive effect of high ambient temperature on body

weight. Supplemental ascorbic acid did tend to reduce weights of the adrenals, bursa, and spleen, but had no effect on heterophil number, lymphocyte number, or H/L ratios. Supplemental ascorbic acid tended to increase surface temperatures of chicks maintained at 23C but had no effect on either core or surface temperatures of heat-stressed chicks. No consistent effects on plasma or adrenal ascorbic acid levels occurred in response to heat stress or supplemental ascorbic acid.

Overall, the results of these studies clearly indicated that skin strength was not associated with the protein, collagen, or fat concentrations of the skin. Moreover, skin thickness, particularly the thickness of the hypodermis, was negatively associated with skin strength. More detailed chemical and histological examination of specific skin layers would be required to elucidate specific physical or chemical factors contributing to skin strength. In addition, the results indicated that C:P ratios of the diet influenced skin strength. The feeding of diets containing higher protein than those commonly used might be beneficial in reducing the incidence of skin tears in commercial broiler production, but the economic implications would have to be carefully considered. The effects of increased supplemental methionine and lysine, which would be more cost effective than increased crude protein, on skin strength

would appear to be a fruitful area for subsequent investigation.

Finally, the results indicated that supplemental ascorbic acid would be of no benefit in reducing the incidence of skin tears in commercial broiler processing. Supplemental ascorbic acid, however, may offer some protection against reduced growth rates associated with high ambient temperatures. Since the present results were inconclusive, further experimentation would certainly be warranted. The physiological role of ascorbic acid in maintaining homeothermy would also be fruitful for future investigation.

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APPENDIX

Appendix Table 1. Mean squares and degrees of freedom of skin breaking strength in chickens among genetic combinations (Chapter I)

Source	df	Breaking strength (106)
Line	4	0.170**
Site	1	0.600**
L*S	4	0.033
Error		0.015

[1]Mean squares of skin breaking strength are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 2. Mean squares and degrees of freedom of body weight (Chapter II)

Source	df	Breaking strength (108)
Age	2	1.320**
Diet	1	0.048**
Sex	1	0.023**
A*D	2	0.001
A*S	2	0.003
S*D	1	0.006
A*S*D	2	0.003
Error		0.002

[1]Mean squares for body weights are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 3. Mean squares and degrees of freedom of skin breaking strength and percent fat, water, protein, and collagen of skin from broilers fed diets with comparatively wide and narrow ratios of calories to protein (Chapter II)

Source	df	Breaking Strength (142)	Fat (144)	Water (144)	Protein (144)	Collagen (144)
Age	2	0.086**	0.107**	0.074**	0.0020**	0.071**
Diet	1	0.233**	0.108**	0.081**	0.0046**	0.051**
Sex	1	0.689**	0.024**	0.003	0.0258**	0.089**
Site	2	2.952**	0.696**	0.377**	0.1201**	0.489**
A*D	2	0.084**	0.027**	0.015**	0.0003*	0.001
A*Sex	2	0.007	0.002	0.001	0.0011**	0.050**
A*Site	4	0.184**	0.007**	0.005**	0.0012**	0.016**
D*Sex	1	0.012	0.001	0.001	0.0003	0.083**
D*Site	2	0.012	0.000	0.001	0.0003	0.000
S*S	2	0.076**	0.029**	0.014**	0.0010**	0.009*
A*D*Sex	2	0.021	0.002	0.002	0.0031	0.003
A*D*Site	4	0.016	0.003	0.003*	0.0002	0.002
D*S*S	2	0.033*	0.002	0.002	0.0001	0.006
A*S*S	4	0.008	0.003	0.003	0.0004**	0.006
A*D*S*S	4	0.042**	0.003	0.003	0.0001	0.004
Error		0.011	0.002	0.001	0.0001	0.003

[1]Mean squares for breaking strength are from log-transformed data.

[2]Mean squares for skin chemical composition are from arc sin transformed data.

[3]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 4. Mean squares and degrees of freedom of total skin thickness, the thickness of the hypodermis, epidermis and dermis, and skin breaking strength in commercial broiler chicks at 8 weeks of age (Chapter III)

Source	df	Total skin thickness (183)	Hypo-dermis (183)	Epidermis + dermis (183)	Breaking strength (63)
Diet	1	2.193**	4.049**	5.195**	0.123**
Sex	1	1.898**	2.144**	0.331*	0.048**
Site	3	38.002**	41.483**	1.619**	0.128**
D*Sex	1	0.345*	0.403*	0.032	0.001
D*Site	3	0.026	0.049	1.074**	0.002
S*S	3	0.275**	0.362**	0.498**	0.001
D*S*S	3	0.453**	0.488**	0.069	0.004
Error		0.066	0.059	0.091	0.002

[1]Mean squares of epidermis and dermis thickness and breaking strength are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 5. Mean squares and degrees of freedom of body weight (Chapter IV)

Source	df	Breaking strength (107)
Diet	1	0.022**
Temperature	2	0.051**
Sex	1	0.063**
D*T	2	0.005
D*S	1	0.001
T*S	2	0.004
D*T*S	2	0.001
Error		0.002

[1]Mean squares of body weights are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 6. Mean squares and degrees of freedom of skin breaking strength and percent fat, water, protein, and collagen of skin from broilers (Chapter IV)

Source	df	Breaking Strength (306)	Fat (319)	Water (320)	Protein (315)	Collagen (284)
Diet	1	0.004	0.006	0.005	0.0015*	0.094**
Temp.	2	0.104*	0.008*	0.009*	0.0015**	0.128**
Sex	1	2.009**	0.041**	0.011*	0.0182**	0.229**
Site	2	2.727**	1.836**	1.023**	0.2841**	0.930**
D*T	2	0.009	0.007	0.004	0.0007	0.006
D*Sex	1	0.014	0.001	0.002	0.0012*	0.075**
D*Site	2	0.062	0.001	0.000	0.0001	0.007
T*Sex	2	0.037	0.007	0.003	0.0009*	0.003
T*Site	4	0.100**	0.001	0.001	0.0004	0.015
S*S	2	0.092*	0.004	0.001	0.0017**	0.000
D*T*Sex	2	0.100**	0.003	0.002	0.0008*	0.064**
D*T*Site	4	0.042	0.002	0.001	0.0001	0.004
D*S*S	2	0.049	0.000	0.001	0.0000	0.002
T*S*S	4	0.011**	0.000	0.000	0.0003	0.018*
D*T*S*S	4	0.019	0.000	0.001	0.0002	0.003
Error		0.023	0.003	0.002	0.0002	0.007

[1]Mean squares for breaking strength are from log-transformed data.

[2]Mean squares for skin chemical composition are from arc sin-transformed data.

[3]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 7. Mean squares and degrees of freedom of core temperatures at 27 and 30 days of age (Chapter IV)

Source	df	Core temperature (days of age) 27 (96)	30 (96)
Temp.	2	9.506**	9.970**
Sex	1	0.910	0.065
Diet	1	0.619	0.490*
Period	1	2.822**	1.057**
T*S	2	0.567	0.242
T*D	2	1.635**	0.019
T*P	2	3.840**	4.385**
S*P	1	0.024	0.000
S*D	1	0.000	0.075
D*P	1	0.277	0.009
T*S*P	2	0.058	0.021
T*D*P	2	0.058	0.131
S*D*P	1	0.023	0.021
T*S*D	2	0.542	0.033
T*S*D*P	2	0.008	0.025
Error		0.273	0.094

[1]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 8. Mean squares and degrees of freedom of broiler body weight at 33, 40, and 49 days of age and skin breaking strength at 49 days of age (Chapter V)

Source	df	Body weight (days of age) 33 (345)	40 (48)	49 (47)	Breaking strength (48)
Sex	1	0.1244**	0.0266**	0.0423**	0.676**
Temp.	1	0.0044	0.0099*	0.0493**	0.081
Diet	2	0.0000	0.0003	0.0000	0.125
S*T	1	0.0001	0.0017	0.0003	0.000
S*D	2	0.0072*	0.0028	0.0020	0.058
T*D	2	0.0016	0.0072*	0.0007	0.082
S*T*D	2	0.0013	0.0001	0.0027	0.008
Error		0.0021	0.0017	0.0023	0.049

[1]Mean squares of body weights and skin breaking strength are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 9. Mean squares and degrees of freedom of adrenal, spleen, and bursa of Fabricius weights of broilers at 49 days of age (Chapter V)

Source	df	Adrenal (48)	Spleen (48)	Bursa (48)	Adrenal/ 100 g body wt. (48)	Spleen/ 100 g body wt. (48)	Bursa/ 100 g body wt. (48)
Sex	1	0.029	0.002	0.005	0.0000	0.0042	0.0011
Temp.	2	0.109	0.305**	0.359**	0.0000	0.0249*	0.0116**
Diet	1	0.101**	0.012	0.047	0.0018**	0.0031	0.0042
S*T	2	0.022	0.036	0.000	0.0001	0.0068	0.0000
S*D	2	0.005	0.019	0.009	0.0000	0.0075	0.0008
T*D	1	0.078*	0.020	0.038	0.0007	0.0066	0.0004
S*T*D	2	0.023	0.000	0.020	0.0001	0.0009	0.0015
Error	2	0.013	0.014	0.020	0.0001	0.0043	0.0015

[1]Mean squares of adrenal, spleen, and bursa of Fabricius are from log-transformed data, and the corrected weights to 100 g body weight are from arc sin-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 10. Mean squares and standard errors of plasma and adrenal ascorbic acid (AA), core and surface body temperatures, and surface/core (S/C) ratio (Chapter V)

Source	df	Plasma AA (45)	Adrenal AA		Temperature		S/C ratio (48)
			Concentration (48)	Content (48)	Core (48)	Surface (48)	
Sex	1	19.20	0.030	24482*	0.014	0.033	0.0000
Temp.	1	39.01	86.862**	3514	1.768**	425.600	0.4838**
Diet	2	20.10	73.527**	2816	0.003	21.152	0.0200**
S*T	1	61.34	0.744	16124	0.020	0.486	0.0006
S*D	2	82.21	3.472	812	0.062	1.443	0.0026
T*D	2	38.50	13.409	7921	0.083	17.163	0.0160*
S*T*D	2	133.21	9.402	9684	0.033	0.546	0.0006
Error		39.93	11.054	5642	0.048	1.508	0.0017

[1]Mean squares of adrenal ascorbic acid concentration and surface/core ratios are from arc sin transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

Appendix Table 11. Mean squares and standard errors of heterophils, lymphocytes, and heterophil/lymphocyte (H/L) ratios in broilers (Chapter V)

Source	df	Hetero-phils (46)	Lympho-cytes (46)	H/L ratios (46)
Sex	1	24.96	24.96	0.047
Temp.	1	0.48	0.48	0.007
Diet	2	96.58*	96.58*	0.163*
S*T	1	24.96	24.96	0.012
S*D	2	44.05	44.05	0.036
T*D	2	51.64	51.64	0.074
S*T*D	2	34.95	34.95	0.066
Error		25.84	25.84	0.042

[1]Mean squares for heterophil/lymphocyte ratios are from log-transformed data.

[2]Error degrees of freedom are presented in parentheses under trait.

*P ≤ .05.

**P ≤ .01.

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SKIN BREAKING STRENGTH

IN BROILER CHICKENS

by

Ilan Kafri

(ABSTRACT)

A procedure was developed to examine factors contributing to skin strength of meat-type chickens. Skin breaking strength was initially measured in lines of chickens divergently selected for high (H) and low (L) juvenile body weight, their reciprocal crosses (HL and LH), and an F_2 generation derived from HL and LH matings. Skin of chicks from the LL line was weakest, that from the HH and LH matings strongest, and that from HL and F_2 matings was intermediate. Percentage heterosis for breaking strength was significantly positive while percentage recombination was not significant.

Skin breaking strength and protein, fat, moisture, and total collagen concentrations of skin from the breast, thigh, and back of male and female commercial broilers were then examined in response to diets containing relatively wide or narrow ratios of calories to protein (C:P). Comparisons were made at 28, 42, and 56 days of age. Chickens fed diets containing wider C:P ratios had weaker

skin than those fed diets with narrower C:P ratios, with the differences being greater at older than at younger ages. Males had stronger skin than females. Among body sites, breast skin was stronger than thigh skin with that from the back being intermediate in strength. Skin breaking strength did not appear to be consistently associated with either the protein, fat, or collagen concentrations in the skin.

Skin from broiler chicks fed diets with differing C:P ratios was also examined histologically. Males had stronger and thinner skin than females, with the differences in thickness due primarily to differences in the thickness of the hypodermis. Regardless of sex, feeding diets containing wider C:P ratios resulted in weaker and thicker skin. The thicker skin was associated with an increase in the thickness of the hypodermis and a decrease in the thickness of the dermis and epidermis. With the exception of differences between back and thigh skin, increases in breaking strength occurring between skin from different sites were associated with a reduction in total skin thickness and in the thickness of the hypodermis. It was concluded that increased hypodermis thickness and/or decreased thickness of the dermis and epidermis reduced skin strength.

Two experiments were conducted in which skin strength was examined in relation to heat stress and supplemental

ascorbic acid. In the first experiment, chicks fed diets containing 0 or 100 mg of ascorbic acid per kg of diet were subjected to either a constant ambient temperature of 23C, a constant ambient temperature of 32C, or a treatment consisting of 32C from 1600 to 1200 hr and 40C from 1200 to 1600 hr (32/40C). Both heat stress and supplemental ascorbic acid tended to increase collagen concentrations in the skin, but differences in breaking strength were inconsistent in response to these treatments. Supplemental ascorbic acid, however, partially alleviated reductions in body weight due to heat stress but did not decrease body temperatures of heat-stressed chicks.

In the final experiment, diets containing either 0, 200, or 400 mg/kg of supplemental ascorbic acid were fed to chicks maintained at ambient temperatures of 23 and 34C. Contrary to previous observations, ascorbic acid failed to ameliorate the depressive effect of heat stress on body weight. Supplemental ascorbic acid tended to reduce adrenal, bursa of Fabricius, and spleen weights but had no effect on heterophil and lymphocyte numbers or ratios. Adrenal ascorbic acid concentrations were not significantly affected by supplemental ascorbic acid and plasma ascorbic acid levels were inconsistent among treatment groups. Neither supplemental ascorbic acid nor heat stress significantly influenced skin strength. It was concluded

that supplemental ascorbic acid was not beneficial in improving skin strength in commercial broiler chicks.