AN ABSTRACT OF THE DISSERTATION OF
Elżbieta Iwona Zakrzewska for the degree of Doctor of Philosophy in Poultry Science
presented on December 11, 1995. Title: Inhibited Feathering, K1 a Sex-Linked Dominant Gene in the Turkey (Meleagris gallopavo), Genetics and Nutrition.

Abstract approved: Redacted for Privacy

Thomas F. Savage

A mutation which inhibits feathering in the turkey has been identified and is inherited as a dominant, sex-linked trait. The gene affects the number, size and structure of the feathers. At hatching, poults with inhibited feathering (IF) can be distinguished by the absence of primary and secondary remiges and coverts. The expressivity of this trait varies in older birds, from almost the complete absence of feathers to nearly a full feather covering. The calamus of the feather is very fragile, barbs may be crossed, and the feathers may be twisted and bent. Several pleiotropic effects of the gene have been identified. The amino acid composition of the feathers, as well as the blood chemistry are modified. The thyroid function is affected and they display some of the symptoms of hypothyroidism. The IF gene also adversely affects reproductive performance. Egg production is reduced (P<0.0001), and male infertility is higher (P=0.030) than in their normal counterparts. Studies to ameliorate the feather condition in IF turkeys were conducted. Methionine supplemented at the level of 1.14% of the diet exacerbated the abnormal plumage (P=0.030) and reduced the weight gain (P=0.040) of IF poults at 8 WOA. In older birds studied (12-20 WOA) methionine supplementation at dietary levels of 0.62% and 1.22% had no effect on plumage or on weight gain. Supplementation of thyroxine at 1 ppm in the diet between 12 and 20 WOA in IF turkeys had no significant effect on feather growth or weight gain. Zinc fortification of the poult diet between 0 and 8 WOA at levels of 114 ppm and 210 ppm improved feather growth (P=0.029 and P=0.006, respectively). A linear enhancement of feather quality was observed in turkeys fed zinc supplemented diets at levels 120 ppm, 240 ppm, and 360 ppm between 12 and 20 WOA. This sex-linked gene expresses numerous pleiotropic effects and may function as a major physiological regulator in the bird. Through proper dietary intervention and
intensive genetic selection it will be possible to improve feather development in IF turkeys to make them suitable for commercial utilization (feather-sexing).
Inhibited Feathering, K' a Sex-Linked Dominant Gene
in the Turkey (Meleagris gallopavo),
Genetics and Nutrition

by
Elżbieta Iwona Zakrzewska

A DISSERTATION

submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed: December 11, 1995
Commencement: June, 1996
Doctor of Philosophy dissertation of Elżbieta Iwona Zakrzewska presented on December 11, 1995

APPROVED:

Redacted for Privacy

Major Professor, representing Poultry Science

Redacted for Privacy

Head of Department of Animal Sciences

Redacted for Privacy

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Redacted for Privacy

Elżbieta Iwona Zakrzewska, Author
Acknowledgments

I would like to express my deepest gratitude to my major professor, Dr. Thomas F. Savage, for his continuous guidance, encouragement, support and patience during my studies at Oregon State University. Being his student was an unforgettable learning experience for me, which will provide me with inspiration for the years to come.

Special recognition is extended to the members of my committee, Drs. H. S. Nakaue, J. Hermes, G. Fisher and D. Thomas, for their advice and critical review of this dissertation.

Among the people who made this dissertation possible, thanks for the invaluable assistance of Dr. Ann Brodie of Department of Animal Sciences and Dr. S. P. Snyder of College of Veterinary Medicine, for preparation of microscopic photographs.

I would also like to thank Ron Scott for his outstanding help with my experiments on the Turkey Farm.

I would like to give a million thanks to Kari Henschel, whose constant moral support, patience and willingness to help at any time allowed me to survive moments of discouragement and to finish my work.

Special recognition goes to Stan Taylor for his professional assistance and his advice that helped me to complete my thesis.

I would also thank the Department of Animal Sciences for financial assistance in the form of a graduate research assistantship, as well as the Pacific Egg and Poultry Association for its scholarship. Additional thanks go to the OSU Poultry Club for partly funding my trips to professional meetings, participation in which greatly helped me in my research.

I would like to extend very special thanks to Mr. Robert Ritz of Monmouth, Oregon, the turkey grower who first found the Inhibited Feathering mutation in his flock. Instead of discarding the unusual bird, Mr. Ritz brought it to the attention of Oregon State University, thus initiating the research which finally resulted in this dissertation. It is people like him who make progress in animal genetics possible. Without you, Mr. Ritz, this work would never have been written. Again, thank you very much.
Finally, I would like to express my deep gratitude to my friends and family. Many thanks go to Judy Lasswell for her friendship, support and help during my stay in U.S. I would like to thank my mother Wieslawa Bajkowska for her unconditional love, for giving me the opportunity of education and for her unending patience. I would also like to express my greatest appreciation to my husband, Radek, for his love, support in difficult times and help with preparing my dissertation.
Table of Contents

1. Introduction
   1.1. Zoological taxonomy of the domestic turkey 2
   1.2. The history of the domesticated turkey 2
   1.3. Genetics in the history of animal breeding 5
   1.4. Analogy and divergence of turkey and chicken genomes 7
   1.5. The focus of this work 8

2. Heredity and Phenotype of Inhibited Feathering Turkey 10
   2.1. Literature review 10
      2.1.1. Avian feather - development and structure 10
         2.1.1.1. Morphology and development of skin and feather follicles 11
            2.1.1.1.1. Skin morphology 11
            2.1.1.1.2. Feather follicle morphology 12
            2.1.1.1.3. Muscle system of feather follicles 14
         2.1.1.2. Formation of the skin 15
         2.1.1.3. Formation of the feather germs 16
      2.1.2. Ptilosis of the turkey 18
         2.1.2.1. Morphology and characterization of the contour feather 18
            2.1.2.1.1. General morphology of the contour feather 18
            2.1.2.1.2. Types of the contour feathers 21
         2.1.2.2. Morphology and characterization of other types of feathers 23
      2.1.3. Feather tracts of the Medium White Turkey 25
         2.1.3.1. Arrangement of feather tracts and apteria 26
         2.1.3.2. Characterization of the major feather tracts 28
      2.1.4. Genetic feather disorders 32
         2.1.4.1. Hereditary feather disorders in the chicken 32
            2.1.4.1.1. Autosomal dominant traits 33
            2.1.4.1.2. Autosomal recessive traits 35
            2.1.4.1.3. Sex linked dominant genes 39
            2.1.4.1.4. Sex-linked recessive genes 39
            2.1.4.1.5. Composed traits 40
            2.1.4.1.6. Traits with unknown mode of inheritance 40
         2.1.4.2. Feather disorders in the turkey 41
            2.1.4.2.1. Autosomal recessive traits 41
            2.1.4.2.2. Sex linked dominant traits 42
Table of Contents (continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.4.3. Feather disorders in the Japanese quail</td>
<td>42</td>
</tr>
<tr>
<td>2.1.4.3.1. Autosomal dominant traits</td>
<td>42</td>
</tr>
<tr>
<td>2.1.4.3.2. Autosomal recessive traits</td>
<td>43</td>
</tr>
<tr>
<td>2.1.4.4. Feather disorders in the guinea fowl</td>
<td>44</td>
</tr>
<tr>
<td>2.1.4.5. Feather disorders in the geese.</td>
<td>44</td>
</tr>
<tr>
<td>2.2. Genetics and the description of the Inhibited Feathering turkey</td>
<td>44</td>
</tr>
<tr>
<td>2.2.1. Introduction</td>
<td>44</td>
</tr>
<tr>
<td>2.2.2. Materials and methods</td>
<td>48</td>
</tr>
<tr>
<td>2.2.2.1. Investigation of the mode of inheritance</td>
<td>48</td>
</tr>
<tr>
<td>2.2.2.2. Methods for the describing Inhibited Feathering poults</td>
<td>49</td>
</tr>
<tr>
<td>2.2.2.3. Biochemical assays</td>
<td>49</td>
</tr>
<tr>
<td>2.2.2.3.1. Blood chemistry analysis</td>
<td>49</td>
</tr>
<tr>
<td>2.2.2.3.2. Feather amino acid analysis</td>
<td>50</td>
</tr>
<tr>
<td>2.2.2.4. Scanning electron microscopy (SEM) of feather structure</td>
<td>51</td>
</tr>
<tr>
<td>2.2.3. Results</td>
<td>51</td>
</tr>
<tr>
<td>2.2.3.1. Mode of inheritance</td>
<td>51</td>
</tr>
<tr>
<td>2.2.3.2. Plumage of the slow feathering poults</td>
<td>52</td>
</tr>
<tr>
<td>2.2.3.3. Biochemical assays</td>
<td>67</td>
</tr>
<tr>
<td>2.2.3.3.1. Blood chemistry</td>
<td>67</td>
</tr>
<tr>
<td>2.2.3.3.2. Amino acid content of the feathers</td>
<td>67</td>
</tr>
<tr>
<td>2.2.4. Discussion</td>
<td>73</td>
</tr>
<tr>
<td>2.2.4.1. Mode of inheritance and phenotype description</td>
<td>73</td>
</tr>
<tr>
<td>2.2.4.2. Biochemical assays</td>
<td>75</td>
</tr>
<tr>
<td>2.2.4.2.1. Blood chemistry</td>
<td>75</td>
</tr>
<tr>
<td>2.2.4.2.2. Amino acid analysis of the feathers</td>
<td>77</td>
</tr>
<tr>
<td>2.3. Reproductive performance of Inhibited Feathering turkeys</td>
<td>78</td>
</tr>
<tr>
<td>2.3.1. Introduction</td>
<td>78</td>
</tr>
<tr>
<td>2.3.2. Materials and methods</td>
<td>79</td>
</tr>
<tr>
<td>2.3.2.1. Management procedures</td>
<td>79</td>
</tr>
<tr>
<td>2.3.2.1.1. Management procedures for breeder hens</td>
<td>79</td>
</tr>
<tr>
<td>2.3.2.1.2. Management procedures for breeder males</td>
<td>82</td>
</tr>
<tr>
<td>2.3.2.2. Determination of the egg production</td>
<td>82</td>
</tr>
<tr>
<td>2.3.2.3. Semen collection</td>
<td>83</td>
</tr>
<tr>
<td>2.3.2.4. Evaluation of incubation performance of the IF line</td>
<td>84</td>
</tr>
<tr>
<td>2.3.3. Results</td>
<td>85</td>
</tr>
<tr>
<td>2.3.3.1. Egg production</td>
<td>85</td>
</tr>
<tr>
<td>2.3.3.2. Semen production</td>
<td>85</td>
</tr>
<tr>
<td>2.3.3.3. Hen fertility and incubation performance</td>
<td>87</td>
</tr>
<tr>
<td>2.3.4. Discussion</td>
<td>87</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3. Studies Regarding Improvement of Plumage of Inhibited Feathering Turkeys</td>
<td></td>
</tr>
<tr>
<td>3.1. Literature review</td>
<td>94</td>
</tr>
<tr>
<td>3.1.1. Role of methionine in organism</td>
<td>94</td>
</tr>
<tr>
<td>3.1.1.1. The importance of amino acids</td>
<td>94</td>
</tr>
<tr>
<td>3.1.1.2. General structure of amino acids</td>
<td>95</td>
</tr>
<tr>
<td>3.1.1.3. Nonproteinogenic amino acids</td>
<td>96</td>
</tr>
<tr>
<td>3.1.1.4. Essential and not essential amino acids</td>
<td>97</td>
</tr>
<tr>
<td>3.1.1.5. Importance of methionine</td>
<td>98</td>
</tr>
<tr>
<td>3.1.1.6. Factors influencing requirements of dietary methionine in poultry</td>
<td>99</td>
</tr>
<tr>
<td>3.1.2. The thyroid</td>
<td>100</td>
</tr>
<tr>
<td>3.1.2.1. Development and the structure</td>
<td>100</td>
</tr>
<tr>
<td>3.1.2.2. Thyroid hormones</td>
<td>102</td>
</tr>
<tr>
<td>3.1.2.3. Functions of the thyroid hormones</td>
<td>103</td>
</tr>
<tr>
<td>3.1.2.4. Malfunction of the thyroid gland</td>
<td>105</td>
</tr>
<tr>
<td>3.1.3. Zinc</td>
<td>106</td>
</tr>
<tr>
<td>3.1.3.1. Zinc as the ion</td>
<td>106</td>
</tr>
<tr>
<td>3.1.3.2. Zinc in the history of the medicine</td>
<td>106</td>
</tr>
<tr>
<td>3.1.3.3. Genetic mutations affecting zinc availability</td>
<td>107</td>
</tr>
<tr>
<td>3.1.3.4. Zinc and enzymes activity</td>
<td>108</td>
</tr>
<tr>
<td>3.1.3.5. Role of zinc in proteins synthesis</td>
<td>110</td>
</tr>
<tr>
<td>3.1.3.6. Interactions of zinc with vitamins and minerals</td>
<td>111</td>
</tr>
<tr>
<td>3.1.3.7. Role of zinc in humeral regulation</td>
<td>113</td>
</tr>
<tr>
<td>3.1.3.8. Role of zinc in reproduction processes</td>
<td>116</td>
</tr>
<tr>
<td>3.1.3.9. Influence of zinc level on central nervous system</td>
<td>116</td>
</tr>
<tr>
<td>3.1.3.10. Zinc deficiency</td>
<td>117</td>
</tr>
<tr>
<td>3.1.3.11. Zinc toxicity</td>
<td>120</td>
</tr>
<tr>
<td>3.2. Effect of methionine on feather development in IF turkeys</td>
<td>120</td>
</tr>
<tr>
<td>3.2.1. Introduction</td>
<td>120</td>
</tr>
<tr>
<td>3.2.2. Materials and methods</td>
<td>124</td>
</tr>
<tr>
<td>3.2.2.1. Experiment 1. The effects of dietary methionine in the brooding period</td>
<td>124</td>
</tr>
<tr>
<td>3.2.2.2. Experiment 2. The effects of dietary methionine in the growing period</td>
<td>125</td>
</tr>
<tr>
<td>3.2.3. Results</td>
<td>129</td>
</tr>
<tr>
<td>3.2.3.1. Results of Experiment 1</td>
<td>129</td>
</tr>
<tr>
<td>3.2.3.2. Results of Experiment 2</td>
<td>131</td>
</tr>
<tr>
<td>3.2.4. Discussion</td>
<td>134</td>
</tr>
</tbody>
</table>
Table of Contents (continued)

3.3. Thyroid gland and its function in feather development of IF turkeys 136

3.3.1. Introduction 136
3.3.2. Materials and methods 140
  3.3.2.1. Experiment 1. Comparative evaluation of thyroid microanatomy of IF and NF turkeys 141
    3.3.2.1.1. Birds and management 141
    3.3.2.1.2. Thyroid collection 142
  3.3.2.2. Experiment 2. The effects of thyroid hormone in the diet on the ratio of feather development of 12 to 20 weeks old turkeys 142
    3.3.2.2.1. Management of the birds 142
    3.3.2.2.2. Evaluation of the thyroxine level in the diet 143
    3.3.2.2.3. Measurements 145
    3.3.2.2.4. Thyroid collection and examination 145
3.3.3. Results and discussion 146
  3.3.3.1. Experiment 1 146
  3.3.3.2. Experiment 2 151

3.4. Role of zinc in feather growth and development in the Inhibited Feathering turkey 157

3.4.1. Introduction 157
3.4.2. Materials and methods 159
  3.4.2.1. Experiment 1. The effects of dietary zinc in the brooding period (0-8 wks) 159
  3.4.2.2. Experiment 2. The effects of dietary zinc in the brooding period (0-10 wks) 161
  3.4.2.3. Experiment 3. The effect of dietary zinc in the growing period 162
3.4.3. Results 164
  3.4.3.1. Experiment 1 164
  3.4.3.2. Experiment 2 165
  3.4.3.3. Experiment 3 165
3.4.4. Discussion 168

4. Final conclusions 175
  4.1. Is the inhibited feathering a new mutation? 175
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2. The effects of the thyroid gland on the reproductive performance of the IF turkeys</td>
<td>176</td>
</tr>
<tr>
<td>4.3. The effects of zinc on the reproduction of IF turkeys</td>
<td>177</td>
</tr>
<tr>
<td>4.4. Zinc and thyroxine interactions</td>
<td>179</td>
</tr>
<tr>
<td>5. Future research and final remarks</td>
<td>180</td>
</tr>
<tr>
<td>Bibliography</td>
<td>183</td>
</tr>
<tr>
<td>Appendix</td>
<td>220</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The original mutant hen at 20 weeks of age</td>
<td>47</td>
</tr>
<tr>
<td>2.2</td>
<td>Comparison of down and feather development in an IF (left) and a NF (right) turkeys</td>
<td>55</td>
</tr>
<tr>
<td>2.3</td>
<td>Down and feather development in an IF poult at 16 days of age</td>
<td>56</td>
</tr>
<tr>
<td>2.4</td>
<td>Down and feather development in a NF poult at 16 days of age</td>
<td>56</td>
</tr>
<tr>
<td>2.5</td>
<td>Comparison of down and feather development in IF (left and center) and NF (right) turkeys at eight weeks of age</td>
<td>57</td>
</tr>
<tr>
<td>2.6</td>
<td>Variations in down and feather development in IF hens at 20 weeks of age</td>
<td>62</td>
</tr>
<tr>
<td>2.7</td>
<td>An IF male at 20 weeks of age</td>
<td>62</td>
</tr>
<tr>
<td>2.8</td>
<td>Primary remiges of an IF (left) and a Normal Feathering (right) turkeys at 30 weeks of age</td>
<td>64</td>
</tr>
<tr>
<td>2.9</td>
<td>Secondary remiges of an IF (left and center) and a NF (right) turkeys at 30 weeks of age</td>
<td>66</td>
</tr>
<tr>
<td>2.10</td>
<td>Rectrices of a NF (left) and an IF (right and center) turkey at 30 weeks of age</td>
<td>68</td>
</tr>
<tr>
<td>2.11</td>
<td>Electron micrograph of the primary remige of a NF turkey at 10 weeks of age</td>
<td>69</td>
</tr>
<tr>
<td>2.12</td>
<td>Electron micrograph of the primary remige of an IF turkey at 10 weeks of age</td>
<td>69</td>
</tr>
<tr>
<td>2.13</td>
<td>Electron micrograph of the primary remige of an IF turkey at 10 weeks of age</td>
<td>70</td>
</tr>
<tr>
<td>2.14</td>
<td>Electron micrograph of the primary remige of a NF turkey at 10 weeks of age</td>
<td>70</td>
</tr>
</tbody>
</table>
## List of Figures (continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.15</td>
<td>Electron micrograph of the primary remige of an IF turkey at 10 weeks of age</td>
</tr>
<tr>
<td>2.16</td>
<td>Electron micrograph of the primary remige of an IF turkey at 10 weeks of age</td>
</tr>
<tr>
<td>3.1</td>
<td>Comparison of the feather development in an eight week old Inhibited Feathering turkey with a feather score of 2 (left) and a Normal Feathering turkey with a feather score of 1 (right)</td>
</tr>
<tr>
<td>3.2</td>
<td>Comparison of the feather development in two eight week old Inhibited Feathering turkeys with a feather score of 2 (left) and with a feather score of 3 (right)</td>
</tr>
<tr>
<td>3.3</td>
<td>Comparison of the feather development in two eight week old Inhibited Feathering turkeys with a feather score of 3 (left) and with a feather score of 4 (right)</td>
</tr>
<tr>
<td>3.4</td>
<td>Feather development in two IF turkeys at 8 weeks of age fed the <em>Control</em> diet containing 0.54% methionine</td>
</tr>
<tr>
<td>3.5</td>
<td>Feather development in two IF turkeys at 8 weeks of age fed the <em>Methionine-1</em> diet containing 1.09% methionine</td>
</tr>
<tr>
<td>3.6</td>
<td>A cross section of the thyroid gland from an IF turkey at 4 weeks of age</td>
</tr>
<tr>
<td>3.7</td>
<td>A cross section of the thyroid gland from a NF turkey at 4 weeks of age</td>
</tr>
<tr>
<td>3.8</td>
<td>A cross section of the thyroid gland from an IF turkey at 9 weeks of age</td>
</tr>
<tr>
<td>3.9</td>
<td>A cross section of the thyroid gland from a NF turkey at 9 weeks of age</td>
</tr>
<tr>
<td>3.10</td>
<td>A cross section of the thyroid gland from an IF turkey at 16 weeks of age</td>
</tr>
<tr>
<td>3.11</td>
<td>A cross section of the thyroid gland from a NF turkey at 16 weeks of age</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3.12</td>
<td>A cross section of the thyroid gland from a NF turkey at 20 weeks of age fed the <em>Control</em> diet</td>
</tr>
<tr>
<td>3.13</td>
<td>A cross section of the thyroid gland from a NF turkey at 20 weeks of age fed a diet containing 1 ppm thyroxine</td>
</tr>
<tr>
<td>3.14</td>
<td>A cross section of the thyroid gland from a NF turkey at 20 weeks of age with feathers removed at 12 weeks of age fed the <em>Control</em> diet</td>
</tr>
<tr>
<td>3.15</td>
<td>A cross section of the thyroid gland from a NF turkey at 20 weeks of age, with feathers removed at 12 weeks of age fed a diet with 1 ppm thyroxine</td>
</tr>
<tr>
<td>3.16</td>
<td>A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet with no thyroxine supplementation</td>
</tr>
<tr>
<td>3.17</td>
<td>A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 1 ppm thyroxine</td>
</tr>
<tr>
<td>3.18</td>
<td>Feather development in two IF turkeys at 8 weeks of age fed the <em>Control</em> diet containing 65 ppm zinc</td>
</tr>
<tr>
<td>3.19</td>
<td>Feather development in two IF turkeys at 8 weeks of age fed the <em>Zinc-1</em> diet containing 130 ppm zinc</td>
</tr>
<tr>
<td>3.20</td>
<td>A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 40 ppm zinc</td>
</tr>
<tr>
<td>3.21</td>
<td>A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 360 ppm of zinc</td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Progeny phenotypes from the matings of Normal Feathering (k\k), homozygous dominant (K\k\k), heterozygous (K\k\k) and hemizygous (K\k-) Inhibited Feathering turkeys</td>
</tr>
<tr>
<td>2.2</td>
<td>Mean plasma chemistry values of IF (Inhibited Feathering) and NF (Normal Feathering) turkey hens measured at 30 weeks of age</td>
</tr>
<tr>
<td>2.3</td>
<td>Amino acid composition of feathers from IF (Inhibited Feathering) and NF (Normal Feathering) turkeys</td>
</tr>
<tr>
<td>2.4</td>
<td>Feeding program for turkeys between 1 day and 25 weeks of age (WOA)</td>
</tr>
<tr>
<td>2.5</td>
<td>Composition of Hen and Tom Diets fed between 31 weeks to 45 weeks of age</td>
</tr>
<tr>
<td>2.6</td>
<td>Egg production of the IF (Inhibited Feathering) and NF (Normal Feathering) turkey females</td>
</tr>
<tr>
<td>2.7</td>
<td>Semen production of the IF (Inhibited Feathering) and NF (Normal Feathering) turkey males</td>
</tr>
<tr>
<td>2.8</td>
<td>Hen fertility and incubation performance of eggs produced by IF (Inhibited Feathering) and NF (Normal Feathering) turkey hens</td>
</tr>
<tr>
<td>3.1</td>
<td>Composition of Control-1 and Methionine-1 diets fed to IF (Inhibited Feathering) and NF (Normal Feathering) poult's from day-old to 8 weeks of age</td>
</tr>
<tr>
<td>3.2</td>
<td>Composition of Control-2, Methionine-2 and Methionine-4 diets fed to IF poult's from 12 to 20 weeks of age</td>
</tr>
<tr>
<td>3.3</td>
<td>Feather score and body weight gain in IF (Inhibited Feathering) and NF (Normal Feathering) poult's fed diets containing 0.54% and 1.09% methionine from day-old to 8 weeks of age (Experiment 1)</td>
</tr>
<tr>
<td>3.4</td>
<td>Feather score improvement index (FSII) and weight gain in IF (Inhibited Feathering) poult's fed diets containing 0.32% (control), 0.62% and 1.22% methionine from 12 to 20 weeks of age (Experiment 2)</td>
</tr>
</tbody>
</table>
### List of Tables (continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>Composition of diets without <em>(Control)</em> and with supplemental thyroxine at 1 ppm <em>(Thyroxine)</em> fed to IF poults from 12 to 20 weeks of age</td>
</tr>
<tr>
<td>3.6</td>
<td>Feather score improvement index (FSII) and weight gain (kg) of the IF (Inhibited Feathering) turkeys fed unsupplemented <em>(Control)</em> and supplemented 1ppm of L-thyroxine free acid <em>(Thyroxine)</em> diets from 12 to 20 weeks of age</td>
</tr>
<tr>
<td>3.7</td>
<td>Composition of <em>Control-1</em> and <em>Zinc-1</em> diets fed to IF poults from day-old to 8 weeks of age (Experiments 1 and 2)</td>
</tr>
<tr>
<td>3.8</td>
<td>Composition of <em>Control-2</em>, <em>Zinc-3</em>, <em>Zinc-6</em>, and <em>Zinc-9</em> diets fed to IF poults from 12 to 20 weeks of age (Experiment 3)</td>
</tr>
<tr>
<td>3.9</td>
<td>Feather score and weight gain in IF (Inhibited Feathering) and NF (Normal Feathering) poults fed a starter containing 65 ppm and 130 ppm of zinc from day-old to 8 weeks of age (Experiment 1)</td>
</tr>
<tr>
<td>3.10</td>
<td>Results of dietary zinc supplementation on feather score of IF (Inhibited Feathering) poults fed a starter containing 65 ppm of zinc <em>(Control-1)</em> or 130 ppm of zinc <em>(Zinc-1)</em> from day-old to 10 weeks of age (Experiment 2)</td>
</tr>
<tr>
<td>3.11</td>
<td>Feather score improvement index (FSII) and weight gain in IF (Inhibited Feathering) poults fed diets containing 40 ppm (control), 120 ppm, 240 ppm and 360 ppm of zinc from 12 to 20 weeks of age (Experiment 3)</td>
</tr>
</tbody>
</table>
Inhibited Feathering, K^1 a Sex-Linked Dominant Gene in the Turkey (Meleagris gallopavo), Genetics and Nutrition

Chapter 1
Introduction

The turkey industry has traditionally been an important part of American agriculture. In 1994, 286 million birds were produced, and the forecast for turkey production in the near future is very optimistic. It is predicted that until the year 2000, the number of turkeys produced in United States will steadily increase. At the same time the prices are expected to decrease even below their current, low level to attract more consumers (Christensen, 1994). Turkey meat is not only an indispensable component of Thanksgiving, Christmas and other national holidays, but it also constitutes a significant portion of the regular diet of a typical American. The continuing growth in the consumption of turkey products is apparent. An average person in the United States consumed 7.83 kg (17.6 lb) of turkey meat in 1990, whereas the average consumption in 1995 is estimated at 8.19 kg (18.4 lb) (Christensen, 1994). Turkey meat has won its place in the every day diet of the average consumer. The popularity of this kind of meat arises from its numerous advantageous characteristics: it is low in cholesterol - only 49 mg in 100 g of light meat, with 0.3 g of saturated fatty acids and lean, and 103 kcal of energy in 100 g of raw breast. The composition of the skinless turkey breast for example is similar to the composition of veal which contains in 100 g of a raw fillet, 84 mg of cholesterol and 0.9 g of saturated fatty acids, and 109 kcal of energy (Holland et al., 1992). The already high consumption of this meat would almost certainly increase if the price of turkey meat products were reduced. Similar to other branches of agriculture, the turkey industry has been recently under increasing pressure to reduce the costs and improve profitability. In today's competitive market, to achieve those goals it is necessary to utilize innovative technologies and scientific analysis. No single science discipline can investigate biological processes without referring to other biologically
related fields. In particular, when geneticists and nutritionists combine their efforts, they may offer the producers results of significant practical value.

This dissertation is devoted to studies describing a new gene mutation in the turkey, which has the potential to dramatically improve economic efficiency of turkey production. This thesis investigates the possibilities of practical applications of a new gene for the turkey industry. To this end, both genetic and nutritional methods are used to investigate and control features influenced by the gene. In addition, histology and ethology are used for analysis of the effects of the gene. This integration of different biological science methods to solve a practical problem is typical to most of advanced industrial applications of animal science.

1.1 Zoological taxonomy of the domestic turkey

The turkey (*Meleagris gallopavo*) is represented by six subspecies which are the ancestors of the modern domesticated bird (Campbell and Lack, 1985). All six of them belong to the order Galliformes, family Meleagridae, together with the ocellated turkey (*Meleagris ocellotae*). One of the subspecies, turkey Mexicana (*Meleagris gallopavo gallopavo*) - has played the most important role in the development of the domesticated turkey.

1.2 The history of the domesticated turkey

According to current knowledge, the turkey as a separate specie originated in North America. The most probable region of origination is Mexico. It is believed that turkeys were common in the Miocene period (about 30,000,000 years ago), even though there is no physical evidence such as fossils from that interval (Wetmore, 1936). Their presence in Pliocene and Pleistocene can be proved without any doubt based on the skeletal remains (Howard, 1950). The turkey was domesticated by the Native Americans
prior to the discovery of America by the Europeans in 1492. During the Cortés expedition in 1519, the members of the crew repeatedly reported that the turkey was widely distributed as a domesticated bird in the area of present day Mexico. Several members of Cortés party described large birds, which they called "gallinas" that were as large as peafowl. In the Mexican territory, turkeys were raised in abundance. There is evidence that the turkey was used as a meat source at least 500 years before the arrival of Europeans to America (Webb, 1938). It is also believed that thousands of turkeys were killed and used every year by Native Americans. Among numerous other examples, Schorger (1966) mentions that every day large number of turkeys were required to feed not only humans but also the carnivores in the Montezuma’s menagerie.

Some Native American tribes used the turkey not only as a source of meat, but also for its feathers. There are indications that some Native American did not maintain the turkey as a domesticated bird. Rather, they used the wild turkey as a source of meat (Feltwell, 1957) or feathers used for adornment and for arrow manufacturing (Mosby and Handley, 1943). Several Native American nations had beliefs which forbade or discouraged eating turkeys. For some of them, turkey was a kind of taboo whereas others did not consider the wild turkey as a valuable prey. The Cherokee tribe, for example, reserved turkeys for young, eight to ten year old children to practice hunting (Wright, 1913).

The turkey was brought to Spain in the early sixteenth century. Until the late 1960’s it was believed that the earliest arrival of the turkeys to Europe occurred between 1518 and 1525. Several records are available that suggest introduction of the turkey to Spain between these years (Schorger, 1966). According to other sources a more probable time of introduction of the turkey to Europe was 1519 or 1520 (Wright, 1913, Wright, 1914). However, in 1960 new documents were uncovered in the Archives of the Indias that shift the day of the first arrival of the turkey to Europe to 1511 or 1512. In particular, two documents have been most often quoted (Schorger, 1966). One of them is an order from the Bishop of Valencia to bring turkeys for breeding purposes. The other document reports arrival of turkeys in Spain. From there turkeys spread to England and continental Europe. Records indicate that the first breeder turkeys were shipped to
Italy in 1524 and probably reached England about the same time. The earliest document describing turkeys in Germany is dated 1530. This suggests an approximate time of introduction of the turkey into this country. Existing documents suggest that this bird was present in France in 1538 (Schorger, 1966). Based on turkeys imported from America several varieties of the bird had been developed in Europe which were later reintroduced to the North America as the domesticated turkey. In the nineteen century the European domesticated turkey was crossed with the wild, American bird. After several years of crossing, the Bronze turkey resulted. This variety of turkey when inbred produced many mutant colors, such as white, gray, and black. There is no agreement on when the white ancestor of the modern white domesticated turkey was developed. Some sources suggest that the white turkey used currently in the United States did not originate from the European white bird but it was independently developed in North America (Mardsen and Martin, 1939). Others (Cline, 1933) indicate that the white variety of turkey was imported to America from continental Europe.

Turkey meat was used by American pioneers as a holiday dish. The practice of using the turkey at Christmas is a European tradition that was later introduced to United States. Historical records suggest that the turkey dish was used in Europe for the first time in France in 1570 by King Charles IX (Cline, 1933). In North America, the turkey was the traditional dish at Thanksgiving: "In 1621, after the first harvest was gathered and it had been proved a good yield, the early Pilgrims instituted a three day festival, the well known forerunner of our present Thanksgiving day. At this first feast, above all they had the turkey, of which they found a 'great store' in the forest" (Wright, 1914).

The turkey has also established its place in American culture in another way. In all parts of United States different places have been named after the turkey. Names such as Turkey Island, Gobbler Knob, Turkey Cock Mountain are very common through the whole country (Mosby and Handley, 1943).

In the dispute around the National Emblem for the United States, Benjamin Franklin spoke for the turkey and against the bald eagle (Franklin, 1987). He said at that time: "... the turkey is in the comparison a much more respectable bird and withal a true original native of America. [...] He is [...] a bird of courage..."
For several years the turkey was used mainly as a feast dish for Christmas, Thanksgiving and other major holidays. Nowadays, the production of turkey meat is high all year around. The main part of the production is sold as so called value added product, whereas only a small part of total production is marketed traditionally as a whole carcass. Imported domesticated turkey served as the "seed" for development of small farms in the New England States. Birds raised in the farm industry served as a trade item between America and Europe. This rather small industry soon spread to the west and became more and more commercially important. In 1890, turkey production appeared for the first time in the census report. Eleven million turkeys were raised that year. Since that moment there could be noticed some fluctuations in the number of the turkey produced in United States. The overall tendency, however, seems to be increasing at that time. Census records indicate, that while decreases were taking place in New England territories -12% and in the east north states by 44%, Pacific states showed approximately a 50% increase in the turkey production during the period 1910-1920. In the overall picture of turkey industry in all states, the increasing tendency could be noticed (Cline, 1933). Between 1980 and 1995, the number of turkey raised in the United States increased by 77%. A similar escalating trend is in the consumption of turkey products. In 1980 each person consumed only 4.45 kg of turkey meat. This amount increased to 8.19 kg in 1994 (Christensen, 1994).

For a long time the turkey production has been concentrated in five states: North Carolina, Minnesota, Arkansas, California and Missouri. This list has not changed lately and these states still supply about 60% of all turkey meat with outstanding leader - North Carolina supplying 21% of the total turkey production (Christensen, 1994).

1.3 Genetics in the history of animal breeding

Genetics is a branch of biology which has been developing very rapidly and extensively in recent decades. Although most of the genetic discoveries took place within last hundred years, the origin of genetics can be traced back to ancient times. The
concept of inheritance was known in Egypt and Mesopotamia several thousand years B.C. At the time when the first animals were domesticated, they were chosen for breeding based on their characters (best looking, healthy and promising individuals). The early genetic work was limited to the selection only. People believed that good parents gave good offspring.

Hippocrates and Aristotle were among those who attempted to explain procreation and heredity. They envisaged the inheritance of specific characters and illnesses. It was thought that the progeny had to resemble their mother and father. The differences observed between parents and their offspring were explained as due to environmental factors, such as injury to the uterus, influence of the surroundings, malnutrition etc. (Aristotle, 1963; Hippocrates, 1964). The problems of character preservation are also addressed in the Iliad by Homer. He indicated how strength and greatness of the father were important for the excellence in his sons, which meant that only the best gave the best (Homer, 1965). The Romans were also aware of the hereditary nature of some characteristics. Some of their theories, however, were very naive particularly the concept of the origination of new species and the concept of hybridization. Their legends contain many examples of relations between human and animals resulting in the creation of the beasts. Nevertheless, the Romans did develop an accurate theoretical foundation of selection and breeding of animals (Stubbe, 1972).

The medieval ages were not very friendly for those studying the nature of the world. European scientists working at that time tried to fit the theories of Aristotle to the axioms of the Christian Church. Consequently, genetics underwent periods of stagnation. In the eighteenth and nineteenth centuries the number of genetic discoveries increased. Both, plant and animal mutants were used as objects of studies. Polled cattle and short-legged sheep became very popular after Felix d’Azara and S. Wight developed these lines of animals (Stubbe, 1972). The achievements of scientists such as Thomas Andrew Knight, Gregor Mendel and others opened the gateway for other discoveries. To date, genetics is inherently connected with breeding of animals and plants.
1.4 Analogy and divergence of turkey and chicken genomes

The increasing public demand for inexpensive turkey meat creates a need for intensified production with lower costs. A portion of the production cost can be reduced through changes in management, but major savings will be obtained by utilizing the most current knowledge of the turkey genome. Through carefully designed breeding programs, it is possible to obtain birds displaying favorable economic traits while lowering production costs.

One source of genetic information for turkeys is the results of research performed on other poultry species, particularly chickens due to genotypic similarities between these species. This similarity of both genotypes can be demonstrated by the possibility to create a hybrid organism using turkey and chicken parents. Warren and Scott in 1937 attempted to produce a turkey-chicken hybrid using artificial insemination, and they were able to obtain embryos. Despite their efforts, however, they were unable to hatch live hybrids. The oldest embryo capable of life, survived to 20 days of incubation. A similar attempt was made two years later by Quinn and co-workers, 1937. Of the incubated eggs, 20.1% were classified as fertile but none of the hybrid embryos hatched. Yet another attempt was made by Asmundson and Lorenz in 1957, with similar results of 119 turkey eggs incubated, only 19.3% were fertile and two embryos died in the advanced stages of development. In spite of the lack of successful results to obtain developed hybrids, other attempts were made in order to hatch turkey-chicken hybrids. Finally, Olsen in 1960 was able to hatch 23 hybrids, four of which survived several weeks. Despite the very low incidence of hybrids surviving, it can be stated that the genetic material of the chicken and turkey is similar (Harada and Buss, 1981). It is therefore possible to consider gene homology in the two species if they have comparable modes of inheritance. It must be emphasized, however, that comparison of the two genotypes has a limited applicability and most research should be performed involving the species of the interest.

The number of genes described in the turkey itself is very limited. In spite of the bird’s popularity, only 35 gene loci have been described (Savage, 1990). The majority
of reported genes influence feather color. There are three neurological and four metabolic mutations that have been described to date. In the history of genetic research involving the turkey specie only two genes have been found to be mutations of the skin, and only three genes are associated with variations of feather development and feather appearance. A complete review of the current status of the turkey genome will be elaborated in a subsequent chapter.

1.5 The focus of this work

Some of the single genes responsible for qualitative traits in poultry are utilized for improving production efficiency. Well documented examples of the application of single genes in the poultry industry are those affecting plumage color and rate of feather development. The former can be utilized to distinguish sex in day-old chickens through the color of the down. The latter gene is used to sex chicks based on the appearance of the primary and secondary wing feathers. Both, autosexing and feather sexing are very common methods of sex identification in commercial chicken hatcheries. These methods are advantageous when compared to traditional vent sexing because they not only save money and time, and they are also less stressful to the birds.

The paucity of genetic information in the turkey does not allow for such sophisticated methods. The only available method of sex determination in day-old poults is vent (cloacal) sexing. This technique which is time consuming and stressful for young poults could be replaced with an autosexing or feather sexing, assuming an appropriate gene is available. Unfortunately none of the currently known genes responsible for the down color in turkeys are appropriate for this purpose. The current commercial demand for colored feathered birds is not practical. Dark pigmented feathers can leave skin on the carcass colored and unpleasant. On the other hand, if a gene affecting feather development is sex linked and is available, it could be applied to commercial turkey production.
The purpose of this dissertation is to investigate a newly discovered gene, K¹, that affects feather development in the turkey. The gene has the potential for the commercial application of feather sexing of newly hatched poults. This activity could have a great economic impact on the turkey industry and would allow producers to lower one of their production costs.
Chapter 2
Heredity and Phenotype of Inhibited Feathering in the Turkey

2.1 Literature review

2.1.1 Avian feather - development and structure

The understanding of feather growth processes is essential to deciphering the mechanism of the gene's action. This part of the dissertation contains a review of the most important facts about the development and morphology of the avian feather with particular emphasis to those of the turkey. The objective is to provide background information that is necessary to appreciate the contents of the following sections of this chapter. First, the development of the skin and of feather follicles is discussed in some detail. The information reviewed was adapted from the chicken since the literature describing turkey embryo development is very limited. The similarities of the chicken and turkey permit descriptive details related to the chicken to be applied to the turkey. The indisputable differences between these two species such as the length of the embryonic development must be recognized. Second, to facilitate the description of mutants birds in comparison with normal ones, the structure and main types of avian feathers are discussed. Finally, some attention is given to the description of normal feather tracts of the Large White Turkey - the strain in which the inhibited feathering gene was first identified and investigated.

The review found in this chapter is by no means a complete description of avian, or even only turkey plumage. What follows is intended as a brief reference, and therefore only aspects relevant to the K' mutation are covered. The objective of this narrative is to establish terminology necessary for the remainder of this dissertation. General information on avian feathers can best be found in the monograph of Lucas and Stettenheim (1972) which also served as the main source for this compilation. The
description of plumage of normally feathered White Medium Turkey in section 2.4 is based by the author’s observations performed on the turkey research facility at Oregon State University and to her best knowledge, cannot be found elsewhere.

2.1.1.1 Morphology and development of skin and feather follicles

This section contains a brief overview of epidermal tissues and the developmental processes that lead to feather formation. An understanding of the morphology of skin and feather follicles will be necessary in the following chapters where the mutant birds will be discussed.

2.1.1.1.1 Skin morphology

The skin of a bird consists of two main layers, the epidermis and dermis. The former is the outer layer, while the latter lies beneath it. The epidermis is composed of two sublayers, the outer sublayer, known as stratum corneum which is keratinized, and the inner, stratum germinativum which creates the lining. An alternative, more detailed description of the epidermis by Cane and Spearman (1967) delineates four layers: a basal germinal layer which is the most inner one, a layer of larger polygonal shaped cells with large nuclei and easily visible nucleoli, a transitional layer of squamous cells with small nuclei where the keratinization takes place and, a stratum corneum, consisting of squamous keratinized cells without nuclei.

The epidermis does not display any vascularization and its nourishment can only be provided by the most inner layer of the skin, the dermis. In the areas where the skin is thin, the nutrition of epidermis is sustained by diffusion processes. In the thick areas, two structural adaptations help to manage the nutrition of the epidermis. First, the surface of connection between the dermis and the epidermis is enlarged by folds. Second,
capillaries from the dermis enter the stratum germinativum and create loops on the apices of the dermal folds (dermal papillae). The epidermis itself may be of variable thickness. On parts of the body that are covered with the feather or otherwise protected from friction movements and injuries, the epidermal layer may be very thin, whereas the beak or feet are covered with a thicker epidermis. In thin areas the stratum germinativum may be formed only by two layers of cuboidal cells. One layer of transitional cells lies between the stratum germinativum and the stratum corneum. The latter consists of many layers of squamous keratinized cells.

Dermis, also called the corium, is located under the stratum germinativum. The most apical part of corium consists of numerous layers of collagen fibers. Fibrous connective tissue comprises a significant portion of the dermis. All blood vessels and arteries as well as nerves are embedded in connective tissue. The dermis is responsible for the maintenance of nutrients for the epidermis. It is connected to loose connective tissue, subcutis, which overlies muscles of the body.

### 2.1.1.1.2 Feather follicle morphology

Feather follicles are located in the dermal and subdermal layers of avian skin. A feather follicle is composed mainly of the epidermal tissue, and can be described as "a tube of epidermis sunken into a dermis" (Ostmann et al., 1963). The wall of the feather follicle is lined with epidermal cells located in the dermal part of the skin. The shape of the follicle resembles that of an onion bulb whose base is filled with loose connective tissue. This area, called the dermal papilla, is characterized by the shape of a lens or a hemisphere and can be easily distinguished from the surrounding tissue. The dermal papilla is one of the structures essential for feather formation. The basic structure of the underlying connective tissue consists of bundles of collagen fibers which anchor their edges firmly to the wall of the feather follicle. The upper part of the papilla has no well defined boundary and it passes out apically as strands of cells which grow into feather pulp. A single artery called the axial artery and a number of veins passing trough the
center of the papilla nourish the feather follicle. The dermal papilla is responsible for feather growth, but it does not determine the feather type. If the papilla is removed from the follicle, the feather will not form.

A collar of the feather follicle germinates from the dermal papilla. It is surrounded by dermal papilla and gives rise to the feather shaft and barbs. The basal part of the collar is comprised of several layers of cells. During the resting period, when the feather is present in the follicle, the main part of the collar becomes keratinized and constitutes the base of the calamus. When the feather is lost, the collar proper develops from the layer of the germ cells. From this structure the new feather develops. The collar proper consists of four main regions:

- germinative layer. This outer layer consists of the sheath cells which are undifferentiated and in contact with the dermis. These cells are small and compressed with dark staining nuclei.

- zone of inner sheath cells. Two types of cells can be found in this zone. Polygonal cells are found in the lower section of the collar - incipient transitional layer, whereas the upper part consists of spindle-shaped cells whose long axes are tangential to the circumference of the collar - outer layer.

- zone of intermediate cells. Several layers of cells accounts for 20 - 30% of the wall of the collar. They are oval and their axes are set radially to the circumference of the collar.

- zone of basilar cells. This is the most inner layer of the collar proper that surrounds the feather pulp. The cells are cylindrical with nuclei aligned near the outer ends of the cells. A thin cellular structure, the basement membrane is found on the inner edge of this region and it separates the collar from the pulp (Lucas and Stettenheim, 1972).

The above described layers give rise to different parts of the feather. The feather sheath develops from the outer layer. The intermediate and basilar cells zones give rise to the rachis and to the barbs. The feather sheath and epidermal lining of the follicle wall create an empty space called the follicle cavity. It has been suggested that since this cavity is present only in some sections, it is only an artifact due to histological processing. Further evidence for that statement is given by presence of tiny keratinized strands connecting the two layers. It is possible that those strands are formed during
shrinkage of the feather and the keratinized layer of the epidermis (Ostmann et al. 1963). Studies performed much later than Ostmann and co-workers (1963), showed that two different kinds of attachments can be observed between the feather and the follicle wall (Angel et al., 1981). In the first kind of connection of feather and feather follicle, the follicle wall is covered with a layer of flattened and keratinized cells that face the feather with their convex surfaces. The feather shaft is also covered with squamous keratinized cells that fit into the shape of the cells that line the follicle wall. Therefore, the cells of the follicle wall "aim" into the cells over the shaft. The other kind of connection of feather and feather follicle consists of "bridges" that link the wall of the follicle and the feather. The combination of both arrangements may be present in the same feather follicle (Angel et al, 1982). These connections may be important in preserving the feather in the follicle (Lucas and Stettenheim, 1972).

2.1.1.1.3 Muscle system of feather follicles

The feather follicles are situated within a network of muscles. Typically, the follicle of a contour feather is surrounded by four muscles called arrectores plumorum muscles which are arranged in a quadrilateral configuration. This network of muscles is arranged between any four neighboring follicles. Connections between muscles and the feather follicles is provided by elastic tendons. The elastic fibers of the tendons are connected with elastic fibers from connective tissue surrounding the feather follicles. Tendons may be attached to each side of the feather follicle through divided attachments or may be connected perpendicularly to the follicle (Ostmann et al. 1963). Normally, there are two muscles which connect each two adjacent feathers. The depressor muscle is attached to the basal end of the posterior or ventral feather and to the upper portion of the anterior or dorsal feather. Contraction of this muscle causes depression of both feathers. The antagonistic elevator muscle runs from the basal part of the anterior or dorsal follicle to the upper part of the other one. Contraction of this muscle causes erection of both feathers. Numerous contraction bands have been observed in the muscles
of the feather follicles. These are a result of smooth muscle cells being caught in a completely contracted stage during fixation and staining. Therefore, they appear as shorter, thicker and darker fibers than the rest of the muscle cells (Ostmann et al. 1963).

The feather follicles are innervated by efferent branches of the thoracicolumbar division of the autonomic nervous system. The nerve fibers which penetrate the smooth muscles can be connected to them in two ways. They continue to course through the muscle as a thin bundle or they branch and continue to course as individual nerve fibers which can terminate as free nerve endings (Ostmann et al. 1963). Numerous nerves are present in the connective tissue stroma of the skin and within the muscles but not in the wall of the feather follicle.

The sensory nerve ending is called the Vater-Paccini corpuscle (Herbst corpuscle). It is an encapsulated nerve ending and is composed of concentric lamellae filled with fluid (Ostmann et al. 1963). Herbst corpuscles are observed in close association with feather follicles, even though they can also be found in the bare skin. Usually there are one or two Herbst corpuscles for a given feather follicle regardless of the area from which the skin originates. The Herbst corpuscles can be located just above the insertion of the multiple smooth muscle bundle on the feather follicle (Winkelmann and Myers, 1961). It is believed, that stimulation of the nervous system surrounding feather follicle promotes feather development (Spearman, 1971).

2.1.1.2 Formation of the skin

The integument arises from two embryonic layers: ectoderm and mesoderm. Epidermis, the outer part of the skin, can be recognized as a single layer of cells on the first day of the incubation of the chicken embryo. On the second day of the incubation, the single layer starts to divide to form epitrichium (a superficial layer of squamous cells) and the layer of cylindrical cells. The latter proliferates and forms stratum germinativum. Throughout the life of a bird the germinative layer produces cells for the cornaceous layer (zone of keratinized cells). A very thin basement membrane forms between the epidermis
and the dermis. At least some components of this membrane's structure are produced by epidermis. During the next 13 days of incubation four multilayer zones of cells are formed. (Dodson, 1967). The dermis originates from the derivatives of the mesoderm. The skin on the dorsal and dorsolateral areas of the body is created from the dermatome (the outer layer of the segments). The ventral and ventrolateral parts of the body are covered by dermis that originated from the layer of mesoderm laying lateral to the somites. Blood vessels and nerves arise from all three embryonic layers and are embedded in the dermis (Lucas and Stettenheim, 1972).

2.1.1.5 Formation of the feather germs

The feather germs appear on approximately the fifth day of incubation of a chicken embryo as a condensation of mesenchyme into a layer of dermis (Holmes, 1935). They usually emerge near to the edge of the future feather tract. The dermis induces epidermal differentiation at a very early stage of feather germ formation, but later the epidermis influences dermis to form a core of pulp (Lucas and Stettenheim, 1972). This suggests that ectoderm induces feather development (Wessels, 1965). The first visible signs of development of feather germs are placodes which are clumps of epidermal cells that become more elongated than the cells surrounding them (Lucas and Stettenheim, 1972). Placodes appear prior to the formation of rows of dense clusters in the dermis. The epidermis surrounding this part of the skin breaks up into lines of feather primordia. One line of feather primordia is formed on each side of the area of dense dermis. Subsequent rows repeat that process and in this fashion the pattern of the feather tracts is laid out in the skin (Holmes, 1935). All feather follicles seem to be formed during embryogenesis even though some of the feather does not form until late after hatch (Lucas and Stettenheim, 1972). The pattern of feather formation is regulated by the distribution of the homeoproteins in the embryo's tissues. Concentrations of homeoproteins vary along the main body axis of the embryo. One of the homeoproteins that has been investigated is X1Hbox 1, whose concentration is highest in the anterior-proximal region and
gradually decreases along the body axis. No expression of X1Hbox 1 was detected in feather buds of the caudal region of the embryo (Chuong, et al., 1990). One of the chemical factors influencing localization of the feather appendages is retinoic acid. The concentration of all-trans and 13-cis retinoic acids influence morphogenic activity. For normal feather development, it is necessary that the concentration of retinoic acid also vary appropriately along the body axis. The absence of a suitable gradient of retinoic acid concentrations prevents formation of the anterior-posterior distribution of Neural - Cell Adhesive Molecules (N-CAM) and affects the orientation of feathers (Chuong, et al., 1992).

The order in which feather germs begin to develop in the tracts is species specific. In the chicken, the feather germs begin to elevate about the eighth day of incubation (Hamburger and Hamilton, 1951). The epidermis and the dermis are involved in this phase of development. On the ninth day of incubation, young feather germs are approximately 0.5 mm long and become macroscopically visible on the outside surface of the skin, afterwards feather development becomes very rapid. During the next 24 hours the feather doubles its size (Bell and Thathachari, 1963). At this stage, cells of the epidermis become rearranged and create a group of barb ridges - rudiments of the barb (Lucas and Stettenheim, 1972). In the twelfth day of incubation, feather follicles and feather germs become more distinct. The feather follicle is visible as a deep narrow cavity with a long cylindrical feather germ inside. The follicle wall has formed and the follicular cavity is developed between the feather shaft and the follicle wall. The feather muscles become more organized although they are not yet attached to the wall of the follicle. In the next step the epidermis starts to proliferate at the base of the feather follicle and the epidermal collar is formed. Almost at the same time, barbulae rudiments become visible at the barb ridges of the feather. In the developing feather within the distal and peripheral parts are older and more developed than their proximal part. The latter, however, represents the later stage of feather structure development. Keratinization of the feather occurs approximately on the 13th and 14th days of the chick embryo’s incubation. By the 16th day, the feather sheath has become fully keratinized but the lining of the feather follicle remains unchanged until it is keratinized at around the 19th
day. Final barb keratinization also occurs at this time. The development of the feather follicle continues after the natal down has formed. After hatching, the feather follicle grows deeper into the skin. This, however, is due more to skin thickening and not to feather follicle development itself.

2.1.2 Ptilosis of the turkey

2.1.2.1 Morphology and characterization of the contour feathers

Feathers that cover the body of a bird are very diverse. They can be assigned to several types based on their size, structure, as well as their location on the body. Usually four types of the feathers can be distinguished on any kind of the bird: contour feathers, down feathers, bristles, and filoplumes.

Contour feathers are the most distinct, and the largest type of the feathers, and they constitute the main part of the bird’s plumage. The majority of the externally visible feathers belongs to this type (Rawles, 1963). Three major groups of contour feathers can be distinguished on a bird: remiges, rectrices, and body feathers. They are all highly specialized and may differ in the macroscopic appearance (Rawles, 1963).

2.1.2.1.1 General morphology of contour feathers

Apart from the differences in the size and shape of the rectrices, remiges and body feathers, the morphology of all contour feather is quite similar. One of their purposes is to protect the thin and delicate skin against mechanical injuries. Contour feathers are also helpful in the thermoregulation. In order to withstand harsh environmental conditions, contour feathers must be characterized by strength and firmness. These requirements are reflected in the morphology of contour feathers.

The typical contour feather consists of two major parts: shaft and vane. The shaft is the long axis of the feather on whose upper part the vanes are located. The proximal
fragment of the shaft which is not connected with vanes is called the calamus. It is a stiff, and rather short structure, attached to the feather follicle. It is not pigmented and usually transparent. The wall of the calamus consists of three layers of the stratified squamous epithelium: the outer layer - sheath, the intermediate layer, and the basilar layer - pulp epithelium. The structure of the inside of the calamus after the growth of the feather reflects the stages of its development. This is well illustrated by feather caps, which resemble transverse partitions. These are formed in the most inner tube built of the basilar layer, and are artifacts of the pulp that fills the calamus during early phases of its growth (see section 2.1). Soon after the pulp is reabsorbed, membranes surrounding the pulp become cornified and form feather or pulp caps.

The distal portion of the shaft is known as the rachis. Its structure is significantly different from that of the calamus. Unlike the calamus the rachis is a solid structure. Its wall is opaque because of the air enclosed inside the cells of the intermediate layer of the epithelium. The rachis is not round in crosssection as is the calamus, but displays a depression on the ventral side called the ventral groove of the rachis which is surrounded by two ventral ridges. The dorsal side of the rachis is oval. Its lateral edges are the sites where the rami grow. In the crosssection, two layers can be distinguished. The inner layer, called the medulla is composed of pith, a spongy tissue consisting of keratinized epithelial cells. The thinner, outer layer called the cortex is formed of the same type of cells as is the wall of the calamus.

The other element of the feather structure is the vane. Two vanes, the outer and the inner are located on two sides of the rachis. Each of the two vanes consists of two major sections that differ in the type of connections between rami and barbules. The most proximal fragment of the vane is called the plumulaceous portion, while the distal area is denoted as the pennaceous portion. Both are described below.

Generally, a vane consists of the rami and barbules equipped with hooklets and cilia that allowing connections between them. The location of the barbules on the vane and presence and type of hooklets and cilia determines morphological differences between the types of vanes. Structures arising from the lateral sides of the rachis are called the rami. In their crosssection, the ramus and the rachis are composed of two similar layers -
cortex and medulla. The outer surface of the ramus is the place for barbulae growth which allow connections between the neighboring, parallel rami, and effectively creates the flight surface of the feather. The dorsal site of the ramus is the site for growth of the distal barbules. Those emerge from the distal ledge located just below the dorsal ridge of the ramus. The proximal barbules are outgrowing from the proximal ledge. The ventral side of the ramus is identified by the presence of its ventral ridge.

There are two main types of barbules - plumulaceous and pennaceous. The difference between them lies in presence or absence of connection devices. Plumulaceous barbules also called downy barbules do not have hooklets or other apparati for connection with each other. Thus, the rami of the plumulaceous (the most proximal) portion of the vane are not connected and do not form a flat surface with general appearance similar to that of down. A plumulaceous barbulae consists of the base, the pennulum, and the nodes. The base is composed of a few cells and is wider than the rest of the barbulae. The pennulum is composed of several long cells arranged linearly one on top of the other resulting in a structure resembling a bamboo plant. The junction between those single cells of the pennulum are called nodes. The structure of a node is characteristic for particular species.

The pennaceous barbules are located on the main part of the vane in the flat and knit section of the feather. Their main characteristic is the presence of the connection apparati. Two different types of pennaceous barbules may be specified according to the arrangement of those appendices, distal and proximal barbules. Distal pennaceous barbules are outgrowths from the distal ledge of the ramus. Each barbule consist of a long base and a pennulum. The terminal end of the pennulum is equipped with a set of cilia and hooklets. On its dorsal side the dorsal cilia are situated while the ventral side is covered with hooklets which transform into ventral cilia toward the distal end of the barbule. The base of the distal barbule is cessated by the ventral tooth. A second type of a pennaceous barbule, the proximal barbule, is characterized by its base being much more complex than its pennulum. The apical portion of the base is wide and furnished with a dorsal flange. Towards the terminal end the dorsal flange becomes the dorsal spine with wavy edges. The ventral tooth is very simple and uniform. The pennulum of a
pennaceous proximal barbule is incomplete and devoid of any hooklets. Cilia, if present, are very delicate, however they usually do not develop.

Apart from the two basic types of barbules, a third category of simplified or reduced barbules is sometimes observed. This term is used for barbules whose structure is simpler than that of the pennaceous or plumulaceous barbules. Four main types of reduced barbules are often distinguished.
- reduced pennaceous barbules; these display less developed hooklets and cilia apparati.
- reduced plumulaceous barbules; they do not have nodes as compared to normal plumulaceous barbules.
- rachidial barbules; these barbules are characterized by a very short base and a small number of pennular cells which are very fragile and often missing.
- stylet barbules; these are characterized by firmness, a thin pointed form, small nodes and paler pigmentation.

Afterfeather is the structure arising from the shaft of the main contour feather. Usually it is composed of elements that are normally present in the regular contour feathers. Afterfeathers arise from the underside of the main feather at the junction of the calamus and rachis. The ventral side of the feather faces the ventral surface of the contour feather (Rawles, 1963). The texture of vane of the afterfeathers is plumulaceous. Afterfeather structure is characteristic to the species of bird and may be used in determination of the phylogenetic relationships among birds (Chandler, 1916).

2.1.2.1.2 Types of contour feathers

*Remiges.* Remiges are the major wing feathers located on the posterior borders of the wings. Their size determines the bird’s ability to fly. Therefore, they usually have very strong rachis and well organized outer and inner vane structures.

Two groups of remiges can be distinguished. The distal posterior edge of the wing is a site of primary remiges. Secondary remiges are located in the proximal region of the
posterior edge of the wing. The difference between both types of remiges is not vast but is easily distinguished.

Primary remiges are very long, asymmetrical, and stiff. The number of primary remiges may vary depending on the species. Usually there are ten primary remiges located on each wing of the chicken (White Leghorn), turkey, common Coturnix and common pigeon. Eleven primary remiges can be found in ducks. The number of primary remiges can be affected by the action of two completely dominant autosomal genes as reviewed by Somes (1990a). These genes are common in the Rhode Island Red, Barred Plymouth Rock, and Nagoya breeds of chicken.

Secondary remiges are slightly more delicate in structure than the primary ones. The number of the secondary flight feathers in the wing can also differ in various species. The Single Comb White Leghorn normally has 17 secondaries, whereas the turkey and White Pekin Duck are characterized by 18 secondaries. The common coturnix and common pigeon each display 15 secondaries on each wing.

Rectrices. Retrices are large feathers located in the tail. They are also flight feathers whose size and structure determines the ability of a bird to fly. Retrices in various species may differ significantly in size and appearance. The largest feathers reported in a wild bird are the median rectrices of the male Crested Argus, *Rheinardia ocellata*, which are 153 mm wide and 1.83 m long (Lucas and Stettenheim, 1972). Yet, the morphologic structure of retrices is similar in all breeds unless altered by a genetic mutation or nutritional factors. The number of rectrices differ between species. The common pigeon has only five rectrices on the each side of the tail, White Leghorn chickens have seven, Bronze turkeys have nine and the White Pekin Duck may display up to ten rectrices on each side of the tail. The number of tail feathers depends not only on the species of bird but can also be modified by genetic factors. In the chicken, the number of tail retrices can be affected by an autosomal recessive gene with incomplete penetrance. Birds homozygous for that recessive allele demonstrate additional tail feathers (Somes, 1990a).

Body feathers. The majority of contour feathers are the body feathers also known as coverts. These feathers are distributed in most of the feather tracts. The morphology
of coverts is quite similar to that of other contour feathers. Body feathers are smaller and more delicate than remiges and rectrices. The shaft of the feather is thinner, and barbules have less complicated hooklets and cilia apparati than those found in flight feathers.

2.1.2.2 Morphology and characterization of other types of feathers

Apart from contour feathers the other types of feathers are semiplumes, down, bristles and filoplumes. Since majority of the discussion in this dissertation is devoted to contour feathers, only a brief description of those other types of feathers is provided here.

Semiplumes. This various in size feathers can be as long as 200 mm or as short as 30 mm. Their shape and size depend on their location on the body. Generally they resemble typical contour feathers with the main difference being the complete absence of the pennaceous portion of the vane. Semiplumes may be thought of an intermediate type of feather between the typical contour and down feathers. Semiplumes can be distinguished from down feathers by the presence of a long rachis. Semiplumes are not found in all feather tracts and apteria. The major body areas where the semiplumes are present are the abdominal part of the trunk including the pectoral, lateral, sternal and abdominal tracts. Some of the semiplumes are also distributed in apteria located on the abdomen. The major region where semiplumes are present on the dorsal side in the turkey is the lateral part of the dorsopelvic tract.

Down. There are two categories of down feathers the first is present on the newly hatch bird and the other can be found between contour feathers of older birds. Both of the down types have some similarities but also differences.

A natal down feather is composed of similar parts as a typical contour feather. The main axis includes the calamus and rachis although they may be modified or may regress to a stage in which they are barely visible. Size of the calamus in a down feather
may differ greatly between different species of birds as well as between different feather tracts of the same bird.

In the water fowl, distinct and long calamus with well defined rachis are present in the natal down. The longest down is located on the lateral apterium and can reach 58 mm. Most of the rami arise from the rachis. They lie strait and do not entangle with each other. The barbules emerge from the apical end of the rami and are reduced in its distal direction (Lucas and Stettenheim, 1972).

Very short calamus and rachis are characteristics for natal down of chickens and turkeys. The typical down feather of the chicken is shorter than 40 mm. The calamus is not longer than 4 mm in the largest down feathers and usually does not exceed 2 mm in length. The rachis of natal down feathers is much shorter than the longest barb.

In birds such as quail or pigeon, the rachis is degenerated and not visible (Jones, 1907). Rami are attached to the upper edge of the calamus (Lucas and Stettenheim, 1972) or to the false calamus (Jones, 1907). The barbules are always of the plumulaceous type regardless of the bird species. The hooklet and cilia apparatus in natal down is always more primitive than in higher organized feathers of the same bird.

Mature down feathers are much smaller and more delicate than contour feathers under which they are typically located. The number and location of the mature down feathers depends on feather tracts and may differ among birds. Down of the mature bird displays a true calamus, the rachis, but may differ among species. Some down feathers may posses their own afterfeather. As in the natal down, the plumulaceous barbules are rather primitive and grow out from the rami.

**Bristles.** Bristles are the type of feathers that are located almost exclusively on the head. They can be easily distinguished from other feathers by their stiff and narrow rachis which is devoid of rami and barbules. Only the proximal end of the rachis is characterized by a meager number of stylet or pennaceous barbules. The calamus is short and may constitute up to a tenth part of the total length of the bristle. However, the length of calamus cannot be measured precisely due to the lack of a well defined border between the calamus and the rachis. Usually most of the bristles do not possess afterfeathers.
Filoplumes. Filoplumes are the only kind of feathers that can be found in all feather tracts. A fully mature filoplume consists of the shaft and barbs. The shaft is very fine and delicate. At the top of the rachis a tuft of very short rami and barbules can be found, usually one to six on each side of the rachis. An immature filoplume contains a group of downy barbs at the basal end of the rachis. Mature filoplumes, however, do not have this feather fragment. The filoplume lengths ranges from 1 mm to 60 mm. The calamus is not very distinct and is approximately 0.5 to 2 mm in length. The rachis is slightly smaller in diameter than calamus but is very long. Crosssections of the filoplume rachis reveals similar morphology to contour feathers. Filoplumes are present in all feather tracts of almost all birds (Chandler, 1916).

2.1.3 Feather tracts of the Medium White Turkey

Feathers in the plumage of any bird are not distributed uniformly on the skin. The surface of a bird may be divided into pterylae and apteria that is feathered and featherless regions. Every species displays its own, unique pattern of pterylae and apteria, which may differ from other species in size of the regions, in number of feather follicles and their size. Therefore, the following feather tracts description is based only on the appearance of turkey skin. The characterization of feather tracts of normal feathered turkey is based on the description of the Bronze Turkey (Lucas and Stettenheim, 1972). The basic information on turkey plumage has been extended in this dissertation with the author’s observations of normal feathered Wrolstad Medium White Turkey at 24 to 30 weeks of age. This type of turkey will serve as the example for normal feathering for the subsequent description of the inhibited feathering genetic disorder. The majority of the names of the feathers, feather tracts, and apteria has been adopted after Lucas and Stettenheim (1972). Sometimes, however, the synthesis of new terminology was
necessary to avoid misinterpretation and confusion. For further references as well as for
the description of feather tracts and plumage of other species, the reader is encouraged
to refer to Lucas and Stettenheim, (1972).

2.1.3.1 Arrangement of feather tracts and apteria

Feather tracts, also designated as pterylae are those regions of the skin which are
densely covered with feather follicles. From these follicles normal full-sized feathers are
formed and supported by the presence of down, filoplumes and bristles. Apteria are
categorized by the absence of contour feathers, however down and filoplumes can be
scattered on the surface of an apterium. The names of the apteria are often derived from
names of neighboring pterylae.

Ventral feather tracts. The largest feather tracts visible on the ventral side of the
body are two symmetrical pectoral tracts that are elongated toward the head as ventral
cervical tracts. Long and situated in the middle are two sternal tracts which without any
noticeable boundary transform into the medial abdominal tracts. The ventral part of the
tail contains the ventral caudal tract. The shank is covered by the femoral tract. The
ventral side of the wing contains several small feather tracts. The cranial region of the
prepatagium is covered by the ventral prepatagial tract whereas its caudal region is
surrounded by the under-forearm tract covering the radial region and the subhumeral
tract above the humerus. In the cranial edge of the wing, elongated up to the elbow
region, a small fragment of the posthumeral tract can be visible. A major part of the
posterior edge of the wing from the elbow region including the hand region is covered
by the ventral postpatagial tract. A small allular tract is located on the terminal phalanx
of digit II.

Ventral apteria. The ventral cervical apterium is located between two elongated
and symmetrical ventral cervical tracts. The large pectoral apterium surrounds the sternal
tracts which in turn enclose the sternal apterium. The lateral body apterium occupies the
region between the pectoral apterium and the crural tract. The very narrow median
abdominal apterium continues from the sternal apterium toward the tail and is enclosed by two medial abdominal tracts. The lateral abdominal apterium is a continuation of the pectoral and lateral body apterium.

**Dorsal feather tracts.** The neck region is covered by a single long dorsal cervical tract which in the interscapular region divides into two symmetrical interscapular tracts. In the prodorsal region, two interscapular tracts fuse into one, wide dorsopelvic tract which covers the majority of the back. The dorsal caudal tract continues from the dorsopelvic tract toward the posterior end of the latter one. The lateral - marginal tail tract can be also described as a fragment of the dorsal caudal tract (Lucas and Stettenheim, 1972). It is visible on the dorsal and on the ventral side of the body. On both sides of the body in the posterior part of the prolatal region, a small fragment of the lateral body tract is visible. The largest fragment of the dorsal side of wings is covered by the upper prepatagial tract. The humeral tract connects parts of the upper prepatagial tract with the trunk. The posthumeral tract, the dorsal postpatagial, tract and the upper allular tract cover the wing extremities. The knee region is covered by a triangular femoral tract. Also visible on this side of the body is also the crural tract.

**Dorsal apteria.** In the neck region, on its sides, two parallel and elongated lateral cervical apteria are present. Their caudal ends transform into the scapular apteria which are located between the wings and the interscapular tract. Four minute apteria are located on the upper side of the wing: the humeral apterium, the upper cubital apterium, the upper hand apterium, and the upper allular apterium. The humeral apterium is situated between the humeral tract and the upper prepatagial tract. The upper cubital apterium covers the tip of the elbow joint. The narrow and slightly curved upper hand apterium connected with the upper allular apterium is located anterior to the dorsal postpatagial tract.

The scapular apterium occupied the region between two sections of the interscapular tracts. The lateral body apterium expands from the lateral body tract up to the femoral tract and the crural apterium. The dorsal border consists of the dorsopelvic tract whereas the ventral boundary is defined as the pectoral tract together with the pectoral apterium. The crural apterium continues as a proximal boundary of the crural
tract, and extends to the intracrural apterium which is located on the medial region of the shank. The lateral caudal apterium separates the dorsal caudal tract from the lateral marginal tail tract.

2.1.3.2 Characterization of the major feather tracts

In this section the typical normal feathering of a Medium White turkey is characterized.

Dorsal cervical tract and interscapular tracts. Feather follicles are arranged in several rows that traverse toward the sides of the neck. The direction of the feather rows is not altered on the boundary of the dorsal cervical and the interscapular tracts. The juvenile feather consist of contour feathers that are similar to each other in size and in structure. As poults grow, contour feathers on the anterior region of the dorsal cervical tract are replaced by short and narrow bristle feathers. The posterior fragments of the dorsal cervical tract and the interscapular tract are covered with body feathers. The length of the feathers decreases toward the head region. The longest feathers at the posterior region of the dorsal cervical tract can be as long as 80 mm, and oval or even round in shape. Those at the anterior region tend to be not longer than 35 mm.

Dorsopelvic tract. Feather follicles are arranged in rows with the medial end being anterior. The distribution of the rows is not as uniform as in the dorsal cervical tracts. Some of the feather follicles are missing, whereas other may be shifted in any direction. Feathers covering the dorsopelvic tract are contour body feathers. These feathers do not differ significantly among young birds. The juvenile plumage type of this region is of medium length and uniform. In mature birds, plumage of this region is influenced by the bird’s sex. Female turkeys have shorter feathers and the majority of them display less of the plumulaceous part of the feather than in males. In other words, a contour feather of a tom is more fluffy at its basal end than that of a hen. A typical contour feather of the dorsopelvic tract is tapered on the apical end and does not have oval appearance.
**Upper prepatagial tract.** Feather follicles are arranged rather uniformly along the upper prepatagial tract. Contour feathers along the anterior border of the wings (upper marginal coverts of the prepatagium) grow parallel to the edge. They are oval and have a dull tip. Their length is usually between 20 and 25 mm. The afterfeather associated with the upper marginal coverts of the prepatagium does not exceed 10 mm in length. Upper minor secondary coverts present in the center of the prepatagium point toward the bases of the remiges rather than toward the hand. Length and width of the major coverts do not exceed 50 mm and 12 mm, respectively. Feathers are oval with blunt point and have curved boundaries.

**Humeral tract.** Feather follicles of the humeral tract are located more densely than at the upper prepatagial tract. Feathers growing here have lengths similar to that of the upper major primary coverts, but the down or plumulaceous portion of the feather may exceed 50% of the feather length.

**Dorsal postpatagial tract.** This tract contains the most prominent feathers on the wing - primary and secondary remiges. Ten primary remiges are accompanied by perpendicularly arranged upper major primary coverts. One row of the upper median secondary coverts marks the boundary between upper major secondary coverts and upper minor secondary coverts and separates the upper prepatagial tract from the dorsal postpatagial tract. Eighteen secondary remiges with matching perpendicular upper major secondary coverts are located on the caudal edge of the forearm. Down feathers are sparsely distributed between remiges of the arm and hand. The primary rectrices are long and stiff. Their length depends on the size of the bird and the location of the feather on the wing and varies between 250 and 400 mm. The usual width is approximately 35 to 50 mm. The outer vane is much more narrow than inner one (usually within 25%). The secondary remiges are usually longer and slightly wider than primary remiges. There are no significant differences in the width of the inner and outer vane of this type of feathers.

**Ventral prepatagial tract.** This minor tract is shielded by under marginal coverts of the prepatagium. Feathers present here are very similar to those present on the upper margin of the prepatagium.
**Under-forearm tract and subhumeral tract.** These tracts are located on the ventral surface of the wing. They are covered with a common type of feathers which resembles that of the under marginal coverts of the prepatagium.

**Femoral tract.** Feather follicles of the femoral tract are located in rows that are perpendicular to the main body axis. The follicles are distributed uniformly throughout the triangular area of the track. The majority of the feathers are contour with the major part of the feather being plumulaceous. Only approximately one-fourth of the feather consists of the fully developed barbules (pennaceous part). Some of the large feathers on the femoral tract are bent and have asymmetric vanes. The length of the feather usually does not exceed 200 mm and the width - 40 mm. Large semiplumes are also present also. The length of semiplumes can reach 130 mm and they are oval.

**Crural tract.** Feather follicles are scattered evenly throughout the crural feather track. The size of the feathers on the legs varies. The longest and widest feathers are located on the upper part of the leg whereas the distal region is covered with very short feathers. The length of contour feathers of the crural tract varies from 35 mm to 70 mm.

**Caudal tracts.** This rather composite area consists of dorsal and ventral caudal tracts. The lateral - marginal tail tract is discussed separately from the dorsal caudal tract for clarity of the description. The anterior part of the dorsal caudal tract is covered with medium sized feathers (approximately 100 mm long and 35 mm wide). They are of the plumulaceous type except for the tip where the vane is pennaceous. The ventral caudal tract is covered with similar type of contour feathers but they are shorter. Similar width of feathers makes them more round.

**Lateral - marginal tail tract.** A large portion of the tract is occupied by rectrices and upper major tail coverts. The number of coverts corresponds with the number of rectrices, and each pair of rectrices has parallel major tail covert and median tail covert. Rectrices located at the center of the tail are the largest (approximately 350 mm long and up to 70 mm wide). They are characterized by a symmetric vane which is rather flat. Lateral retrices are smaller with the inner vane wider and the vane tending to be asymmetrical.
**Pectoral tract.** Feather follicles are rather evenly distributed in the pectoral tract. A typical feather is pointed and is approximately 125 - 160 mm long and 25 - 40 mm wide. The vane is asymmetric with the inner vane being the wider. This tract contains the largest semiplumes whose length may exceed the length of contour feathers in this area (170 - 200 mm).

**Sternal and abdominal tract.** Follicles of these tracts are situated rather evenly in a few rows that are parallel to the sternum. The length of the contour feathers depends on the location on the feather tract. Feathers located in the anterior portion tend to be shorter than those located on the abdominal tract. Length and width of feathers of the sternal tract usually do not exceed 65 and 30 mm, respectively. In the abdominal tract, feathers can be up to 120 mm long and about 35 mm wide. The vanes are oval but it is difficult to describe the tip of the feathers due to wearing out of the distal fragments of the feathers. Normally, most of feathers in the abdominal tract are broken at the transition zone between pennaceous and plumulaceous parts of the feather. The major part of the feather is formed by plumulaceous barbules. Semiplumes are also present.

**Ventral cervical tract.** Feather follicles are arranged in several rows that traverse toward the sides of the neck but unlike in the dorsal cervical tract they are separated by the ventral cervical apterium. Feathers located on this tract are very similar to those described in the dorsal cervical tract, however they tend to be slightly longer. The longest feathers of the anterior part may exceed 100 mm in length, while toward the posterior part they are rather short - 25 mm. The width of the feathers is similar to those located at the dorsal part of the neck.

**Lateral tract.** This rather small feather tract carries large semiplumes whose follicles are uniformly scattered through the tract. The length of feathers found here usually does not exceed 100 mm.
2.1.4 Genetic feather disorders

The development of skin appendages and the appearance of the feathers can be affected by various factors including nutrition, diseases, skin parasites, and environment. In poultry breeding several feather and plumage disorders have been described which have genetic origins. A number of them have been identified and investigated in the chicken while only few are known in the turkey. Several feather mutations have been reported in the pheasant, Japanese quail, duck, and other birds. To better understand the mechanism for the inhibited feathering trait investigated in this dissertation and to relate the new mutation to those previously described in literature, a brief discussion of various plumage disorders is provided. Some genetic variants of the birds' plumage have been appreciated and are of economic importance to the commercial poultry industry. Others are recognized only by poultry fanciers and are bred in small flocks for their attractiveness and beautiful appearance. Birds displaying genetic disorders are maintained for research purposes to allow for studies of the hereditary mechanisms.

The remaining portion of this section contains description of those plumage disorders which may be relevant in interpretation of studies of inhibited feathering in the turkey. The majority of those mutations are in the chicken, however, similarity of the two species and of their genomes make the knowledge of chicken genetics very useful in studying hereditary traits in the turkey. For the same reason some hereditary feather disorders in the Japanese quail and in the guinea fowl are also discussed.

2.1.4.1 Hereditary feather disorders in the chicken

Various genes responsible for plumage appearance have been described in the chicken. They can be classified in different ways but in this presentation the mode of inheritance is used as the most natural classification method.
2.1.4.1.1. Autosomal dominant traits.

**Crest.** One of the earliest feather mutations in the chicken that was described, is "Crest". Experiments with birds characterized with this condition revealed that the single gene responsible for the disorder is incompletely dominant and is located on an autosomal chromosome (Davenport, 1906). Crest belongs to the same linkage group as frizzling and the dominant white genes (Warren, 1936). The symbol \( Cr \) was designated for the gene responsible (Dunn and Jull, 1927). The disorder can be described as the presence of long feathers on the top of the bird's head. A few feathers may be raised or the whole head may resemble knob like structure (Somes, 1978). Each feather has a normal structure with extended barbs in the distal part of the feather. The probable cause of the unusual length of the feathers is its prolonged growth because the skin surrounding the follicles of mutated feathers is unusually thick and well nourished (Davenport, 1906). The trait is present in many fancy breeds such as: Silky, Polish, Houdan, and several others. This trait has no commercial significance but it is acknowledged by hobbyists.

**Naked Neck.** This condition was first described in the early 1900's, and was found to be an effect of a single incompletely dominant gene (Davenport, 1914; Warren, 1933a). The symbol \( Na \) has been assigned to the gene (Somes, 1978). Birds which have the \( Na \) gene in the homozygous state do not develop feather follicles which results in the absence of the feathers in the area ranging from the ear lobes down to the clavicle at the ventral side of the neck. Chickens heterozygous for this gene develop reduced feathers in this area. Other feather tracts are also affected by this gene. Feather number is also reduced in several feather tracts on the back area (dorsopelvic and dorsal caudal tracts), as well as on the breast - pectoral tract (Somes, 1978). Lateral pelvic and sternal apteria, which are usually covered by dispersed feathers are wider than in the wild type of the bird (Classen and Smyth, 1977).

**Henny Feathering.** The presence of this gene in the genotype of a cock results in the female type of the plumage. The feather follicles develop the henny type of the feather as a response to altered levels of estrogen or androgen (Somes, 1978). Initially it was thought that the gene responsible for this disorder was dominant. The results of
the first matings were inconclusive to whether the mutation was a result of single gene or if modifiers existed (Morgan, 1920). Later, it was confirmed that the mutation was a result of the single gene with incomplete dominance (Punnet and Bailey, 1921). The idea of modifying factors possibly involved in henny feathering was reported also by Jull and Quinn (1931), but Punnet (1937) rejected that theory implying that the only modifying factor could be the amount of testosterone produced by the bird. The origin of the mutation is thought that the gene was originally carried on the W chromosome and it was dislocated to an autosome in a henny feathered male (Punnet, 1937). The difference between homozygous and heterozygous birds can only be recognized by the differences in the activity of aromatase (Somes, 1990a).

**Flightless.** This condition is controlled by an incompletely dominant gene (Warren, 1937). Chickens which appear normal at hatch develop defective feathers by four weeks of age. The feathers of affected birds become so brittle that they break very easily. Flight feathers develop irregularly in size and shape. Additionally, some growth retardation and distortion of the beak and toenails can be observed. The homozygous condition may be lethal during embryonic development or in the first few weeks of life (Warren, 1932). Those homozygous birds that survive develop fragile beaks and toenails, and are featherless (Somes, 1978). The symbol $Fl$ was assigned to the gene (Hutt and Lamoreux, 1940). A very interesting observation was made by Marlow and Caldwell (1934) regarding the amino acid content of the feathers of abnormal chickens compared to the normal feathered birds. The cystine content of the normal feathers was found to be 22.7% higher and the amount of phosphorus was less than half that in mutant feathers. This suggested that significant differences existed in protein metabolism between normal and mutant birds (Marlow and Caldwell, 1934).

**Apterilosis.** The condition was first observed in the Rhode Island Red breed and it is caused by a dominant autosomal gene (Sturkie, 1942). Homozygous and heterozygous birds are difficult to distinguish. Several feather tracts (pterylae) are absent or reduced in size. Mutant birds differ in the degree of nakedness. The mortality among affected birds is considerably higher than in normal chicken, with the highest death rate (54.1% - 57.9%) observed during the first 10-15 days of life. The degree of nakedness
is not causally related to the death rate. The condition does not alter the reproductive characteristics of the mutants, such as age of the sexual maturation and the egg production (Sturkie, 1942). Ap is the symbol designated for that condition (Hutt, 1949).

**Surplus flight primaries.** This feather condition was described in the Rhode Island Red, Barred Plymouth Rock, and Nagoya breeds. Affected birds are characterized by the presence of more than 10 primary remiges. Two dominant, autosomal genes Sf1 and Sf2 are involved in the inheritance of this mutation (Somes, 1990a).

**Long filoplumes.** Mutants with this feathering disorder are characterized by elongated filoplumes protruding beyond the tips of the feathers. This condition has not been thoroughly investigated although it is postulated that the condition is due to an autosomal dominant gene, with the symbol, Lf (Somes, 1990a).

**Hypoplasia of the rectrices.** This feathering disorder was discovered in the Ingie breed. It is inherited as an autosomal dominant trait (Somes, 1990a) and it does not affect the number of tail feathers, but their feather structure is altered. They look frizzled, thin, and unusually long. The microscopic structure of tail feathers is abnormal with no hooklets present at barbules (Hashiguchi et al., 1978).

### 2.1.4.1.2 Autosomal recessive traits

**Silkiness.** The Silky breed was first mentioned in thirteenth century literature by Marco Polo (Davenport, 1906). The presence of the gene causes a lack of barbules and hooklets. The feather of the Silky chicken is more delicate and wavy than in normal birds. All contour feathers are affected with tail feathers being transformed the most. All primary and secondary flight feathers as well as contour feathers of the body are downy in nature (Davenport, 1906). Several studies confirmed that the silkiness is due to an autosomal recessive gene (Jones, 1921; Dunn and Jull, 1927) with the symbol h (Hutt, 1949).

**Retarded feather growth.** Two variants of this condition are known as tardy and retarded feathering. In birds affected with the retardy gene only three secondary remiges are present at the time of hatch, while normally feathered chicks usually develop six of them. The feather development is also stunted in the tail area, however, no differences
in the plumage of mature retardy and normal chicken can be noticed (Warren, 1933b). The other allele, called tardy feathering affects feather development more acutely (McGibbon and Halpin, 1946). At hatch, chicks with this condition display no secondary feathers. As the birds mature, some of the feathers develop but significantly more slowly than in retardy condition. At six weeks of age the birds back and tail feathers are still missing (McGibbon and Halpin, 1946). Both feather mutations are due to the action of two genes located at the same autosomal locus (Jones and Hutt, 1946). Tardy, the more severe condition is recessive to retardy (Warren 1933b) and both are recessive to the wild type plumage (Jones and Hutt, 1946).

**Frayed feathers.** In this condition the structure of the rectrices and remiges is affected. The anterior hooklets and barbules of those feathers are missing or altered such that they are no longer connected. The condition can be observed at 6 - 12 weeks of age, even though it is not distinguishable at hatch and is rather difficult to recognize in older birds. It is believed, that the gene responsible is autosomal (Warren, 1938).

**Ragged wing.** Due to the action of this gene several or even all flight feathers on the wing are missing (Hutt et al., 1944). Some reports indicate a milder variant of the condition in which wing feathers are present but are significantly shorter (Juhn and Shaffner, 1962). The condition can be recognized only in mature birds. Several researchers suggest that the gene is common in genetically diverse populations of chicken (Potemkowska et al., 1977). Available data suggest that the condition is caused by an autosomal recessive gene with incomplete penetrance (Hutt et al., 1944).

**Ropy.** This condition can be easily noticed at hatch as chicks appear wet and sticky. No major effects in mature birds can be observed except for the cleft on the underneath of the remiges. The gene causing this disorder is autosomal and recessive (Warren, 1949).

**Stringy-2.** At hatch, chicks affected by this feathering disorder resemble the rropy condition. In adult birds rectrices and remiges are characteristically shortened with absent or altered barbules (Buss et al., 1950). Elongated premaxilla, crossing of the beaks, and abnormally long toenails are also reported in mutants. The egg production is not affected by the gene. Results of crosses between mutants with stringy-2 and rropy mutations
revealed that the two genes are located on different loci (Warren, 1949). The symbol \textit{st-2} was assigned to this recessive gene (Somes, 1978).

\textbf{Wooly}. This hereditary feather disorder is recognizable at hatch. Chicks with a mild expression of the gene may display shorter down and in more severe instances, there is no down feathers in the dorsal and abdominal tracts. Down feathers in other tracts are rolled into the shape of small nodules. The skin on the feet and shanks is unnaturally shiny and smooth in all mutant chicks (Jones and Morgan, 1956). In adult birds, all feathers are present however their proximal barbules are missing which contributes to the rough appearance of the feathers. The gene significantly increases mortality of embryos and hatched chicks. Body weight and egg weight are affected as well. The gene responsible for this disorder is autosomal and recessive (Jones and Morgan, 1956).

\textbf{Sunsuit}. At the time of hatch this condition is quite similar to stringy-2 (Buss \textit{et al.}, 1950) but as the mature plumage develops the differences between these two mutations become apparent. The primary remiges are twisted and tangled. In the completely mature chicken, the number of remiges and rectrices is significantly reduced although the number of the feathers follicles is not diminished. Feathers on the head and neck are not affected whereas those located on humeral, alar and femoral tracts display the most severe effects of the mutation. Also elongated and bent beaks and abnormally long toenails can be observed in mutant birds. Hatchability of mutants is not different than that of normal chicken. Results of matings suggest an autosomal recessive character of the gene (Hutt and Long, 1950).

\textbf{Ottawa naked}. This feather disorder has been known since 1956. Chicks affected by this mutation lack most of the down and in some situations may have no down at all. The feather follicles are absent in the affected areas which remain naked throughout the lifespan of the bird. Mutant birds can be raised only if special care is taken since they display other deformities such as edema, kidney defects, skeleton deformities and fusion of the third and fourth toes (Crawford \textit{et al.}, 1982; Fulton \textit{et al.}, 1987). The symbol \textit{nk} is used for this autosomal recessive gene (Etches and Hawes, 1973).
**Scaleless.** This condition can be observed at the time of hatching. Chicks may either completely lack the down or only very limited areas are covered. Adult birds are naked with only a few undifferentiated feathers developed irrespective of the body location. Scales and spurs do not develop and the skin on the shanks has a smooth and shiny appearance which is characteristic for these mutants. The nails, beak, comb and wattle are not affected. It has been suggested that this feather condition is the expression of an autosomal recessive gene (Abbott and Asmundson, 1957).

**Porcupine.** This mutation affects down feathers as well as mature plumage of the bird. Day old mutant chicks are covered with spiky quills. Observation of the embryos reveals that the mutation can be recognized at the beginning of feather formation in the embryo. Mature birds are also covered with spike-like feathers. The porcupine mutation influences the egg production and reduces fertility despite artificial insemination. It is believed that a recessive gene is responsible for the disorder (Waters, 1967).

**Wiry.** Stringy down feathers with altered down morphology is the expression of an autosomal recessive. In adult mutants, remiges and rectrices are also affected even though less than in newly hatched chicks. The width of the rachis is reduced and there are alterations in the histology of the barb and feather follicle. The reproductive capacity of the birds is not affected (Fiser et al., 1973). The symbol assigned for this gene, is "wi" (Somes, 1978).

**Dysplastic remiges.** This plumage abnormality is not recognizable at the time of hatch. In adult birds the wing and tail feathers are missing. The lack of rectrices and remiges becomes apparent by four weeks of age. The affected feather follicles appear empty or develop defective feather. In some cases the wing coverts also become modified (Urrutia et al., 1983).

**Edema.** This condition, described by Savage et al. (1986) affects feather growth in day-old as well as in adult birds. The edema is observed as sacs of fluid located in the thigh region which separate the epidermis from dermis and does not permit proper feather follicle development. Adult birds have missing feathers in regions where the edema lesions were present. The mutation increases embryonic mortality during the third week of incubation (Savage, personal communication).
2.1.4.1.3 Sex linked dominant genes

*Slow feathering.* Several genes situated at the same locus control the rate of feathering in chicks. Affected birds are characterized at hatch by the easily recognizable lack of some or all flight feathers. Serebrovsky (1922) discovered that normal feathering can be affected if the dominant gene, K is present. This trait was studied further by Warren (1925) who confirmed the sex-linked nature of the gene. Extremely slow feathering, \(K^a\), an allele at the K locus was reported by Somes (1969). Primary and secondary remiges are absent in day old chicks carrying that gene. As adults, the birds develop some feathers and can be distinguished from slow feathering (KK or Kk) birds. This finding was followed by the discovery of yet another allele at the same locus by McGibbon (1977) with the proposed symbol \(K'\). There are significant differences in the ratio of feathering between different slow feathering mutants. Almost no feather development was observed in young and mature birds possessing the \(K^a\) allele. Carriers of the \(K^s\) allele feather well but there is no growth of primary and secondary remiges and rectrices. The milder variant of the slow feathering chicken are those with the K allele. At hatch, only the remiges are affected by the \(K^s\) gene and they are shorter than wing coverts. This gene affects comb development and causes enlargement of uropygial gland (Somes, 1969).

2.1.4.1.4 Sex-linked recessive genes

*Sex-linked naked.* This is the only sex-linked and recessive gene affecting feather development that has been described to date. This mutation is expressed at the time of hatching. Although feather follicles are present in the pterylae, there is no feather growth (Somes, 1978). The expressivity of the condition may vary from birds covered with wiry and curled down to those almost naked ones. The replacement of the down by juvenile feathers is very slow and the juvenile chicken remains naked. The condition is also
evident in the mature birds because of the rough appearance and the absence of some of the rectrices and all of the remiges. The featherless areas usually show broken feathers stubs. The mutation influence mortality around the 20th day of incubation as well as within the first 6 weeks after hatching. Egg production is not adversely affected by the gene (Hutt and Sturkie, 1938).

2.1.4.1.5 Composed traits

*Frizzling.* This hereditary feather condition has been known for centuries and is maintained in populations mainly for exhibition purposes. It is controlled by two interacting genes. The major gene $F$ exhibits incomplete dominance (Hutt, 1930; Landauer and Dunn, 1930). The other gene $mf$, also autosomal but recessive can modify the result of the dominant gene. The plumage of dominant homozygote birds is very curled with rachis of all feathers being affected. If, however, a homozygous bird carries the recessive gene the plumage is less affected. In heterozygotes for the $F$ locus, the modifying gene results in plumage that was barely affected whereas chickens without the modifier display moderately affected plumage (Hutt, 1936; Landauer, 1933). Birds with frizzling condition are characterized by accelerated metabolism and increased production of adrenaline and thyroxine (Landauer and Abarle, 1935).

2.1.4.1.6 Traits with unknown mode of inheritance

*Congenital baldness.* This disorder is observed in some newly hatched chicks that are characterized by bald areas on the head. The condition is rather common in many breeds and in genetically diverse flocks. The baldness is due to separation of the dermis and epidermis on the embryos' head between eight and fifteen days of incubation. The tissue separation which prevents feather follicles formation (Sturkie, 1941). The inheritance of this condition is not fully determined (Jull, 1952). It is suspected that the gene responsible for the baldness is recessive (Somes, 1978).
**Stringy.** This feather disorder was first reported by Kessel (1945). Chicks at hatch display down with a stringy appearance. The barbs are twisted and glued together. The degree of feathering varies from almost complete lack of down to a moderate feather cover. The mode of inheritance is unknown. It is possible that an autosomal recessive gene is involved but a sex-linked gene cannot be excluded (Kessel, 1945). The symbol *st* is assigned for this gene (Somes, 1978).

### 2.1.4.2 Feather disorders in the turkey

Only three mutations which affect feather appearance or development in the turkey have been described. Two of these mutations are located on the autosomal chromosomes and one was determined to be on the Z chromosome.

#### 2.1.4.2.1 Autosomal recessive traits

**Hairy.** This mutation was the first plumage deviation discovered and described in the turkey (Smyth, 1954). All feather tracts are affected by this disorder. The gene influences the structure of barbs causing a lack of hooklets in the pennaceous portion of the feather. This results in the lack of proper vane. The mutation can be detected as early as three days prior to hatch. The transformed plumage is noticeable at hatch, as well as during the course of live of the bird. Hatchability of mutants is not negatively affected but significantly higher mortality within 12 weeks of life may be observed. The symbol *ha* is designated for this mutation.

**Naked.** In birds affected by this condition, feathers and feather follicles are missing except for posthumeral and humeral tracts, upper marginal coverts of the prepatagium and fragments of pectoral tracts. Mutant birds display translucent and smooth skin. No scales are present on shanks and feet. The beak and toenails are not affected. Several structural abnormalities of legs such as additional hind toes and severe
leg twisting, are common in virtually all mutants. The gene is autosomal recessive and symbol $na$ has been proposed (Poole and Mardsen, 1961).

2.1.4.2.2 Sex linked dominant traits

_Late feathering_. This feathering disorder can be recognized as early as at 16 days of incubation due to the shorter than usual down and the presence of areas devoid of down. On the day-of-hatch, primary and secondary remiges of the affected poultis are severely underdeveloped. Down feathers are not affected in day-old-poults. In eight-week-old poults, however, feather development may vary significantly. The poults may be almost naked or may also lack only some feathers. In adults, wing feathers are either missing or their structure altered. The fertility and hatchability of eggs from the mutant is not different from those of normal birds. The rate of growth is slightly reduced (Asmundson and Abbott, 1961).

2.1.4.3 Feather disorders in the Japanese quail

2.1.4.3.1 Autosomal dominant traits

_Defective feather disorder_. This is the only autosomal dominant gene affecting feather development that has been identified in the Japanese quail. The effects of this gene are modified by another gene which is autosomal and recessive. The results of the presence of the dominant gene are evident only if the recessive gene is homozygous (Fulton _et al._, 1983). The mutant is characterized by altered feather structure. There is no connection of the hooklets with the barbules and the arrangement of the vane is defective (Cheng and Brush, 1984). In the dominant homozygous condition this disorder is lethal.
2.1.4.3.2 Autosomal recessive traits

**Downless.** In birds affected by this disorder, feather tracts are affected at hatch. Mutants differ in the degree of the expression of the condition from reduction of down feathers in several feather tracts to complete nakedness. Plumage of adult quails is affected in similar matter. Tracts on the neck and the back of a bird are influenced less severely than the rest of the body. Body weight and egg production are reduced by the mutation. The trait is controlled by two autosomal recessive genes (Savage and Collins, 1971).

**Rough-textured.** This mutation is apparent at hatch. The down feather is shorter and rough to the touch. Feather structure in the mature quail is also altered due to misalignment of the barbules and lack of connection between hooklets and barbules (Roberts and Fulton, 1979). Due to maternal effect from mutant females, increased embryonic mortality may be observed (Cheng and Brush, 1984). The trait is controlled by a single autosomal recessive gene (Roberts and Fulton, 1979).

**Porcupine.** This mutation controlled by an autosomal recessive gene is characterized by defective feather structure on the wings and in dorsopelvic tract (Fulton et al., 1982a). The feather vane does not unfold properly and remains covered by the sheath (Cheng and Brush, 1984). The mutant is characterized by poor egg production, low fertility and elevated embryonic and posthatch mortalities (Fulton et al., 1982a).

**Short barb.** This autosomal recessive gene affects fully developed feathers in birds at 2 weeks of age resulting in a ragged, stringy appearance of the feathers. Those most affected are located in the region of dorsopelvic tract. The expressivity of the gene varies between birds (Fulton et al., 1982b).

**Ruffle.** Cheng and Kimura (1990) briefly describe the condition in which the barbs of the inner vane are affected and fold onto the vane itself. There is a large variation in expressivity of the disorder between affected birds.
2.1.4.4 Feather disorders in the guinea fowl

Only two feather mutations have been identified and described in the guinea fowl. One, aprotopteria, is autosomal recessive. Homozygous recessive birds have no flight feathers present on the wings and tail. Other feathers are not affected by aprotopteria (Somes, 1990b).

The other mutation, located on the Z chromosome was described by Somes (1990b). The gene results in similar feather disorder as slow feathering gene in the chicken, and affects the growth of flight feathers on wings.

2.1.4.5 Feather disorders in the geese

Sebastopol. This gene generates elongated feathers in all feather tracts except for the head and the neck regions. The structure of the feathers are fragile and are vulnerable to mechanical factors. Haves (1990) reports that although there is no doubt to the inherited nature of the disorder, the mode of inheritance of this trait has not been proven. It has been suggested nevertheless, that the trait is probably dominant and incomplete (Hawes, 1990).

2.2 Genetics and the description of the Inhibited Feathering turkey

2.2.1 Introduction

Discovery of every new gene in a commercial bird species significantly increases the general knowledge of poultry genetics and may also be very important from the practical point of view. Information about newly described genes may be utilized to improve quality of production, to lower costs of feeding and breeding of animals, and to reduce human labor. A gene that is to be used in the industry must demonstrate
practical features that can be utilized by breeders. At the same time the gene should not express any deleterious effects in the animal and should not interfere with breeding or production goals. Several of the so-called marker genes are widely used in commercial chicken production. A marker gene manifests itself through an easily identifiable feature with no production significance but is associated, probably through closeness on a chromosome with some important quantitative effects. The effects related to a marker gene can be both positive or negative from the producer’s or breeder’s point of view. This is true, for example, in case of the chicken "naked neck" gene described by Davenport in 1914 and Warren in 1933. Birds homozygous or heterozygous for this gene perform better in high temperature environments than those with both wild alleles present in the genome (Merat, 1986). At the same time, however, increased embryonic mortality - up to 10% in the pure lines - was observed in carriers of the "naked neck" allele (Crawford, 1977 and 1978).

A good example of a simple gene that is already widely used in the poultry industry is a gene responsible for late feathering of chickens. The dominant gene located on the Z chromosome is responsible for reduction of the primary feather length in day-old chicks carrying the K (slow feathering) allele. This feature allows efficient segregation of males from females. This method, called feather sexing has been known since the early 1920’s and is commonly used in broiler production (Warren, 1930). Early separation of males and females allows significant savings through differentiation of management and feeds provided to both sexes. Feather sexing allows this without a high cost and stress for the alternative vent sexing process. Although the K gene may have slightly negative effect on the growth rate (Merat, 1970) and egg production (Lowe and Garwood, 1976), it is still practical and economical to use.

With production costs in the turkey industry continually increasing, it is important to minimize costs. One of the areas that still has not been improved is segregation of males from females at hatch. Currently, the only method of sexing day-old turkey pouls is vent sexing - a stressful procedure. Moreover, this method requires highly skilled persons and is time consuming. A possible alternative to vent sexing would be to utilize a sex-linked gene affecting pouls that could be easily recognized at hatch, without
interfering with productive traits. Such a gene must be located on the Z chromosome be
dominant and whose gene action must be easily recognizable.

Current knowledge of the turkey genome and of genes influencing feathering is
very limited (Savage, 1990). Today, only 35 turkey genes have been identified. The
largest number of currently described genes in the turkey are those influencing plumage
color and feather appearance. There are two genes influencing plumage color in the
turkey that are sex-linked. Unfortunately, colored birds are not accepted in commercial
flocks which prevents their utilization as sex markers in the turkey. Consumers insist on
white skin in the birds, therefore colored turkeys with melanin pigmentation present in
the skin (like in Narraganset turkeys) and feather follicle coloration (as in Silver Auburn
turkeys) would have lower commercial value. Genes described in the literature that affect
feather growth and development are also not suitable for feather sexing purposes.
"Naked", described in Beltsville Small White line of turkeys (Poole and Mardsen, 1961)
and "hairy", investigated by Smyth (1954) are both autosomal and recessive. Only one
gene, late feathering (K), described by Asmundson and Abbott in 1961 was sex-linked
and dominant. The gene displayed pleiotropic effects adversely affecting production and
therefore, the researchers advised against the commercial use of this gene. With intensive
selection against negative pleiotropic effects, it would probably have been possible to use
that slow feathering gene in an industrial practice as suggested by Savage (1990).
Unfortunately, in early 1960’s the potential practical significance of that discovery was
not apparent and the line carrying the K gene was terminated after only a brief
investigation.

This chapter is devoted to the characterization of a new sex-linked, dominant gene
with possible application to feather sexing process. This new gene was first observed in
1990, in a single female within a flock of 5000 commercial Nicholas male line hens.
That hen was characterized by a lack of feathers at 8 weeks of age. As the hen matured,
the plumage improved, however the feather appearance was still very unusual. Growing
feathers were silky while the primary and secondary feathers on the wings were altered.
Some of them were absent and existing remiges were narrow, twisted and bent. No tail
feathers were present (Figure 2.1). This unique female was raised to maturity and mated
Figure 2.1 The original mutant hen at 20 weeks of age
with normal feathered Medium White males. Some of the male offspring obtained, demonstrated similar characteristics of the plumage which suggested a hereditary nature for the condition. The new line of the turkeys has been established by crossing abnormal feathered poults with those with typical feather development.

2.2.2 Materials and Methods

2.2.2.1 Investigation of the mode of inheritance

To determine the mode of inheritance, several matings were performed, between birds with inhibited feathering (IF) and with normal feather development (NF). The original IF female was artificially inseminated with the semen pooled from four unrelated NF males. Among the offspring from this mating, only the males exhibited the IF plumage disorder. They were individually wing-banded at hatch and raised to maturity and they were subsequently mated with normal feathered females. Of the progeny that hatched, 16 IF poults displaying the feather anomaly were saved and subsequently sex was determined based on the appearance of the secondary sex characteristics. In the following year, the matings consisted of IF females and males that were mated together. Poults selected for further breeding were sexed when the secondary sex characteristics were apparent. From each generation a portion of the offspring was saved and used for further research. The following are the types of mating that were performed in the investigation:

IF females x IF males
IF females x NF males
NF females x IF males.

The data were collected in four consecutive years 1991-1994.

Sex of the poults was determined either by examination of gonads of euthanatized birds (McLelland, 1990) or by appearance of the sexual characteristics in older poults.
The individual and pooled chi-square values for goodness of fit for the observed data were calculated (Snedecor and Cochran, 1967) to test the hypothesis that inhibited feathering condition was the expression of a dominant, sex-linked gene.

2.2.2.2 Methods for describing the Inhibited Feathering poults

Eggs were collected from IF hens, artificially inseminated with semen from IF homozygous toms. Thirty eggs from the IF line and 15 eggs from NF line were collected, incubated in single-stage, horizontal, transferless incubators (Savage et al., 1991). All the eggs were removed from the incubator at day 16 and down development of the embryos macroscopically examined.

The description of the IF poults appearance was based on comparisons to the feather growth pattern of NF poults with particular emphasis at the time of hatching, and at 7, 8 and at 10 weeks of age. Poults older than seven weeks of age varied in the expressivity of the phenotype and were categorized to groups of birds with similar plumage appearance prior to description. The seven, eight, and ten-week-old poults were given plumage scores of 1 through 5. A score of 1 was reserved for poults with normal feather development. The higher the numeric category score, the more sparse the plumage. The feather scores were based on subjective grading of the quality and quantity of the plumage, the area of the skin covered by feathers, the number of flight feathers, the proportion of feathered and naked skin, and the number of pin feathers.

2.2.2.3 Biochemical assays

2.2.2.3.1 Blood chemistry analysis

Fourteen IF and six NF hens were randomly selected from a flock of 30 week old breeder hens prior to photostimulation. Blood was collected by puncture of the vena
humeri profunda in the left wing (Koch and Rossa, 1973). Five milliliters of blood were collected from each hen into evacuated glass tubes containing sodium heparin and were subjected to analysis\(^1\). The following chemistry assays were performed: creatinine phosphokinase, alkaline phosphatase, lactate dehydrogenase, aspartate amino transferase, alanine amino transferase, glucose, uric acid, calcium, phosphorus, total protein, albumin, cholesterol, triglyceride, sodium, potassium, and chloride.

### 2.2.2.3.2 Feather amino acid analysis

Feathers used for amino acid determination were collected from turkeys at 20 and 22 weeks of age. All IF birds with some development of secondary remiges were selected. Fifteen IF birds of each sex were randomly selected. In addition, fifteen males and fifteen females with normal plumage were also randomly selected as a control group. Both groups of birds were provided the same diet and management while being housed in the same facility.

The third, fourth, and fifth primary remiges on the left wing were cut with scissors about 15 mm above the feather follicle to avoid blood contamination. Each feather sample was individually washed in 60°C water containing a neutral pH detergent. Feather samples were rinsed in warm water until the rinse water was free of debris. A final rinse was performed using distilled water followed by immersion of the samples in 95% ethanol to accelerate desiccation of the feather samples. All samples were then dried in a 37°C oven for 3 h, cooled to the room temperature, and subsequently ground using an electric feed grinder. Pooled samples consisted of ground feathers obtained from five individuals. Three such pooled samples were prepared for each sex and feather type. A total of 12 samples containing feathers from 60 birds were analyzed for amino acid composition as previously described (Savage et al., 1986a).

---

\(^1\)Good Samaritan Hospital Laboratory, 3600 NW Samaritan Dr., Corvallis, OR 97330
2.2.2.4 Scanning Electron Microscopy (SEM) of feather structure

Primary and secondary remiges were collected from 10 week old IF and NF turkeys and prepared for SEM as previously described (Savage et al., 1986b). The feathers were not washed prior to SEM preparation since the washing procedure could alter their structure. Therefore, only feathers that were clean and free of debris were collected for examination. Approximate 1 cm$^2$ samples of feathers were cut from the upper region 1/4 of the feather vanes.

2.2.3 Results

2.2.3.1 Mode of inheritance

The results of the completed matings used to determine the inheritance, are presented in Table 2.1. In the initial mating of the original mutant female with normal feathered unrelated males, all male progeny exhibited the feather disorder whereas all of the females were normal. In the next generation IF male progeny were mated with NF females. Of 79 poults, 42 were normal and 37 demonstrated the abnormal feathering. Due to mortality, sex was determined for only 16 IF poults - half were males. The results from the other matings are also summarized in Table 2.1. Six IF females mated with four homozygous IF males, resulted in 46 males and 41 females and all expressed inhibited feathering. No NF poults were observed from these matings. Three NF females, when mated with one homozygous IF tom produced 37 IF poults, of which 24 were sexed. The sex ratio observed 13 males and 11 females was not significantly different from the expected (1:1). Matings of twelve IF females with six NF toms resulted in 22 NF females and 20 IF males. Matings of five heterozygous IF males with 15 NF hens, resulted in 323 poults. Of these, 153 were NF (79 males and 74 females) and 170
displayed the IF disorder (86 males and 84 females) which was in agreement with the expected segregation ratio. Ch-square = 1.07 (P > 0.1).

2.2.3.2 Plumage of the Inhibited Feathering turkeys

Intensive feather growth occurs at specific ages during the life of the bird. The first such period occurs during embryonic development between days 11 and 16 of incubation. At day 16, down development has progressed to such a stage that major developmental defects may be detected (Asmundson and Abbott, 1961). The second intensive phase of feather development occurs between the time of hatch and 10 weeks of age (Lucas and Stettenheim, 1972). It was assumed that the actual feather growth could best be observed in these periods. The feathering of the mutant birds at day 16 of incubation and at the time of hatch was characterized. Subsequently, feather development was observed at seven, eight and at ten weeks of age. Final evaluation of the feather growth was performed when birds reached physical maturity (more than 20 weeks of age).

The feathers of the mature Gallinaceous bird are represented by five categories: [1] remiges and rectrices, [2] coverts, [3] down feathers, [4] filoplumes, and [5] bristles. It was observed that down, filoplumes and bristles were not altered in IF turkeys. Therefore, only the description of visibly altered feathers by the IF gene, that is primary and secondary remiges, wing coverts and rectrices - the major tail feathers are reported. The classification of the feather types was based on the description of Chandler (1916).

Sixteen day embryos. Down development in 15 normal and 15 mutant embryos was not different. It has been reported that a sex-linked gene affecting the rate of feathering in the turkey (Asmundson and Abbott, 1961) influenced down development in embryos by inhibiting formation of the down in the skin and reducing the down length. This was not observed in IF embryos, and therefore results imply that the feather mutation described herein is different from that described by Asmundson and Abbott.
Table 2.1 Progeny phenotypes from the matings of Normal Feathering (k\k), homozygous dominant (K\K'), heterozygous (K\k) and hemizygous (K\-') Inhibited Feathering turkeys

<table>
<thead>
<tr>
<th>Parental genotypes</th>
<th>Progeny phenotypes</th>
<th>Female progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Feathering</td>
<td>Inhibited Feathering</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Initial mating</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>k/k     K'/-</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Second mating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K'/k     k/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other matings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K'/K'   K'/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K'/K'   k/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>k/k     K'/-</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>K'/k     k/-</td>
<td>79</td>
<td>74</td>
</tr>
</tbody>
</table>

* not sexed
** only 16 poults were sexed
Newly hatched poults. At the time of hatch, all IF birds had uniform down similar to that of NF birds. The IF poults differed from NF poults only by the absence of flight feathers on their wings (Figure 2.2). As the IF poults grew, differences between the IF and normal birds became apparent. At two weeks of age, NF poults had very well developed remiges (Figure 2.3) whereas IF poults had only one or two primary feathers present (Figure 2.4). The IF poults lost their down at an older age and the feather development was delayed by approximately 2 weeks when compared to normal birds. Between 3 and 6 weeks of age, all IF poults were devoid of feathers on the body, with only down remnants present. By the fourth week of age, small feathers (remiges) began to appear on the wings of some of IF poults. Until this age, there were no apparent differences between individual IF birds.

Seven week old poults. At 7 weeks of age, noticeable differences in plumage of individual IF poults were becoming apparent (Figure 2.5). It was possible to classify IF poults with respect to the extent that their feather development was inhibited. The IF birds were assigned to one of five categories based on the rate of feathering. This classification was performed independently for birds of different ages, resulting in five classes of feathering for each described age group.

Class 1. Poults in this class were normal feathered and used as a control reference in description of inhibited feathering poults. These birds had fully developed feathers in all feather tracts.

Class 2. Poults of this class had down present on the head. The remiges were 1 - 2 cm long, and the upper major primary coverts were 2 - 5 cm in length. Some feathers were noticeably twisted and bent. Feathers about 2 cm long covered the pectoral and femoral tracts. The crural tract, and in particular its distal portion was also covered with feathers. Only the abdominal, proximal section of this tract might lack feathers or be covered with "pin-like" feathers.

Class 3. Birds in this class had down remnants on the head and tail and were also characterized by presence of remiges I - VII and upper primary coverts I - VII on the wings. The remiges and the primary coverts were 1 - 2 cm and 1.5 - 4 cm in length, respectively. The feathers on the dorsopelvic tracts were present only on their lateral
Figure 2.2 Comparison of down and feather development in an IF (left) and a NF (right) turkeys. Wings of a day old poults
Figure 2.3 Down and feather development in an IF poult at 16 days of age

Figure 2.4 Down and feather development in a NF poult at 16 days of age
Figure 2.5 Comparison of down and feather development in IF (left and center) and NF (right) turkeys at eight weeks of age
parts and were about 1 cm in length. On the dorsal and ventral cervical tracts the "pin-like" type of feathers could be observed even though in some lower (poorer-feathered) classes there were birds with better feathered cervical tract. Much longer and better developed feather had grown on the femoral tracts (approximately 3 cm long). Single "pin-like" feathers were noticeable on the distal parts of crural tracts. Feathers about 1 cm in length were visible on the pectoral tract.

Class 4. These birds were characterized by only remnants of down on the head (capital tract). They had I - VII primary remiges up to 0.5 cm long and I - VII primary coverts 1 - 2 cm long. Feathers had begun to develop on the borders of the dorsal and ventral cervical tracts, the interscapular tracts, the anterior part of the dorsopelvic tract, and the border between the pectoral tract and the lateral apterium. On the shanks, sparse "pin-like" feathers had begun to grow in the distal parts of the crural tracts. The longest feathers (3 - 4 cm long) were observed on the femoral tract. The under marginal prepatagium and the posthumeral tracts remained devoid of feathers.

Class 5. Birds in this class were considered featherless. Some poults might have the fourth primary coverts in the wings although very short ones (0.5 - 1.0 cm). At this age no feathers were observed on the body of the poults. The down was present in the capital and ventral cervical tracts, the lateral body apteria, the crural tracts and medial abdominal tracts, the lateral abdominal apterum and on the upper and under tail tracts. Some birds might also have "pin-like" feather growing between the dorsopelvic tract and the lateral body apterium. These birds had less down than the others from this class and the down was present only on the head (the capital tract) and partially on the intracrural apteria.

Eight week old poults. Class 1. This class was reserved for normal feathered poults and was used as a reference in description of slow feathering poults. These birds were characterized by all feather tracts containing fully developed feathers.

Class 2. Poults in this class had down on the head. The remiges were approximately 1.5 - 7 cm long and the upper major primary coverts were approximately 1.5 - 10 cm long. They were noticeably twisted and bent. Very long and well developed feathers covered the pectoral tracts (3 - 5 cm long), the femoral tracts (5 - 10 cm), the
medial parts of the interscapular tracts, and the anterior parts of the dorsopelvic tracts were also covered with feathers. The posterior parts of these tracts were either naked or a few feathers might exist on the border between the pelvic tracts and the lateral pelvic apterium. Feathers were well developed on the crural tract, particularly on its distal part. Only the abdominal proximal section of this tract was naked or covered with "pin-like" feathers.

Class 3. Birds belonging to this category had remnants of the down on the head and tail. They had remiges I - VII and the upper primary coverts on the wings. The remiges were 1.5 - 3 cm long and primary coverts were 3 - 7 cm long. Feathers on the dorsopelvic tracts were present not only on the lateral parts of these tracts where the feathers were 1 - 3 cm long, but also close to the dorsal apterium where the feathers were small (0.3 - 0.5 cm) and "pin-like". The same type of the feathers, i.e. "pin-like", were present on the borders of the ventral cervical tract, and on the sternal tract. Much longer and better developed feathers had grown on the femoral tracts (approximately 3 - 5 cm) and on the ventral, distal parts of the crural tracts. Single "pin-like" feathers might exist on the under marginal prepatagium and on posthumeral tracts of a few birds from this class.

Class 4. Turkeys classified to this category had down remnants only on the head (capital tract). These birds had I - VII primary remiges 0.5 - 1 cm long and I - VII primary coverts 1 - 3.5 cm long. The latter were bent and twisted. The feathers had started to grow on the interscapular tracts on the anterior parts of the dorsopelvic tracts, and on the medial pectoral tracts i.e. on the border of the pectoral tract and of the pectoral apterium. On the shanks, feathers had begun to grow on the femoral tracts and on the crural tracts starting from the distal parts of these regions. The under marginal prepatagium and the posthumeral tracts remained naked.

Class 5. This class consisted of virtually featherless birds. Some of them might have the third or the fourth primary coverts, even though they were very short (0.5 - 1 cm). At this age there was no growing feathers observed on the body of the poult's. The down remained on the capital tracts, the ventral cervical tracts, the lateral body apteria, the crural tracts, the medial abdominal tracts, the lateral abdominal apteria and on the
upper and under tail tracts. There were also birds which at 8 weeks of age had signs of growing feathers on the posthumeral tracts or on the border between the dorsopelvic tract and the lateral body apterium. These birds did not have as much down as the others from the same class. The down remained only on the head (the capital tract) and partially on the intracrural apteria.

**Ten week old poults.** Class 1. Birds were characterized by complete feather development in all feather tracts.

Class 2. These poults had well developed feathers in the dorsal cervical, interscapular, humeral, the anterior part of the dorsopelvic and the femoral tracts. The shanks were covered with well developed feathers. Only the proximal part of the crural tract was shielded with "pin-like" feathers. The primary remiges I - IX were partially developed (4 - 9 cm in length) with the majority of them twisted and bent. The upper major primary coverts were also twisted and bent and up to 20 cm in length and. Some of the upper major secondary coverts and secondary remiges were missing although not necessarily symmetrical with respect to the body axis. One to five upper major secondary coverts were 5 - 7 cm in length. The remaining upper major secondary coverts if present, were less than 4 cm long. Secondary remiges were 1 - 3 cm long and some of them were frequently missing. No fully developed coverts were present in the tail. Some IF birds might have "pin-like" feathers or very short upper median coverts present in the tail, while there were no feathers on the upper part of the prepatagium. The ventral cervical and pectoral tracts were covered with well developed feathers. Less developed feathers might be observed in the sternal tracts. Minimal feathering was observed under the prepatagium and in the ulnar region.

Class 3. Birds in this category had the dorsal cervical and the edges of the dorsopelvic tracts covered with feathers about 9 to 12 cm long. The humeral, crural and femoral tracts were covered with well developed feathers and with no differences in these tracts between class 2 and class 3. Some poults might have short upper median coverts in the tail area. The upper major primary coverts were up to 15 cm long. The primary remiges were about 5 cm long and shorter than those of normal birds. The upper prepatagium was not feathered. The ventral cervical and pectoral tracts were covered
with normal, fully developed feathers, while the other ventral tracts were featherless, except for a slightly visible line of "pin-like" feathers in the sternal tracts.

**Class 4.** This category consisted of birds with one to six primary remiges no longer than 2 cm, and upper major primary coverts about 4 - 10 cm long. One to six secondary remiges and one to six upper major secondary coverts were also present, although short (2 to 3 cm). The upper marginal and under marginal coverts of the prepatagium were absent. Feathers about 1 cm long were present on the boundaries of the ventral and dorsal cervical tracts. The same type of feathers covered the distal parts of the crural tracts, whereas on some of the birds the proximal parts of these tracts were covered with "pin-like" feathers. Slightly longer feathers (about 1.5 cm) were present on the borders of the anterior part of the dorsopelvic tract. The longest feathers (about 10 cm long) were present on the femoral and pectoral tracts. On the sternal tract feathers were either missing or very short (0.5-1 cm).

**Class 5.** This class consisted of birds with only one to five primary remiges no longer than 1 cm with upper major primary coverts about 3 - 7 cm long. Only one to four secondary remiges and one to four upper major secondary coverts were present, though very short (less than 1 cm). Absent were the upper marginal and under marginal coverts of the prepatagium. Short (about 0.5 cm long) feathers mixed with "pin-like" feathers existed on the boundaries of the ventral and dorsal cervical tracts, constituting four separate feathered strips along the neck. The same type of feathers covered the distal parts of the crural tracts whereas the proximal parts of these tracts were naked. The borders of the anterior part of the dorsopelvic tract of some birds were covered with "pin-like" feathers while they were lacking on other poult's. A few feathers (about 5 cm long) were present in the femoral and pectoral tracts. There were no feathers on the sternal tract.

**Mature turkeys.** When the IF birds were 24 weeks of age, it was no longer possible to classify them into distinct categories with respect to feather development. Mature IF birds displayed considerable diversity of feathering with different tracts affected in different birds - Figures 2.6 and 2.7. Generally, IF males are more feathered than IF females. Here, only a general qualitative description how the $K^1$ gene typically
Figure 2.6 Variations in down and feather development in IF hens at 20 weeks of age

Figure 2.7 An IF male at 20 weeks of age
affects different feather types in mature turkeys is provided. It should be kept in mind that combinations of features described below could be present on individual turkeys.

The primary remiges are the largest feathers of the posterior edges of the wings and were most affected by the IF gene. Generally, remiges are characterized by their length, size, stiffness and strength. In normal feathered mature turkeys the average length of the flight feathers is between 20 and 25 cm. The largest feathers on the wings, the primary remiges, are located in the region of phalanges - digits III and IV (Lucas and Stettenheim, 1972). Considerable variation in the shape and the appearance of remiges of IF turkeys does not allow a simple and uniform description. About a half of the observed IF birds displayed fragments of the remiges or were missing all of them. Some IF turkeys developed remiges but their appearance differed greatly from those formed on the wings of the normally feathered turkeys. Primary remiges present in the feather tracts of IF birds are those numbered 1-6 (Lucas and Stettenheim, 1972) and the terminal and basal phalanges of the digit III are not covered by feathers. Those primary remiges that developed were significantly narrower and shorter than those on NF turkeys. The typical width of the primary remiges measured at the widest portion of the vane is about 3.5 cm for normally feathered hens and about 4.7 cm when measured on normal males. The average width of the primary remiges was about 8 mm and 17 mm on IF females and males, respectively. The length of the primary remiges present on IF turkeys also varied greatly. The length of some IF turkey feathers did not differ from the length of feathers of normal feathering turkeys whereas in the most extreme situations, feather length did not exceed 0.5 cm. The shape of the primary remiges also differed greatly from what was considered as normal (Figure 2.8). Primary remiges present on the wing of the inhibited feathering turkey were bent and twisted. The direction of the bending was consistently towards the bird’s body. The inner part of the vane was not a flat surface but had an undulating curve. The vane texture also showed noticeable variations. The proximal region of the pennaceous portion of the vane was closely knit and appeared as a firm and uniform surface. The veneer (surface) of the distal part of the vane, however, was wavy due to bending and did not appear as flat and smooth. The barbs located in this area were not parallel but crossed each other (Figures 2.11; 2.12 2.13; 2.14; 2.15 and
Figure 2.8 Primary remiges of an IF (left) and a NF (right) turkeys at 30 weeks of age
In such a situation the proximal pennaceous barbules were no longer attached to the distal barbules by the set of cilia and hooklets. In severe situations, a large portion of the barbules were absent or visible only as short immature buds formed on barbs. The calamus of the altered feather was not different from that of the normal feather. There were no major differences on the proximal half of the rachis of the primary remiges when compared with normal appearing feathers. Often, the distal region of the rachis was twisted between 60 and 90 degrees and the ventral groove of the rachis was present on the side of the feather rather than on its ventral surface.

Effects of the IF gene on secondary remiges also varied among IF birds. In many situations, several of the secondary remiges were missing. Usually the feather follicle of the absent feather existed in the skin, however it appeared empty. Similar to the primary remiges, there were major differences in the number, quality, and quantity of the secondary remiges between inhibited feathering turkeys. Even when the feathers were present in the feather follicles, they usually differed significantly from the corresponding feathers in normal birds. A significant narrowing of the secondary remiges was noticeable, and growing remiges tended to twist in the proximal region of the shaft, resulting in reversal of the feather vane by 90 to 180 degree (Figure 2.9). Similar to the primary remiges, these feathers might bend toward the inner side of the feather causing defects in structure of the vane, such as curling of the surface. Some of the barbs in affected areas were disconnected and the hooklets absent (Figures 2.12; 2.13; 2.15 and 2.16). Severely affected secondary remiges resembled long and narrow strings. In some birds several secondary remiges might appear normal, whereas others would be totally missing. In normally appearing feathers, barbules were arranged parallel and hooklets were present in their normal configuration. These feathers, if separated from the bird could not be distinguished from feathers collected from the turkeys with normal plumage.

In the majority of the IF turkeys observed, fully developed rectrices were absent. The feather follicles responsible for their growth existed as large empty cavities. Some IF birds, particularly males, developed rectrices which could be assigned to one of three types based on their appearance. The first type of the rectrices resembled a string which was not wider than 1.5 cm, and the length varied between 5 and 15 cm (Figure 2.10).
Figure 2.9 Secondary remiges of an IF (left and center) and a NF (right) turkeys at 30 weeks of age
The second type was small - not longer than 2.5 cm and not wider than 10 mm (Figure 2.10) while the third could be described as shorter than 8 cm. For comparison, the average length of normal rectrices was about 25 - 35 cm. The width of these feathers was not affected. The average width of the widest locus of affected feathers was 41 mm compared to the same measurement obtained on normal bird feathers, which was approximately 45 mm (Figure 2.10).

All tail feathers of IF turkeys were characterized by fragile and easily cracked calamuses. Unlike normal growing feathers, it was difficult to remove tail feathers from the feather follicle without fracturing the calamus wall. Growing rectrices were noticeably bent and predisposed to twisting up to 180 degrees resulting in a disturbed vane texture and disconnection of barbules. Fragments of the vane were missing in majority of the feathers.

### 2.2.3.3 Biochemical assays

#### 2.2.3.3.1 Blood chemistry

The data comparing blood plasma chemistry of IF and NF hens at 30 weeks of age are summarized in Table 2.2. The concentrations of alkaline phosphatase, sodium and glucose were higher in IF than in NF hens with estimated statistical significance of P < 0.05, P < 0.01, and P < 0.05, respectively. The cholesterol concentration was lower (P < 0.01) in the IF females.

#### 2.2.3.3.2 Amino acid content of the feathers

The amino acids levels in feathers collected from NF and IF birds are summarized in Table 2.3. There were no significant differences between males and females in NF or IF turkeys. The data were pooled for NF and IF birds. Concentrations of alanine, aspartic acid, glycine, leucine, tyrosine were lower (P < 0.01) in IF feathers as was
Figure 2.10 Rectrices of a NF (left) and an IF (right and center) turkey at 30 weeks of age
Figure 2.11 Electron micrograph of the primary remige of a NF turkey at 10 weeks of age. Note the well developed barbules and barbicells. Bar represents 100 μm

Figure 2.12 Electron micrograph of the primary remige of an IF turkey at 10 weeks of age. Note the disconnected barbules. Bar represents 100 μm
Figure 2.13 Electron micrograph of the primary remige of an IF turkey at 10 weeks of age. Note the disconnected barbules. Bar represents 1000 μm

Figure 2.14 Electron micrograph of the primary remige of a NF turkey at 10 weeks of age. Note the well developed barbules and barbicells. Bar represents 10 μm
Figure 2.15 Electron micrograph of the primary remige of an IF turkey at 10 weeks of age. Note the degenerated barbules. Bar represents 100 μm

Figure 2.16 Electron micrograph of the primary remige of an IF turkey at 10 weeks of age. Note fusing of barbules and barbicells. Bar represents 10 μm
Table 2.2 Mean plasma chemistry values of IF (Inhibited Feathering) and NF (Normal Feathering) turkey hens measured at 30 weeks of age

<table>
<thead>
<tr>
<th>Plasma variable</th>
<th>IF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>NF&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine phosphokinase (U/L)</td>
<td>9113 ± 1063</td>
<td>9350 ± 2569</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>1032&lt;sup&gt;a&lt;/sup&gt; ± 252</td>
<td>818&lt;sup&gt;b&lt;/sup&gt; ± 52</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>643 ± 72</td>
<td>639 ± 120</td>
</tr>
<tr>
<td>Aspartate amino transferase (U/L)</td>
<td>638 ± 60</td>
<td>506 ± 35</td>
</tr>
<tr>
<td>Alanine amino transferase (U/L)</td>
<td>4.3 ± 0.3</td>
<td>6.5 ± 2.7</td>
</tr>
<tr>
<td>Glucose (g/dl)</td>
<td>354&lt;sup&gt;a&lt;/sup&gt; ± 4</td>
<td>334&lt;sup&gt;b&lt;/sup&gt; ± 8</td>
</tr>
<tr>
<td>Uric Acid (g/dl)</td>
<td>7.3 ± 0.6</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Calcium (g/dl)</td>
<td>12.2 ± 0.2</td>
<td>11.9 ± 0.2</td>
</tr>
<tr>
<td>Phosphorus (g/dl)</td>
<td>5.7 ± 0.2</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.70 ± 0.11</td>
<td>4.48 ± 0.14</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.01 ± 0.06</td>
<td>1.92 ± 0.11</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>148&lt;sup&gt;A&lt;/sup&gt; ± 3</td>
<td>167&lt;sup&gt;B&lt;/sup&gt; ± 6</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>159 ± 12</td>
<td>233 ± 49</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>170.5&lt;sup&gt;A&lt;/sup&gt; ± 0.8</td>
<td>164.8&lt;sup&gt;B&lt;/sup&gt; ± 1.5</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>4.96 ± 0.13</td>
<td>4.93 ± 0.18</td>
</tr>
<tr>
<td>Chloride (mEq/l)</td>
<td>120 ± 1</td>
<td>119 ± 2</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± SEM for n = 14  
<sup>2</sup> Mean ± SEM for n = 6  
<sup>a</sup><sup>b</sup> values with different superscripts within a row differ significantly (P < 0.05)  
<sup>A</sup><sup>B</sup> values with different superscripts within a row differ significantly (P < 0.01)
phenylalanine ($P < 0.05$). The concentration of isoleucine was statistically higher in IF feathers ($P < 0.01$).

2.2.4 Discussion

2.2.4.1 Mode of inheritance and phenotype description

The data presented in Table 2.1 are consistent with the hypothesis that inhibited feathering is the expression of a dominant sex-linked gene. Comparison of the IF mutation described herein with other hereditary feather disorders reported in the literature suggests that this is a new mutation. Among several feather mutations previously described in the turkey, only "late feathering" was sex-linked and dominant (Asmundson and Abbott, 1961). Due to the same mode of inheritance and similarity of observed gene effects, phenotype of IF turkeys in the present study were compared with the description of late feathering turkeys provided by Asmundson and Abbott (1961). Despite similar modes of inheritance these mutations are different. Description of embryo development in Asmundson and Abbott’s late feathering turkeys suggests that down formation is delayed. In the current study, there were no differences in the length of down between IF and NF turkeys either on the 16th day of incubation or at the day of hatch. It can be concluded that the down development in IF turkeys during embryonic development is not affected. Growth of mature feathers on the body of IF poults appears to be similar to what was described in late feathering (Asmundson and Abbott, 1961), however, in the present study, tail feathers (rectrices) of the IF birds do not develop or are altered. In late feathering turkeys, rectrices were not affected and birds developed full size tail. Therefore, based on the phenotype, the current mutation it is distinct from the late feathering mutation described by Asmundson and Abbott (1961). Since the gene for the late feathering in the turkey is no longer available, determination of allelism was not possible.
Table 2.3 Amino acid composition of feathers from IF (Inhibited Feathering) and NF (Normal Feathering) turkeys

<table>
<thead>
<tr>
<th></th>
<th>IF</th>
<th>NF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.25±</td>
<td>4.33±</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.36±</td>
<td>5.45±</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.96±</td>
<td>5.57±</td>
</tr>
<tr>
<td>Cystine</td>
<td>6.27±</td>
<td>6.16±</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7.89</td>
<td>7.98±</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.28±</td>
<td>6.20±</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.33±</td>
<td>0.32±</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.83±</td>
<td>3.48±</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.08±</td>
<td>6.84±</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.86±</td>
<td>0.85±</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.18</td>
<td>0.16±</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.02±</td>
<td>4.43±</td>
</tr>
<tr>
<td>Proline</td>
<td>10.02±</td>
<td>10.19±</td>
</tr>
<tr>
<td>Serine</td>
<td>9.90±</td>
<td>10.00±</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.06±</td>
<td>4.02±</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.28±</td>
<td>2.65±</td>
</tr>
<tr>
<td>Valine</td>
<td>6.41±</td>
<td>6.51±</td>
</tr>
</tbody>
</table>

1 Mean ± SEM for n = 6

\( ^{a,b} \) values with different superscripts within a row differ significantly (\( P < 0.05 \))

\( ^{A,B} \) values with different superscripts within a row differ significantly (\( P < 0.01 \))
2.2.4.2 Biochemical assays

2.2.4.2.1 Blood chemistry

There were major differences between the blood chemistry of the IF and NF hens. The level of the alkaline phosphatase was significantly higher in IF hens when compared to NF females ($P < 0.05$). It has been suggested in literature that the level of alkaline phosphatase may be influenced by: genetics (Singh and Balaine, 1983b; Wilcox, 1963; Wilcox et al., 1963), age (Singh and Balaine, 1983a), onset of egg production (Savage, et al., 1970) and housing system (Singh and Balaine, 1983a,b). Activity of the alkaline phosphatase decreases with age (Banerjee, et al., 1973; Tamaki et al., 1975), and can differ as much as 10 times between immature birds and adults (Bell, 1960). Opposite results, however, were obtained in a study involving offspring of four lines of White Leghorn hens, where an essentially linear increase with age was observed (Singh and Balaine, 1983a). Since those studies were performed on different strains of birds, the contradictory results may suggest that activity and the concentration levels of alkaline phosphatase is genetically determined and varies between strains of birds. Consequently, the differences between the concentration of the alkaline phosphatase in the blood of IF and NF turkey hens may suggest more complex differences in their physiology other than only ratio of feathering. According to Banerjee et al. (1973) a lowered activity of alkaline phosphatase is associated with lower egg production, so that it is possible to increase egg production by selection for high level of alkaline phosphatase in serum (Gutowska et al., 1943), however, other authors imply the opposite effect (Rako et al., 1964; Singh and Balaine, 1983b). Egg production in IF turkeys was significantly lower than that of NF hens. Phenotypic correlation between alkaline phosphatase and egg mass were estimated in chicken as high and negative (Wilcox, 1963), whereas, genetic correlation between activity of the alkaline phosphatase and egg weight was high and positive, even though it decreased with age (Singh and Balaine, 1983b). Indeed, the eggs laid by IF hens were heavier than those from NF hens. Nevertheless, the total weight of
eggs laid by IF hens is much lower than the total weight of eggs laid by normal feathered hens. Because numerous factors may influence the activity of the alkaline phosphatase, the data cited in the literature cannot be used in direct comparison of birds. However other results may be used as guidelines of what may be expected.

It has been suggested that the bird's environment as well as nutrition may be a factor influencing alkaline phosphatase (Bell, 1961; Bell and Siller, 1962). Also level of nutrient levels will affect the level of this enzyme in serum. Starvation may also be a factor reducing alkaline phosphatase level in White Leghorns, while never reaching the level from the period prior to fasting when regular feeding was resumed (Bide and Dorward, 1970). The activity of alkaline phosphatase in the inhibited feathering birds was not affected directly by the environmental conditions or diet. All birds were housed in the same open sided building and received the same feed. It has been suggested that the alkaline phosphatase level may be altered in stress conditions (Singh and Balaine, 1983a). Inhibited feathering may be a factor that induces stress in poults. Nervousness and increased activity of the hens may indicate that the stress threshold may be much lower for inhibited feathering birds than it is for normal feathered turkeys (section 2.3).

An increased plasma sodium concentration was observed in the IF hens. Hypernatriemia can be associated with elevated serum osmolality. In severe diarrhea, vomiting, or renal failure the water loss exceeds loss of the sodium and potassium, therefore an elevated serum sodium level is an indication of a relative water deficit (Carlson, 1989). No water loss due to diarrhea was observed in the line of IF turkeys. The water loss may be also a result of increased evaporation through the skin. Since feathers provide good insulation, the bare skin with a depleted feather cover may result in increased sensitivity to the higher temperature of the environment (Peguri and Coon, 1993). The increased nervousness and activity observed in IF turkeys could result in panting and could cause evaporative respiratory water loss. Salt toxicity as a cause for the hypernatriemia (Padovan, 1980; Pearson and Kallfetz, 1982) was discounted in our study because all IF and NF hens were fed the same diet. The most probable cause of the difference in the sodium level would be continued cutaneous and respiratory insensible water loss.
Cholesterol level was significantly lower in the inhibited feathering line of turkeys when compared to those with normal feathering. Cholesterol is considered as one of the most commonly occurring steroids in animals and plays an important role in evaluation of the vascular diseases (Bartley, 1989). At the same time, it is known that the cholesterol level decreases steadily as a reaction to thyroxine replacement therapy (Kaneko, 1989b). Therefore it has diagnostic importance in thyroid disorder evaluation (Lowe et al., 1974). Hypocholesterolemia due to zinc deficiency has been demonstrated in rats (Lefevre et al., 1985; Schneeman et al., 1986; Koo and Lee, 1988), pigs (Burch et al., 1975), and humans (Sandstead et al., 1980). It is suspected that in IF turkeys, the absorption of zinc from the diet as well as zinc metabolism may be severely affected since zinc deficiency is known to cause dermatitis (Garrets and Molokhia, 1977; Krieger and Evans, 1980).

The glucose level in the serum of the slow feathering hens was significantly higher when compared with normal feathered hens at the same age. The blood glucose concentration depends on many factors including the equilibrium between the quantity of the insulin and glucagon in the blood. Also, if the renal reabsorptive capacity for the glucose is exceeded, an additional loss of glucose from the system may occur (Kaneko, 1989a). Change of the glucose level may suggest essential differences between the metabolism of the IF and NF turkeys.

2.2.4.2.2 Amino acid analysis of the feathers

The differences in the amino acid composition of feathers can partly result in abnormal feather structure. Since the amino acid contents of barbs and rachis are different, variations in the mass ratio of barbs to calamus may result in variation of amino acid content (Murphy et al., 1990). Feathers of IF turkeys contained significantly less leucine than NF feathers. Nitrogen content in feathers which is closely related to content of the keratin (dominant protein) and is estimated at about 16.5% of dry mass of a whole feather (Harrap and Woods, 1964; Murphy et al., 1990). Low concentrations
of the branched-chain amino acids may result in feathers that are fragile and more susceptible to deformations.

2.3 Reproductive performance of Inhibited Feathering turkeys

2.3.1 Introduction

Several genetic mutations, affecting plumage appearance which have been described in poultry have also been associated with reduced egg production and impaired semen quality. Decreased egg production has been reported in the wooly feather condition observed in the chicken (Jones and Morgan, 1956). Porcupine chickens, in addition to displaying abnormal feather condition are characterized by extremely low egg production. Semen production was also affected in the mutant since the majority of the eggs were infertile despite using artificial insemination (Waters, 1967).

Several mutations affecting feather growth and development which have been investigated in Japanese quail, have been also reported to cause problems with reproductive performance. Savage and Collins (1971) observed that impaired egg production was but one pleiotropic effect of the downless feather condition. Reduced egg production and fertility have been reported in porcupine Japanese quail (Fulton et al., 1982a). Several reports also provide examples of feather mutations that affect hatchability. High mortality in embryos and newly hatched chicks was reported for wooly mutants (Jones and Morgan, 1956). Japanese quail displaying the rough-textured feather condition are characterized by poor fertility and hatchability results (Cheng and Brush, 1984).

Preliminary observations of the reproductive performance of Inhibited Feathering turkeys revealed that the sex-linked gene responsible for impaired feathering modifies reproduction efficiency. High reproductive performance is necessary for the lines of birds that may possibly be incorporated into the commercial crosses. Therefore characterization of the reproductive traits of the mutant turkeys is necessary.
2.3.2 Materials and methods

2.3.2.1 Management procedures

Day old poults were placed on concrete floors covered with fir shavings litter and brooded until 9 weeks of age. Then, they were transferred to open, grass covered ranges with shelters. At 20 weeks of age females were separated from males and maintained on separate ranges. Feed and water during the brooding and rearing periods were provided *ad libitum*. The feeding program is summarized in the Table 2.4.

2.3.2.1.1 Management procedures for breeder hens

At 25 weeks of age hens were assigned to pens containing fir shavings litter and located in the open sided breeder house. Each pen contained four trap nests located on the inside wall of the pen with the nest openings facing the open side of the building. The nests were located 65 cm above the floor and were easily accessible for the NF hens. Based on the experiences with the IF hens, the decision was made to locate the trap nests on the floor of each pen which housed IF hens to allow better nest access. Twenty-four IF hens were randomly assigned to two pens (each pen contained initially 12 hens). Twenty four NF hens were randomly assigned to three pens (each pen contained initially 8 hens). The justification for increased initial number of the IF hens per pen when compared to the NF hens, was previous experiences suggesting that much higher percentage of IF hens would be unresponsive to photostimulation. In each pen, water and feed was provided *ad libitum*. Starting at 31 WOA all hens received the same diet containing 15.4% CP and 2963.5 kcal/kg ME with 2.42% Ca (Table 2.5). Hens were photostimulated by a daily light exposure to 14 hours of light (natural light supplemented with incandescent lights).
Table. 2.4 Feeding program for turkeys between 1 day and 25 weeks of age (WOA)

<table>
<thead>
<tr>
<th>Nutrient level in feed&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Age (WOA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 8</td>
</tr>
<tr>
<td>CP&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>28.1</td>
</tr>
<tr>
<td>ME&lt;sup&gt;2&lt;/sup&gt; (kcal/kg)</td>
<td>2712</td>
</tr>
</tbody>
</table>

<sup>1</sup> in mash form  
<sup>2</sup> calculated
Table 2.5 Composition of Hen and Tom Diets fed between 31 weeks to 45 weeks of age

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Hen Diet</th>
<th>Tom Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, Yellow (8.9%CP)</td>
<td>76.25</td>
<td>80.74</td>
</tr>
<tr>
<td>Soybean meal (47.5%CP)</td>
<td>9.33</td>
<td>13.80</td>
</tr>
<tr>
<td>Meat and bone meal (50%CP)</td>
<td>5.08</td>
<td></td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>4.14</td>
<td>0.60</td>
</tr>
<tr>
<td>Fish meal (65%CP)</td>
<td>2.54</td>
<td></td>
</tr>
<tr>
<td>Alfalfa meal, dehydrated (17%CP)</td>
<td></td>
<td>2.50</td>
</tr>
<tr>
<td>Defluorinated phosphate (32% Ca; 18% P)</td>
<td></td>
<td>1.36</td>
</tr>
<tr>
<td>Fat (blended, animal)</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Vitamin premix 1</td>
<td>0.53</td>
<td>0.35</td>
</tr>
<tr>
<td>Monocalcium phosphate (16%Ca 21%P)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Salt (Iodized)</td>
<td>0.41</td>
<td>0.50</td>
</tr>
<tr>
<td>Trace minerals premix 2</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Biotin (200.4 mg/kg)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Selenium (200.2 mg/kg)</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Calculated analysis:

<table>
<thead>
<tr>
<th></th>
<th>Hen Diet</th>
<th>Tom Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>15.40</td>
<td>14.06</td>
</tr>
<tr>
<td>Met.Energy, kcal/kg</td>
<td>2963</td>
<td>2892</td>
</tr>
<tr>
<td>Ca, %</td>
<td>2.42</td>
<td>0.64</td>
</tr>
</tbody>
</table>

1 Supplied per kilogram of hen diet: vitamin A (retinyl acetate), 8745 IU; vitamin D₃, 2915 IU; vitamin E (dl-α-tocopherol acetate), 2.92 IU; vitamin B₁₂, 14.58 μg; riboflavin, 8.75 mg; niacin, 58.3 mg; d-pantothenic acid, 14.58 mg; menadione bisulfite complex, 1.46 mg; folic acid, 583 μg; choline chloride, 583 mg; and ethoxyquin, 165.36 mg.

Supplied per kilogram of tom diet: vitamin A (retinyl acetate), 5781 IU; vitamin D₃, 1925 IU; vitamin E (dl-α-tocopherol acetate), 1.93 IU; vitamin B₁₂, 9.63 μg; riboflavin, 5.78 mg; niacin, 38.5 mg; d-pantothenic acid, 9.63 mg; menadione bisulfite complex, 0.96 mg; folic acid, 385 μg; choline chloride, 385 mg; and ethoxyquin, 109.20 mg.

2 Supplied per kilogram of hen diet: Mn, 12 mg; Zn, 4 mg; Fe, 4 mg; Cu, 0.4 mg; Co, 0.04 mg; I, 0.24 mg.

Supplied per kilogram of tom diet: Mn, 6 mg; Zn, 2 mg; Fe, 2 mg; Cu, 0.2 mg; Co, 0.02 mg; I, 0.12 mg.
2.3.2.1.2 Management procedures for breeder males

At 25 weeks of age males were transferred to pens with fir shavings litter. Each pen initially housed 30 males. IF and NF males were maintained in separate pens. From 28 WOA the males were fed a standard breeder male diet containing 14% CP and 2892 kcal/kg ME (Table 2.5). All males were photostimulated starting at 28 weeks of age. During the experiment they were maintained on a daily photoperiod of 14L:10D. The natural day light was supplemented with incandescent light.

2.3.2.2 Determination of the egg production

Eggs were collected four times daily and marked with the hen and pen numbers. Prior to storage, eggs were hand cleaned when necessary and fumigated with formaldehyde gas. Cracked, soft-shelled, or other eggs unsuitable for incubation were recorded with appropriate notes and then discarded. Eggs laid on the floor within a pen were recorded as floor eggs and marked with the pen number only. Eggs weights were determined just prior to incubation. Hen-day egg production records for NF hens were initiated when hens were 34 weeks of age. Hen-day egg production records for IF hens were initiated when hens were 36 weeks of age. The two-week difference between the start of egg collections in IF and NF lines was due to the delayed onset of the egg production in the IF turkeys. Similar delays in onset of the egg production of IF hens was also observed in prior seasons. Hen-day egg production was measured weekly from 34 to 43 weeks of age for NF hens and from 36 to 45 weeks of age for IF hens. Duration of egg collection was determined by production period for the IF hens which was 10 weeks (after tenth week, egg production declined precipitously). To obtain comparable egg data, egg collection in NF hens was also stopped after the first ten weeks.
of production. Ten weekly measurements of the egg production was performed based on
the formula:

\[ E (\%) = \frac{N_e}{n_{h-d}} \times 100 \]

\( E \) - hen - day egg production for one week,
\( N_e \) - number of eggs produced in one week
\( n_{h-d} \) - number of hens housed in the pen in one week (North and Bell, 1990)

Collected data were evaluated by employing analysis of variance - ANOVA (Snedecor
and Cochran, 1967). Since there were no differences between individual pens within the
feather type, data from individual pens was pooled and the differences between NF and
IF turkeys performance evaluated.

### 2.3.2.3 Semen collection

The semen was collected at 35 and 37 weeks of age by the method of abdominal
massage (Nilipour, et al., 1988). Males that failed to produce semen were eliminated
from the breeder flock and not included in the statistical calculation.

The difference in the ratio of the males that failed to produce semen in IF and NF
group of males was analyzed using a permutation test (Ramsey and Schafer, 1994). The
statistics whose permutation distribution was used to find the p-value was the difference
in the respective ratios of the infertile males in both groups, with the null hypothesis
being that those ratios are the same. The reason why a non-parametric permutation test
is a better approach than any of t-test based methods is that the binary data characterizing
fertility or infertility of the males are not even approximately normally distributed. It is
worth noting that in general, permutation tests require extensive calculations that they are
impractical for even modest sizes of experimental groups and normal approximations for
t-statistics distribution are used instead. However, in the special case of binary
measurements (males are either fertile or infertile) the permutation test becomes
computationally feasible (as discussed in the Appendix) and gives the exact answer to the
question, what would be the chance of as large or larger difference in the infertility incidence if this difference were caused purely by randomness.

Additionally, the average volume of semen production of IF and NF males was compared statistically. Because volume measurements are continuous numbers for which normal approximation may apply, the standard t-test was used to assess the difference between the two groups.

2.3.2.4 Evaluation of incubation performance of the IF line

Two hundred and six eggs from IF females and three hundred and eighty eggs from NF females were collected and stored in an egg holding room (50-55°F and 80-85% RH) up to fourteen days prior to incubation. Three egg settings were incubated in single-stage horizontal units (Savage et al., 1991). All eggs were candled on day 10 of incubation and eggs without visible embryonic development were removed. The contents of the removed eggs was examined macroscopically. All eggs that did not hatch were also examined macroscopically to determine the age at which the embryos died and the cause of death.

As all the records regarding individual eggs have a binary nature (fertile or infertile, hatch or no-hatch, etc.) the t-test based methods are not quite suitable for analysis of such data. Therefore, the data was analyzed by employing a permutation test (Ramsey and Schafer, 1994) described in the Appendix. To be able to use the permutation test an assumption was made that all eggs may be treated as independent from each other. The type of the feathering by egg set number interactions were not significant (P > 0.05), and the data for egg set number were pooled.
2.3.3 Results

2.3.3.1 Egg production

Inhibited Feathering hens initiated egg production approximately two weeks later than NF hens. Such a late onset of egg production Inhibited Feathering hens suggest that the inhibited feathering gene may have a significant effect on the time of oviposition. The duration of egg production of IF lines was limited. Egg production by hens from the IF line was extremely low (18.38%). Several hens were removed from the pen after they failed to respond to the photostimulation and displayed symptoms of broodiness (pale and dry vent area, inaccessible for insemination). Examination of the vent area of other IF hens did not reveal any characteristics typical for non-productive hens, however those hens most probably never laid eggs. None of these hens demonstrated a tendency toward natural incubation such as remaining in the trap nest. Further, IF hens did not enter the nests and laid all eggs on the floor, consequently, egg production could only be evaluated by the pen.

The results of the egg production are summarized in Table 2.6. Due to lack of the symptoms of the broodiness of IF hens that were not producing eggs, it was impossible to evaluate the true number of hens in production. Only hens that expressed characteristics typical for broodiness were transferred to a separate pen and the hen-day egg production was adjusted. The data indicate that egg production of IF hens is poor when compared to normal feathered hens. Reduced egg production in the IF hens is an effect of late onset of the egg production, low hen-day egg production measured on the weekly basis, and early cessation of egg production.

2.3.3.2 Semen production

Semen production data are summarized in Table 2.7. Sixty two NF males and twenty IF males that were producing semen are included in the summary in the Table
### Table 2.6 Egg production of the IF (Inhibited Feathering) and NF (Normal Feathering) turkey hens

<table>
<thead>
<tr>
<th>Hen phenotype</th>
<th>Hen - day egg production (%)</th>
<th>SEM</th>
<th>Egg weight (g)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF</td>
<td>18.38&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.18</td>
<td>86.17&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.40</td>
</tr>
<tr>
<td>NF</td>
<td>57.25&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.02</td>
<td>75.32&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<sup>A B</sup> Different superscripts within columns indicate significant differences P < 0.0001

### Table 2.7 Semen production of the IF (Inhibited Feathering) and NF (Normal Feathering) turkey males

<table>
<thead>
<tr>
<th>Tom phenotype</th>
<th>Semen volume (ml)</th>
<th>SEM</th>
<th>Toms producing semen %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF</td>
<td>0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03</td>
<td>66.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NF</td>
<td>0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
<td>93.94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a b</sup> P = 0.0305
<sup>c d</sup> P = 0.0007
2.7. All infertile males were excluded from the semen volume results in Table 2.7. The presence of the K\textsuperscript{1} gene in the genome of the turkey significantly reduced the volume of those producing semen (P = 0.03). The number of males that failed to produce semen after photostimulation was significantly higher in the group of IF breeders (P = 0.0007). Four males (6.06\%) with normal feathering (NF) were not producing semen and ten males (33.33\%) with inhibited feather development (IF) were infertile. All these males were excluded from the results summary in the Table 2.7.

### 2.3.3.3 Hen fertility and incubation performance

Incubation results for eggs collected from NF and IF for three consecutive hatches are summarized in Table 2.8. The presence of the IF gene did not significantly affect the majority of the observed incubation characteristics. The percent of fertile eggs laid by IF and NF hens were 88.66\% and 93.94\%, respectively. The difference was significant at P = 0.03. The portion of dead embryos within the first 10 days of incubation (D\textsubscript{10}) for IF and NF lines was 12.36\% and 13.23\%, respectively. The portion of embryos that did not survive from 20 to 28 days of incubation was 13.20\% and 9.21\% respectively. The incidence of pipped embryos was 11.02\% and 9.04\% of eggs laid by IF and NF hens, respectively. None of the above differences were statistically significant (P > 0.05). Finally, 63.34\% of all fertile eggs laid by IF turkey hens hatched, and 68.26\% of eggs hatched in the NF line. The difference was significant (P = 0.05).

### 2.3.4 Discussion

Low egg production has been previously observed in several mutations affecting feather development in various birds and was briefly reviewed in the introduction to this section (2.3.1). A closer investigation of the reports describing genetic mutant birds reviewed in section 2.1, reveals that in each of the described cases when the impaired egg production is correlated with a feather disorder, the gene responsible for the feather
Table 2.8 Hen fertility and incubation performance of eggs produced by IF (Inhibited Feathering) and NF (Normal Feathering) turkey hens

<table>
<thead>
<tr>
<th>Hen phenotype</th>
<th>Incubation performance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertility</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>IF</td>
<td>88.66ᵃ</td>
</tr>
<tr>
<td>NF</td>
<td>93.94ᵇ</td>
</tr>
</tbody>
</table>

D₁, Early embryonic deaths (days 0 - 10 of incubation)
D₃, Late embryonic deaths (days 20 - 28 of incubation)
ᵃᵇ P = 0.03
ᶜᵈ P = 0.05
disorder was autosomal and recessive (Jones and Morgan, 1956; Waters, 1967; Savage and Collins, 1971; Fulton et al., 1982a, Cheng and Brush, 1984). Often, these types of mutations may be observed in inbred populations. It is then difficult to conclude, whether the affected egg production in mutant birds can be associated with the particular feather condition, or if this is a secondary effect of a close relationship between members of the observed population. The IF gene described herein is the first sex-linked dominant trait that affects the feather growth as well as reproductive traits in the turkey. The hens used in the experiment were offspring of IF dams and IF sires and the IF line has been maintained for only three generations using unrelated matings, therefore low reproductive performance should not to be attributed to inbreeding.

It is difficult to establish a single cause of the low egg production in any situation. Usually the problem may be rather complex and a number of factors can add to the cumulative result. It must be stated that in this study, several of the non-producing hens failed to demonstrate symptoms of broodiness, such as persistent nesting, remaining in the nest, and dry, pale vent area. One of the possible explanations of low egg production in IF line of turkeys may be stress resulting from insufficient thermal and mechanical insulation because of poor feather cover. Stress may cause accelerated release of hormones from the pituitary and adrenal glands and may be damaging to the animal. The General Adaptation Syndrome (G.A.S.) has been defined as the sum of non-specific responses to a systemic stressor (Seyle, 1937). G.A.S. is characterized by several physiological changes in the organism. The changes may include enlargement of the adrenal, higher level of the cortical steroids or degeneration of the thymus as well as other lymphatic organs (Garren and Shaffner, 1952; Garren and Shaffner, 1954). Often, enlargement of the pituitary gland can be observed (Garren and Shaffner, 1956; Garren and Barber, 1955). In some cases adrenal hypertrophy and hyperplasia is the major response to the stress conditions (Brown, 1959; Garren and Shaffner, 1956; Siegel, 1960; Siegel, 1959a; Siegel, 1959b). Decreased level of cholesterol with simultaneously increased level of the corticosterone were observed in the turkey (Brown, 1961) and chicken (Flickenger, 1961; Siegel, 1960) as a response to the stress. Similarly, low cholesterol level was observed in blood of IF hens (see section 2.2.). Anxiety is a factor
that may influence the immunological system. Decreased number of lymphocytes and increased number of heterophiles may be observed in birds raised in stressful conditions (Morita and Nishida, 1956; Newcomer, 1958; Schindler, 1962). None of these parameters were measured in the IF turkeys, but other complex deviations from the norm were observed in this interesting line of birds.

A significant modification of the thyroid gland activity was observed in IF turkeys. This problem was extensively discussed in section 3.2 of this dissertation. Such prominent differences may be another sign of the G.A.S. since the mechanism is very complex and not fully understood. It is believed that the anterior pituitary gland releases increased amounts of the adrenocorticotropic hormone (ACTH) when bird is placed under stress conditions. ACTH causes increased production of glucocorticoid by the adrenal gland, which is the main reason of enlargement of this gland. Studies have shown that during stress conditions, the hypothalamus secretes a substance identified as a peptide - corticotrophin releasing factor (CRF), which is similar to vasopressin (Schindler, 1962). Any systemic stress acts on the hypothalamus via the central nervous system. Neurosecretory tissue of the hypothalamus releases CRF which in turn affects the hypophysis. An elevated level of ACTH is produced when the anterior pituitary gland is stimulated by CRF. The ACTH then induces overproduction of the glucocorticoid by the adrenal cortex. Glucocorticoid will affect the condition of the lymphoid tissue causing its involution and degeneration (Brown, 1967). It cannot be ruled out that the observed alterations of the thyroid gland function are not one of the effects of G.A.S. Moreover, it has to be stated that usually only a few symptoms of G.A.S. occur simultaneously, so one should not expect all of its symptoms in IF turkeys. The reaction of the organism to a stress usually depends on the type of stressor and on the amount of the ACTH synthesized. In milder stress conditions, the level of ACTH may be sufficient to increase the level of corticosterone but not high enough to enlarge the adrenal gland. Susceptibility of the bird to the stress is probably influenced by genetic factors according to the theory that it should be possible to select a line of animals that will be less susceptible to stress (Brown, 1967). This would also suggest that already existing lines and strains of birds react to the stress with different growth, feed conversion rates, sexual
behavior, reproductive performance, nervousness, etc. In case of IF hens, the stressful conditions may be for example, weather conditions such as temperature and humidity. An inadequate coverage of the skin by feathers will cause demand for more energy, as thinner layer of the plumage may not be sufficient to prevent thermal losses.

Several other difficulties have been revealed regarding egg production by IF females. These hens displayed several other characteristics that may be correlated with low egg production such as hesitation among IF females to enter nests. As mentioned earlier, the nests in the pens with IF hens had to be moved to the floor level. In previous years, serious difficulties were experienced during the laying season and the majority of hens with inhibited feather development were not able to use trap nests. These difficulties were attributed to the height at which the roosts and trap nests were mounted. In this experiment, nests were moved to the floor level, prior to housing of the hens. It was observed in the experiment that majority of the IF females were avoiding entering the nests despite nests were easily accessible. Even the hens that were laying eggs consistently, preferred not to lay eggs in the nests. Accidental entrances into a nest by the IF hen resulted in panic attacks of the animal. It is not clear at this point whether such a reaction should be explained by physiological or mechanical reasons. It is possible that the skin sensitivity may be different for IF and NF birds. The gene causing the impaired feather development may also influence development of nerve synapses in the skin of IF turkey. This, in turn may cause a bird to be more sensitive to the touch of the walls of the pen or of the trapping nest. High sensitivity of the naked skin as well as fear of the small dark place such as a trapping nest could be an explanation of problems experienced with floor eggs of those few IF hens that did laid eggs.

The problems of floor eggs and insensitivity of egg production have been reviewed (Appleby et al., 1983; 1986; 1989). Floor laying is generally considered a serious problem when flocks are housed in pens with nest boxes. In some flocks 30% of the total egg production are floor eggs (McGibbon, 1976). Moreover, the number of eggs produced on the litter is smaller when compared to the production of the birds in cages (Appleby et al., 1988). Nevertheless, none of the reasons suggested in the literature can account for such a big differences in egg production between the two lines of birds.
Serum alkaline phosphatase activity has been associated with the egg size and egg production. Lower activity of alkaline phosphatase has been associated with lower egg production in some reports (Gutowska et al., 1943; Banerjee et al., 1973), however, other authors imply the opposite effect (Rako et al., 1964; Singh and Balaine, 1983b). Phenotypic correlation between alkaline phosphatase and egg mass were estimated in chicken as negative high (Wilcox, 1963). In the line of IF turkeys, egg weight was significantly higher than that of NF hens. Moreover, the IF hens were delayed in egg production, finished egg production earlier, and eggs were laid less frequently. Significantly higher activity of alkaline phosphatase was found in the blood of IF hens (as discussed in section 2.2). Despite the average weight of an individual egg laid by IF hen was higher (Table 2.6), the egg mass was much lower than those of NF hens. One possible explanation of these results may be the that when eggs are laid less frequently, they spend more time in the oviduct absorbing larger amounts of egg components.

Low semen production can probably be attributed to stress factors similar to egg production. Stress may affect the function of the pituitary gland (Garren and Shaffner, 1956; Garren and Barber, 1955). Since the pituitary gland is one of the organs controlling the testes, variation in its functions may lead to impaired activity of the testes and consequently may halt or reduce production of the semen (Lofts and Murton, 1973). As discussed previously, stress may affect production of several hormones and result in various unpredicted effects. Excess of androgens causing regression of testicular activity in birds was elaborated by Lofts (1962). Interpretation of the low semen production in IF males may be only speculative at this point, since neither hormone activity measurements nor observations of testes development were performed. On the other hand, it was reported that the yield of semen collected by abdominal massage may be a hereditary characteristic. Birds that ejaculated low volume of the semen were not necessarily low semen producers and more semen might have been available in the distal region of the ducti deferentes (Cecil and Bakst, 1984). Further, Cecil and Bakst, (1986) found no differences the testosterone level of low and high semen producers if the semen was collected using abdominal massage method. It is then probable that IF males are less suitable for this method of semen collection. A simple explanation of this fact may be
the lack of the tail feathers. This prevents proper grasping of the birds tail and may cause the palm of the semen collector to slip.

Significant differences were observed only in the percent of fertility and hatchability, \( P = 0.03 \) and \( P = 0.05 \), respectively. None of the other hatching parameters measured in this experiment were found to differ significantly in IF and NF birds \( (P > 0.05) \). Very interesting, however, is the trend observed in all values. The percent of fertile eggs, number of embryos dead in the final stage of incubation \( (D_3) \), number of pipped eggs, and the number of successfully hatched poults seem to be slightly lower in eggs laid by IF hens. It is difficult to establish whether the significant difference in the size of the eggs could be the factor. Landauer (1967) reports some studies that suggests that the egg size can be associated with incubation results and that larger eggs may be characterized by reduced hatching quality. The above statement is true for eggs larger than the average for the particular hen and not necessarily higher that the flock average (Landauer, 1967). It is also probable that eggs of average size are laid by hens that are more fit for the reproductive purposes than females which produce larger eggs (Lerner and Gunns, 1952).
Chapter 3
Studies Regarding Improvement of Plumage of Inhibited Feathering Turkeys

3.1 Literature review

3.1.1 Role of methionine in animals

3.1.1.1 The importance of amino acids

Amino acids are one of the simplest but essential organic substances found in any living organism. Although more than 500 amino acids have been recognized in nature, only 22 are regularly found in proteins (Scott et al., 1984). After discovery of the oldest fossil microorganisms, it became apparent that α-amino acids existed on Earth about 3 billion years ago. The presence of amino acids was detected not only in living organisms. Alanine and glycine were found also in samples of the moon rocks, and precise chromatographic techniques allowed to confirm their existence in the meteorites (Hoppe and Martens, 1985). Amino acids were the original building blocks that lead to development of first grains of proteins.

The extremely long period of evolution from the particles of proteins to the living organism resulted in variety of different proteins synthesized in nature. It is estimated that living cells of various organisms can produce about $10^{11}$ different proteins. Despite tremendous work of several laboratories the sequence of only small portion is known so far. The investigation of the proteins and amino acids was initiated in 1806 when the first amino acid asparagine was isolated from asparagus juice and described by Vauquelin and Robiquet (Degussa, 1984). In 1923, methionine was isolated from casein as the twentieth amino acid (Mueller, 1923). This was followed by separation of threonine from fibrin in 1935 (Degussa, 1984).
3.1.1.2 General structure of amino acids

Amino acids are organic compounds that are diverse in the structure. They all, however, have one common feature - the presence of the amino group and the carboxyl group bound to one chiral carbon. Those two groups allow them to act as buffers in water solutions and make possible to exists in betaine form (structure of inner salt). With only one exception - glycine - all α-amino acids are chiral compounds, present in stereoisomeric forms D or L. Only L forms of amino acids can be incorporated into the proteins of the higher living organisms. The majority of animals usually cannot utilize the D form of an amino acid efficiently (Scott, et al., 1984) despite of the presence of the D-amino acid oxidases in their organisms (Degussa, 1984). Presumably, D-amino oxidases in evolutionary higher organisms exist only as a relict without serving any major role, or they may have protective role in detoxication from the bacteria (Hoppe and Martens, 1985).

The presence of the D-form of the amino acids is harmful for the animals, since D-amino acids can inhibit absorption of L-amino acids and may have other negative effects on amino acid metabolism (Linder, 1991). Small amount of D-amino acids have been detected in animal tissues, e.g. in mice, but typically D-amino acids occur in animal organisms only in rare cases. They can be found in bacterial cell walls (Degussa, 1984).

Among many D-amino acids, methionine seems to be the only exception from the above rule. An organism is capable to utilizing D-methionine by transforming it to L-methionine by process of oxidative deamination. The α-carbon is oxidized in presence of D-amino acid oxidase. Then the amine group is transferred from glutamine or asparagine to α-ketoacid and L-methionine is formed (Dibner, 1983). Also, branched amino acids may be utilized as amine donors (Gordon and Sizer, 1965). In theory, all D-methionine can be transformed into the L-form and then utilized by the cell. In practice, however, less than 100% of the D-form can be converted into the L-methionine (Degussa, 1989). Therefore, DL-methionine used as a feedstuff is slightly less effective than the L-methionine (Noll, et al., 1984).
Functions of any amino acid in the living cell, depends on its side chain structure. All amino acids can be classified according to their different side chain composition (Guthrie, 1971). Characteristics and functions of amino acids depend on the size and the character of the side chain. Therefore the usual classification of amino acids is based mainly on the type of the side chain. We can distinguish neutral, acidic and basic amino acids, present in proteins and peptides in living organisms. Four groups of neutral amino acids are distinguished. Those with aliphatic side chain are: alanine, glycine, isoleucine, leucine, and valine. Aliphatic neutral hydroxy amino acids are serine and threonine. There are three sulphur containing amino acids: cystine, cysteine and methionine. Group of neutral imino acids consists of proline and hydroxyproline. Basic amino acids are lysine, arginine and histidine. Two acidic amino acids and two their hemiamides are aspartic acid and asparagine, and glutamic acid and glutamine. Finally three aromatic amino acids has been found so far: phenylalanine, tyrosine and tryptophan (Hoppe and Martens, 1985).

### 3.1.1.3 Nonproteinogenic amino acids

Many of the nonproteinogenic amino acids are rare and can be isolated only in microorganisms and in some plants. Some of them, however exists in abundance in any organism performing diverse functions. They may be present either in a free form or bound with substances other than proteins. A good example of such an amino acid is β-alanine present in pantothenic acid and in coenzyme A. The main product of the thyroid gland - thyroxine is another example of a nonproteinogenic amino acid present in living organisms. Several amino acids used as antibiotics, such as cycloserine, penicillamine (D), and azaserine, are produced by fungi. A nonproteinogenic amino acid - homocysteine - is an intermediate product between the synthesis of cysteine from methionine. It differs from cysteine in the number of carbon atoms present in the chain. Homocysteine is also present in several fungi. Some of the nonproteinogenic amino acids
may be toxic and can inhibit some stages of synthesis of the amino acids in the organism (Degussa, 1984).

Generally, the nonproteinogenic amino acids are similar to the proteinogenic amino acids in their physical and chemical characteristics such as solubility, thermal stability and electrical conductivity (Degussa, 1984).

### 3.1.1.4 Essential and not essential amino acids

Generally, only microorganisms and plants are capable of synthesizing all necessary amino acids. Animals and humans have to rely on availability of many of the amino acids from the feed, however some of the amino acids may be synthesized in their organisms. Escher in 1876 formulated the first hypothesis on the essential amino acids. Amino acids that can be synthesized by the animal from those available in the feed are considered as nonessential, whereas those that have to be included in the diet are declared as essential (Guthrie, 1971). Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine cannot be produced by most of the animals and are designated as essential. Their absence or inadequate proportion leads to reduction of the value of the dietary protein. Two other amino acids which usually can be synthesized in some animal organism but whose production is limited are arginine and histidine. They are semiessential for turkey, broiler, and layer but essential for the young chicken, rat and cat. The number of limiting amino acids depends greatly on the main source of protein in the diet (Robel, 1977; Dunkelgod and Winkelman, 1982). Dunkelgod and Winkelman (1982) indicated that a significantly reduced concentrations of amino acids in blood of the turkey poult's can be an indicator of inadequate supply of limiting amino acids in their feed. In some specific conditions cystine, glycine, tyrosine, and proline may became essential as well (Degussa, 1984). The essential amino acids limit the utilization of the protein as a building substance and therefore limit the value of the protein. The mechanism of the limitation can be illustrated as a dam on the river that is composed of segments each of them only as high as the amount of each of the essential
amino acids. The dam is only as effective as is the height of the lowest element. The amino acid with the lowest concentration is indicated as limiting, and only a proportional amount of the dietary protein is utilized for body protein synthesis. The remainder is used mainly as a source of energy (National Research Council, 1994).

The first two limiting amino acids in the poultry are methionine and lysine and usually the feed for the poultry is supplemented with synthetic forms of both of these amino acids (Scott et al., 1984). Only methionine can be used in its DL-form since it is the only amino acid that can be utilized in D-form. Supplementation of limiting essential amino acids allows the animal to better utilize proteins in the diet.

3.1.1.5 Importance of methionine

After 117 years since the discovery of the first amino acid methionine was isolated from the casein (Mueller, 1923). Soon after its structure was determined.

Methionine is usually the first limiting amino acid in the feed for animals. Its presence in the diet is critical because methionine functions are not limited to being only one of the "building blocks" for protein synthesis. Methionine is serving as the starter amino acid of all protein chains via its derivative N-formyl methionine. Therefore it is essential in any protein synthesis even if it is not a part of the particular protein chain. In case of methionine deficiency, the synthesis of all tissue proteins is affected but what is even more important the enzymes and protein hormones production may be influenced as well. This may explain the significance of the methionine and all possible effects that may be observed in organisms lacking this only, single amino acid.

Methionine can influence the content of the other very critical amino acid containing sulphur - cysteine. Besides serving as one of the components of the structural proteins, cysteine performs a special function in the formation and stabilization of the spatial structure of the protein. Lack of cysteine in a protein chain affects the secondary, tertiary, and quaternary structure of the protein. The spatial image of the protein is extremely important for the proper function of the structural proteins as well as for
appropriate enzymatic function. In most cases the catalytic function of an enzyme relies on the proper spatial configuration of the apoenzyme and any alteration of this configuration will affect the effectiveness of the enzyme. Cysteine can be very easily synthesized in the organism from methionine via S-adenosyl methionine, homocysteine and cystathionine, therefore methionine deficiency will affect the amount of cysteine available for the organism (Scott et al., 1984). Since methionine cannot be synthesized from cysteine, feed supplementation with the former is more important and more effective than with the latter.

Another function of methionine in a living organism is its participation in the biosynthesis of non-protein compounds, such as adrenalin, creatine, and choline. It supplies methyl groups for several metabolic processes such as lipid metabolism in the liver (Linder, 1991). Supplementation of the methyl group could be taken over by choline, but on the other hand choline cannot perform any of other functions of methionine (Tsiagbe et al., 1992). Therefore close attention has to be paid to the proper level of this critical amino acid (Scott et al., 1984).

Methionine plays an extremely important role in processes of detoxication of aflatoxins that may be present in the feed. It is an important component of the glutathione - a tripeptide responsible for red-ox processes in the liver. The results of several studies performed on broilers indicate that harmful effect of aflatoxin and mycotoxin on the growth of the birds can be reduced significantly by methionine present in the diet (Degussa, 1989). Toxic effects of the tannin present in sorghum on the performance of layers and broilers can be decreased by increased amount of the DL-methionine in the feed (Degussa, 1989).

3.1.1.6 Factors influencing requirements of dietary methionine in poultry

The requirements for methionine or any other essential amino acid depend on several factors. Age and the growth rate of the animal are the two most important elements. The environmental conditions also play a role. Presence of heavy metal ions
or aflatoxin in the feed may alter the requirements for the methionine substantially (Degussa, 1989). Genetic factors cannot be excluded while evaluating requirements for methionine. Metabolic differences were observed between breeds of chicken by McDonald (1957; 1958). The results of the experiments indicated major differences between Australorps and White Leghorns in the conversion rate of methionine to cystine (McDonald, 1957; 1958). Variations between the same strains of birds in the conversion of methionine to cystine were observed later as well (Miller et al., 1960). Major differences in the metabolism of methionine were also found between vent-sexed and feather-sexed chickens indicating that feather-sexed birds are more sensitive to the level of the dietary methionine than vent-sexed chicks (Tsiagbe et al., 1992). The lines of chicken selected for low and high content of fat in the carcass differ in the requirements for the methionine as well. Lean chickens require higher concentrations of the sulphur amino acids than birds from the fat line, however part of this difference could be explained by lower feed intake in leaner birds. Birds from the fatty line utilize sulphur amino acids less efficiently than those from the lean line (LeClercq et al., 1993).

Birds with longer growth periods demand quite different levels of methionine from those with fast growth. Among all poultry species, the turkey requires the highest level of sulphur amino acids. The required levels are so high that they cannot be fulfilled by using natural feedstuffs only. Therefore, turkey feed usually includes synthetic methionine since the effectiveness of such a supplementation has been established (Donovan et al., 1955) and confirmed by others (Pepper and Slinger, 1955; Fergusson et al., 1957).

3.1.2 The thyroid

3.1.2.1 Development and the structure

The thyroid gland originates embryonically from the primitive gastrointestinal tract as a thickening of the epithelial floor of the pharynx. It is a bilobed structure located
on the lateral surface of the trachea. It is one of the endocrine organs that serves exclusively this function. The thyroid gland is surrounded by an external capsule of connective tissue. Below that capsule, an internal, true capsule is located. The internal capsule divides the tissues of the gland into several lobes and lobules (Lesson et al., 1985b). The gland consists of a great number of spheroidal follicles which vary in size between 20 and 250 um. Each follicle is surrounded by a single layer of epithelium (follicular cells). The type of epithelium depends on the age of the animal, on the phase of the gland activity, and can be anything between squamous and high cuboidal or even columnar (Lesson et al., 1985a; 1985b). In an active thyroid gland, follicular cells become more columnar and the lumen of the follicle is smaller. In the inactive stage, thyroid follicles become enlarged with distended colloids since there is no utilization of the colloid. The epithelium surrounding the follicles is stretched and of the squamous type. The follicular cells are polar and secrete products only into the follicle. They are typical secretory cells with well developed organella. Their extensive profile consists of rough endoplasmic reticulum and a large Golgi apparatus in the cytoplasm, which allows synthesis of substantial amounts of protein and transport into lumen of the follicle. The cell membrane between the interior of the follicular cell and the follicle is characterized by the presence of several microvilli which increase the cell surface and provide better cell product transport into the follicle. The interior of the follicle is filled by a colloid which contains thyroxin. The colloid is clear and viscous. The irregularity and the color of the colloid depend on the activity of the gland. In active follicles, the colloid stains basophilic with the standard H&E stain and several vacuoles may be present. Inactive follicles stain weakly basophilic or even acidophilic (Lesson et al., 1985b).

The other type of epithelial cell within the mammalian thyroid gland are called parafollicular cells also known as light, clear or C cells. They are larger than follicular cells and are not in contact with the follicle’s lumen. The nucleus is located centrally and the cytoplasm contains numerous membrane-bound dense granules. The function of C cells is to synthesize calcitonin (Lesson et al., 1985b; Lissitzky, 1990). In birds the parafollicular cells are separated from the thyroid gland (Astier, 1980; Hodges, 1981).
A small number of reticular, collagenous, and elastic fibers can be identified within the tissues of the gland.

The thyroid gland is highly vascularized and nourished by capillaries laying in close contact with the bases of follicular cells. The average blood flow is 4 to 6 ml/min/g of the tissue (Lissitzky, 1990) but the structure of the blood vessels indicates that there are fluctuations in the amount of blood supplied to different regions of thyroid (Lesson et al., 1985b).

The innervation of the thyroid suggests that regulation of the gland’s function may be achieved by the neural system. Several unmyelinated nerve fibers (vasomotor in function) are present in the walls of the thyroid arteries. Sympathetic fibers terminate at the bases of follicular cells, and may have direct effect upon the follicular cells affecting the thyroid function (Lesson et al., 1985b).

### 3.1.2.2 Thyroid hormones

All substrates necessary for synthesis of thyroid hormones such as amino acids and carbohydrates, are transported to the follicular cells with blood. Thyroglobulin is a high molecular weight glycoprotein synthesized in ribosomes of rough endoplasmic reticulum of the follicular cells. Synthesized thyroglobulin is immediately packaged into apical vesicles and extruded to the lumen of the follicle. All further stages of synthesis take place in the follicular lumen. In the apical surface of the follicular cell, iodine is bound to thyrosyl residues in thyroglobulin (Ekholm and Wollman, 1975). Synthesized monoiodotyrosine and diiodotyrosine are further combined to form the active hormones: thyroxine ($T_4$), and triiodothyronine ($T_3$) (Lissitzky, 1990).

**Mechanism of thyroid hormone secretions.** The secretion of the thyroid hormones is triggered as a response to pituitary thyrotropin (TSH) secretion. A negative feedback control mechanism is responsible for triggering synthesis of TSH. A decrease in thyroid hormone level in the circulating blood is detected by neurosecretory neurons in the hypothalamus. The hypothalamus secretes the thyrotropin releasing hormone
(TRH) which acts on the hypophyseal portal circulation. This in turn triggers the synthesis of TSH which then binds with specific receptors on the basal surface of the follicular cell in thyroid gland and increases the reaction rate of thyroid hormones biosynthesis and secretion (Carayon et al., 1979; Lissitzky, 1980). TSH also binds to the apical surface of the follicular cell acting on the microvilli. Increased levels of TSH in the circulation initiate rapid elongation of microvilli on the apical surface of the follicular cells. The microvilli phagocytize some of the colloid fluid and enclose it within the vacuole (Dumont, 1971). The content of the vacuole is fused with lysosomes and then $T_3$ and $T_4$ are activated and released into the blood system. Thyroxine or $T_4$ in the blood is transported as a complex of the hormone and protein bound to albumin and to three globulin fractions. $T_3$ is also transported as a protein-hormone complex bound to albumin and one globulin fraction. The cessation of the thyroid hormone synthesis occurs as a response to the increase of the level of thyroid hormone within the system. The pituitary thyrotropin secretion diminishes at the same time, allowing microvilli to shorten and rest.

3.1.2.3 Functions of the thyroid hormones

Generally the thyroid hormones act upon many different tissues in the organism except the brain tissue in adults (Lissitzky, 1990). Thyroxine and triiodothyronine are responsible for an increased metabolic rate (Lissitzky, 1990) and for all processes that lead to increased glucose levels in the blood in order to maintain the high metabolic rate such as gluconeogenesis, glycolysis, and glucose absorption from the intestine. The thyroid hormones also manage stimulation of protein synthesis and may increase lipid metabolism by activation of lipoprotein lipase. They also act on cholesterol metabolism allowing its conversion to bile acids stimulate the heart rate as well as blood flow. Finally, they also induce neuronal development and stimulate neural transmission (Capen, 1993).

Generally, it is very well known that the thyroid gland affects the basal metabolic rate. It mobilizes glucose to achieve the satisfying level for the increased metabolism.
Due to thyroid action, glycolytic reactions are accelerated and the rate of the gluconeogenesis is increased. The absorption of glucose from intestines is enhanced as well. The other domain of metabolic reorganizations occurs in the protein and lipid metabolism (Leak, 1970). The protein synthesis is more rapid and the rate lipolysis increases when the thyroid hormones levels rise (Capen, 1993).

The ratio of protein synthesis and the metabolism regulated by the action of the thyroid gland affect a large number of processes that require them for proper performance. Reproduction, feather or hair growth and development, neural transmission, cerebration and neuronal development in young animals depend greatly on the proper level of thyroid hormones (Capen, 1993). Any deviations of the activity of the thyroid gland from the level required in a given stage of development lead to gross abnormalities in the affected organism.

The participation of the thyroid hormones in metabolism regulation and particularly in the protein synthesis plays a major role in growth and maturation of the organism. Reproductive performance of birds depend on the correct action of the thyroid gland and its products. Thyroid action is very complex and differs not only between species but also between sex and even the age of the bird. Thyroid hormones are crucial for induction and maintenance of egg production in turkey hens however, the effects of thyroidectomy vary between hens in distinct phases of egg production (Lien and Siopes, 1989a). Thyroid hormones are also necessary for testes development and are required for semen production in some birds (Snapir et al., 1982) even though they are not necessary for water fowl (Jalleageas and Assenmacher, 1979).

The effects of the thyroid hormones on the organism’s growth and differentiation have been well investigated (Lissitzky, 1990). Although the early development of the embryo is independent and controlled only by genes, the later stages of prenatal as well as postnatal growth depend greatly on the proper function of the thyroid gland. This gland affects mitotic activity and cell populations and influences growth and differentiation of the skeleton, lungs, endocrine glands (Cody, 1980).

Proper development of the central nervous system requires the presence of thyroid hormones as well. Numbers of neurons in the cortex, myelinization processes, and
vascularization of the brain are affected by a thyroid deficiency. In extreme deficiency cases, growth of neurites and formation of microtubules is imperfect and cretinism may develop (Fellous et al., 1979).

3.1.2.4 Malfunction of the thyroid gland

Nearly all symptoms of thyroid malfunctions occur during increased or depressed hormone production.

**Hyperthyroidism.** The stage of enhanced thyroid synthesis is called hyperthyroidism. It is believed that hyperthyroidism manifests itself in populations with a genetic predisposition for the disorder (Lissitzky, 1990) although another cause may be exposure to excess iodine, such as contact with iodine containing disinfectants (Russel, 1977). The characteristic signs of the disorder may include: nervousness, palpitation, sweating, increased sensitivity to heat, weight loss, weakness, tachycardia, and goiter (Williams, 1946; Ingbar, 1985). Extremely high levels of circulating thyroid hormones which coincide undetectable levels of TSH may be an indication of the disease.

**Endemic goiter.** An increased volume of the thyroid gland with the corresponding elevated TSH level and somewhat lower concentration of thyroid hormones in the circulation are typical for this disorder. Overproduction of thyroglobulin and its storage in follicles are the causes of increased gland size. The causes of endemic goiter are well known and involve dietary deficiency of iodine (Lissitzky, 1990).

**Simple goiter.** The indication of this disorder is an increased size of the thyroid gland. The pathogenesis of simple goiter remains unknown. The histopathological picture of the gland is characterized by the presence of different types of follicles - active and not active which are congregated in separate nodules. It is probable that epithelia of different types are of polyclonal origin and differ in metabolic properties (Studer and Ramelli, 1982).

**Hypothyroidism.** There are several causes of glandular hypofunction. Genetic deficiency in iodine transport or other defects in the metabolic pathway of the synthesis
of the active thyroid hormones are a few between many. Some of the hypothyroid stages occur in pituitary malfunctions and insufficient stimulation by TSH. Characteristic signs of hypothyroidism include skin lesions, decreased ovulation, edema of face and eyelids, weight gain, memory impairment, cretinism, goiter (Lissitzky, 1990).

3.1.3 Zinc

3.1.3.1 Zinc as the ion

Zinc is a metal ion with an atomic weight of 65.37, an atomic number of 30, and has the symbol Zn. There are 13 isotopes of zinc, five of them with the numbers of 64, 66, 67, 68 and 70 which are stable. The other eight are radioactive with half-life ranges from 1.48($^{61}\text{Zn}$) to 245 days ($^{66}\text{Zn}$). Zinc is a bluish-white, shiny metal, and a fair conductor of electricity. At room temperature zinc is rather brittle and between 100 and 150 C it is malleable. Zinc has numerous applications in the modern civilization, however only a fraction of its unusual electrical, thermal, optical, and chemical properties have been investigated and determined (Weast et al., 1987).

3.1.3.2 Zinc in the history of the medicine

The medical use of zinc have been well documented since pre-Christian times. At that time, preparations containing zinc oxide were used for treatment of burns, egzemas, and other skin irritations. Calamine and zinc oxide ointment are reported in Papyrus Ebers (1931). In the eighteenth century, zinc was used for the successful treatment of epilepsy and other neural disorders (Hoogenraad, 1984) as well as alleviating symptoms of vaginal infections. Treatment with zinc was used to reduce the symptoms of beriberi disease in China. This was probably effective because the level of the zinc in epidermal appendages of beriberi patients is reduced by half (Eggleton, 1939).
3.1.3.3 Genetic mutations affecting zinc availability

**Adema disease.** This disorder was described in the Danish Black Pied cattle breed. The gene affecting absorption of zinc is autosomal and recessive. Homozygous recessive calves appear unaffected at birth but the condition progresses with age and the animals die before the age of four months. Several lesions found through clinical and histological examinations such as alopecia, hyperkeratosis of the skin, and cutaneous infections indicate death due to a severe zinc deficiency even though the level of zinc in the diet appears to be normal. Zinc supplementation above the level required for the non-affected animal reverses the changes in the organism and allows survival (Andresen et al., 1973).

**Lethal milk mutation.** This genetic disorder is autosomal and recessive. Females homozygous for this allele produce milk in which the zinc level is reduced by 34%. At the same time the volume of the milk produced is lower than in normal females maintained under the same conditions. The offspring of affected females die within 5 to 10 days if nursed by mutant females. Those affected exhibited stunted growth and acute dermatitis. Offspring of affected females nursed by normal females do not exhibit the condition. Offspring of non-affected females nursed by mutants display all the lesions characteristic for the malady (Piletz et al., 1987). A significant reduction of zinc in the liver in mice fed milk produced by mutant females has been observed (Lee et al., 1993). The most probable cause of death was low mean plasma zinc level in pups nursing on abnormal females through the first 10 days of the life.

**Acrodermatitis enterophathica (AE).** This rare genetic disorder in which zinc absorption is affected (Cunnane, 1988) is due to a single autosomal recessive gene. Organisms homozygous for the recessive allele display acute symptoms shortly after weaning from the breast milk. The condition is characterized by low zinc plasma levels. The first symptoms are alopecia and diarrhea. Neural signs include mood changes, color blindness and lack cone vision (Mortimer et al., 1984; Moynahan, 1976). The immune system is affected as well by reduced cell mediated immunity (Chandra, 1980; Endre et
The lesions that develop may include dermatitis of the extremities and perioral, perianal and genital regions. The condition is generated by insufficient absorption of zinc from the diet (Atherton et al., 1979). The alteration of zinc excretion in the disease seems to be uncertain (Cunnane, 1988). The condition is lethal if not treated. There are two kinds of remedy known to improve the disease. One is didoquin (diiodohydroxyquinoline), which enhances zinc absorption (Aggett, 1989). The other method is supplementation of the diet with zinc, which seems to be successful by increasing the total zinc available (Atherton et al., 1979).

**Lethal acrodermatitis of bull terrier dogs.** This autosomal recessive trait causes parakeratotic skin lesions and retarded growth in homozygous dogs. An impaired immune system does not protect animals from infections and affected individuals have higher incidences of respiratory infections. Behavioral changes have been reported in affected dogs. The pathogenesis of the disease is not recognized however reduced serum alkaline phosphatase level was observed in afflicted animals (Jezyk et al., 1986).

### 3.1.3.4 Zinc and enzymes activity

In living organisms, zinc is a catalytically active metal ion present in more than 300 metalloenzymes and metal-enzyme complexes. In metalloenzymes, the zinc atom is bound rather firmly to the structure. In metal - enzyme complexes, the zinc is bound more loosely (Forbes, 1984; Vallee and Falchuk, 1993). Enzymes with zinc incorporated into their structure are involved in various biochemical reactions and play major roles in protein, carbohydrate, and fat anabolism and catabolism. They also take a part in coordination of RNA and DNA synthesis. Generally, zinc has three functions in zinc enzymes: catalytic, coactive, and structural (Vallee and Falchuk, 1993). In the catalytic interactive form, the zinc ion is an active element participating in the reaction catalyzed by the enzyme. Inactivation of the enzyme can be achieved by removing the zinc ion by chelation. In some cases, zinc ions can be replaced by other metal ions. The activity of the enzyme is usually affected. Enzymatic activity is completely abolished in
aminopeptidase (Haeggström et al., 1990). Zinc metalloenzymes with cobalt inserted in
the zinc site retain their activity but the magnitude of the enzyme's activity can be
increased or decreased (Vallee and Falchuk, 1993). Examples of the enzymes with
catalytic types of zinc function are: alcohol dehydrogenases (Brändén, et al., 1975;
Chhabra and Arora, 1993), aspartate transcarbamylase (Honzatko et al., 1982),
carboxypeptidase A, B and DD (Rees et al., 1983; Dideberg et al., 1982), alkaline
phosphatase (Kim and Wyckoff, 1989), RNA polymerase (Gierdoc and Coleman, 1986)
and carbonic anhydrases I and II (Kannan et al., 1975; Liljas et al., 1972).

Another category of zinc sites is called coactive when more than one zinc ion is
required in an enzyme activity. One zinc ion has previously described as a catalytic
function. Additional zinc ions (or other metals) assist in forming a ligand bridge between
two metal ions by an amino acid (Vallee and Falchuk, 1993). Alkaline phosphatase has
two metal ion forming bridges - zinc and magnesium (Kim and Wyckoff, 1989).

The third role of zinc in enzymes is a structural function. In such a case, zinc is
required for the structural stability of the enzyme and does not play major role in the
enzymatic reactions. It simply stabilizes the quaternary structure of the protein. These
quite rare structural zinc atoms have been found in a few enzymes by X-ray
crystallographic analysis: alcohol dehydrogenase (Brändén et al., 1975), aspartate
transcarbamylase (Honzatko et al., 1982), RNA polymerase (Gierdoc and Coleman,
1986), and protein kinase (Hubbard et al., 1991). In some enzymes such as collagenses,
the inactive structural form of the zinc ion may be activated and form an active,
functional site of the enzyme. The enzyme is synthesized as zymogen, an inactive form
and is activated when needed. In collagenase, the zinc ion is activated by removing of
the "activation peptide" with cysteine. In inactive form of the enzyme, this amino acid
attaches to the zinc atom with an SH group and blocks its catalytic properties.
Dissociation of "activation peptide" changes structural zinc to the catalytic form (Van

The activity of zinc containing enzymes can be affected by zinc depletion.
Lieberman and Ove (1962) observed DNA inhibition when zinc ions formed chelates with
ethylenediamine tetraacetate (EDTA). The effect of zinc inadequacy can have diverse
effects and depend on the place from which the enzyme was collected. Activity of alkaline phosphatase from intestine and serum decreases in zinc deficient rats (Kfoury et al., 1968; Reinhold and Kfoury, 1969; Kirschgessner et al., 1977). Reduced activity of this enzyme was also observed in bone and kidney (Hsu and Anilane, 1967), pancreas and thymus (Prasad and Oberleas, 1971), and testis (Oberleas and Prasad, 1969). The activity of alkaline phosphatase remained unaltered in striated muscle, lung and heart (Hubber and Gershoff, 1973), liver and brain (Hsu and Anilane, 1967). The effect of zinc depletion on the enzymes involved in nucleic acids metabolism is diverse. RNA polymerase (Sandstead, et al., 1971) and thymidine kinase (Prasad and Oberleas, 1974) activities were reduced in zinc deficient animals. The activities of ribonuclease acid from testes (Somers and Underwood, 1969) and kidney, thymus and bone (Prasad and Oberleas, 1973) was enhanced in the absence of zinc ions. Also, the activities of alcohol dehydrogenase of the liver, retina and intestines of goats supplemented with dietary zinc was significantly higher than in control animals (Chhabra and Arora, 1993). The activity of acid and alkaline deoxyribonucleases in testis, kidney, thymus and bone remained unchanged (Prasad and Oberleas, 1973). Since the activities of other zinc-enzymes may not always reflect low level of zinc in the organism, there are suggestions that primary zinc depletion is correlated with altered activity of alkaline phosphatase and zinc deficiency can be detected by measuring alkaline phosphatase activity (Cunnane, 1988; Weismann and Hoyer, 1985).

3.1.3.5 Role of zinc in proteins synthesis

There are two families of proteins that are known to contain zinc ions: metallothionein and gene regulatory proteins. Metallothionein is a protein of molecular weight 6300 - 6600 daltons. (Cunnane, 1988). The role of metallothioneins is not very well understood. It probably plays role in detoxification of heavy metals, stabilizes membranes, and regulates zinc and copper metabolism (Vallee and Falchuk, 1993). The amount of metallothionein synthesized in the intestine depends on the amount of zinc
present (Cousins, 1979). In severe zinc deficiency, metallothionein synthesis is halted and after zinc administration it can be detected immediately (Grider and Erway, 1986).

Some scientists have postulated that zinc proteins are a source of zinc ion for newly synthesized apoenzymes and may participate as regulator molecules in gene expression (Kägi and Schäffer, 1988; Kägi, 1991). Several nucleoproteins contain zinc atoms in their structure. It is postulated that zinc participates actively in nucleoprotein synthesis. In zinc deficient rats, thymidine incorporation into DNA is inhibited (Williams and Chesters, 1970). By increasing activities of ribonucleases in organisms deficient in zinc ions, the level of RNA is reduced (Somers and Underwood, 1969).

It is impossible to neglect the role of zinc in protein and nucleic acids metabolism. As a structural and catalytic component of enzymes, zinc has a dominant role in the amino acid and protein metabolism. Zinc deficiency increases oxidation of amino acids and the catabolism of proteins. The amount of methionine, lysine, leucine and glutamine oxidized in organisms deficient in zinc ions was significantly higher than in animals with normal consumption of zinc in the diet (Theuer and Hoekstra, 1966; Hsu et al., 1969). Cysteine was also affected by an insufficient level of zinc in the diet. Increased excretion of cysteine in the urine and decreased incorporation of this amino acid into skin proteins was found to be correlated to insufficient zinc in the diet (Hsu and Anthony, 1971). Surprisingly, cysteine metabolism in the liver and testes tissues was not affected (Hsu, 1976). Zinc deficiency may result in an altered protein content of the body. It has been demonstrated that acute zinc deficiency may also lead to reduced protein synthesis as well as elevated levels of protein degradation (Sandstead et al., 1972).

### 3.1.3.6 Interactions of zinc with vitamins and minerals

Zinc deficiency in a living organism affects biological activity of other essential nutrients such as vitamins, macroelements and trace minerals. One of the vitamins that is affected by insufficient zinc in the body is vitamin A. In many reports, animals depleted of zinc in the diet had lower levels of vitamin A and retinol-binding protein in
serum. The level of vitamin A in the liver was not altered, however the level of retinol binding protein in the liver was depressed (Smith et al., 1976). Supplementation of diet with zinc helps to transport vitamin A from the liver to the blood (Ette et al., 1979).

The interactions of vitamin D and zinc also involves calcium. It is known that increased calcium levels elevate the amount of zinc excreted. Supplementation with vitamin D prevented loss of zinc from the organism (Fleischman et al., 1968) but had no effect on zinc absorption (Abu-Hamdan et al., 1985).

Zinc and vitamin E are antioxidants that can stabilize membranes in a presence of free radicals (Bettger and O'Dell, 1981). Vitamin E deficiency symptoms are more acute in animals depleted of dietary zinc (Cunnane, 1988).

The symptoms of zinc deficiency can be similar to those caused by insufficient amounts of vitamin B₆ in the organism. Depletions of zinc and vitamin B₆ may cause dermal lesions, slowed growth, alopecia, hypophagia, and depression of the immune system (Cunnane, 1988). These similarities are the results of parallel associations with metabolism of essential fatty acids (Cunnane et al., 1984; Cunnane and Horrobin, 1985) and protein utilization (Son and Magee, 1986). Pyridoxal phosphate kinase is the enzyme that catalyze conversion of the inactive form of vitamin B₆ (pyridoxal, pyridoxin and pyridoxamine). It is activated by the zinc ions (Neary and Divan, 1970; Twomey and Baxter, 1973). The activity of this enzyme can be affected in zinc deficiency.

Similarity of symptoms between biotin and zinc deficiency have been studied in the rat. Growth retardation, dermatitis lesions and alopecia caused by zinc deficiency may be reduced by supplementing the feed with biotin (Alaoui et al., 1985).

The folic acid content in rat liver (Williams et al., 1973) and in human plasma (Milne and Gallagher, 1983) were reduced with zinc deficiency. Milne with coworkers (1984) observed increased zinc excretion when the diet was supplemented with folic acid while Ghishan with coworkers (1986) observed decreased absorption of zinc from diet with folic acid.
Zinc and vitamin C interactions in mature human have also been studied. Normal levels of vitamin C consumption had no significant effects on zinc absorption (Solomons et al., 1979).

Very interesting interactions have been found between zinc and metal ions. These interactions can be divided into two categories: antagonistic and competitive. Calcium is the example of the mineral that has an antagonistic correlation with zinc. Increasing calcium will increase zinc ion excretion (Plishker, 1984; Yamaguchi et al., 1981). Lesions, such as parakeratosis, which is usually associated with zinc depletion were observed in pigs fed high calcium levels suggesting impaired absorption of available zinc by calcium ions (Berry et al., 1966).

Sodium plays an important role in zinc absorption in the kidney. A sodium deficiency causes greater zinc excretion (Matustik et al., 1982). On the other hand, the abundance of zinc in the intestines decreases sodium absorption in humans (Steinhardt and Adibi, 1984) whereas zinc deficiency is associated with abnormally higher level of sodium (Bettger et al., 1981).

The interactions of zinc and copper are also well known in the literature (Bremner et al., 1976; Gipp et al., 1974; Hill et al., 1983). Excessive dietary supplementation with zinc affects serum copper (Sandstead, 1978). The interaction of copper and zinc influence the metabolism of essential fatty acids. Zinc stimulates the metabolism of linoleic acid to arachidonic acid, therefore excess zinc can lead to reduction of the arachidonic acid and increased amounts of oleic acid. A paucity of zinc will cause elevation of arachidonic acid and reduce oleic acid (Cunnane, 1981; 1982; 1988). Low iron concentration in the liver and tibia, and low hematocrits have been associated with high levels of zinc in diets fed to chicks (Pimentel et al., 1992).

3.1.3.7 Role of zinc in humeral regulation

The effects of zinc on the biological activity of hormones is well established (Cunnane, 1988). The following examples illustrate the complex relationships of zinc and
several of the hormones as well as the intricate control of zinc metabolism by hormones. The relationships are very complex and often they are not fully understood (Henkin, 1980).

High concentrations of zinc were found in extracts of pituitary gland (Cunnane, 1988); corticotropin and adrenocorticotropic hormones (Kocsis et al., 1953), and the examination of crystalline structure of insulin has revealed that it also contains zinc (Scott, 1934; Scott and Fisher, 1935). Zinc may affect the action of various hormones: steroids and polypeptides. Blood levels of adrenal steroids tend to become elevated when there is a zinc deficiency (Quarterman, 1974). On the other hand, elevated levels of adrenal steroids in the blood, whether in a disease such as Cushing’s disease (Henkin, 1974) or by the injection of the hormone in healthy rats (Lui et al., 1986) caused decreased zinc serum and increased urine zinc excretion (Henkin, 1974; Jacob et al., 1984). Meager concentration of adrenal steroids in the blood caused plasma zinc to increase (Henkin, 1984). Effects of zinc concentration on the level of testosterone were also investigated. Serum testosterone was lower and serum LH and FSH higher in zinc deficient rats than in normal animals (Lei et al., 1976). Low levels of testosterone were also observed in children with low serum zinc and significantly increased testosterone concentrations were obtained after zinc treatment (Favier, 1992a). Zinc deficiency affects the pituitary gland and its release of pituitary gonadotrophins rather than the gonads themselves (Henkin, 1976). In vitro observations of bovine pituitary extracts incubated with large amounts of zinc resulted in elevated levels of GH, TSH, LH, FSH and ACTH (LaBella et al., 1973). Testosterone injections increased serum zinc levels in immature rats (Millar et al., 1957) and depression of zinc in serum of hypophysectomized is observed if no testosterone injections are provided (Henkin, 1980). In another study performed on patients with overproduction of GH, decreased levels of circulating GH after hypophysectomy have been associated with increased serum zinc concentration. The level of zinc in the urine decreased significantly. Low serum zinc levels with high amounts of the ion in the urine were observed in patients treated higher doses of GH (Henkin, 1974).
Administration of estrogen has no influence on the serum and urine content of the zinc ions in the rat (Sato and Henkin, 1973). However, decreased serum zinc concentrations have been reported in humans after oral estrogen treatment (Briggs *et al.*, 1971). Large amounts of progesterone may lower serum zinc concentrations in rats (Sato and Henkin, 1973) and humans (Halsted and Smith, 1971), the mechanism of this relationship however is not very well known (Henkin, 1980).

Zinc can form a complex with insulin in the imidazole and N-terminals of the insulin molecule (Summerell *et al.*, 1965; Tanford and Epstein, 1954). If zinc ions are abundant, they may inhibit the time of insulin activity and prolong the hypoglycemic phase. Zinc also affects release of insulin from the Islets of Langerhans (Maske, 1957) and it has been reported that rats deficient in zinc display an increased rate of insulin degradation (Henkin, 1980).

Elevated amounts of parathyroid hormone in the blood may induce increased urinary excretion of zinc. In contrast, surgical treatment of hyperparathyroidism leads to a decrease in urinary zinc as a side effect of low parathyroid hormone levels (Mallette and Henkin, 1976) which may be explained by increased bone turnover associated with an elevated parathyroid hormone level. Bone is one of the main zinc storage sites in the body and with increased turnover of this tissue, zinc ions are released to the urine (Jowsey, 1966; Riggs *et al.*, 1972; Underwood, 1971).

Reduced thyroid hormone levels observed in hypothyroidism have also been associated by increased zinc concentrations in blood cells (Weatherall and McIntyre, 1967) and decreased levels of zinc in serum, saliva (Swaminathan *et al.*, 1976) and in the brain (Weatherall and McIntyre, 1967). Low serum zinc levels are responsible for decrease in plasma thyroxine, however sometimes the thyroid hormone level may remain unchanged in low zinc serum children (Favier, 1992a). Hypothyroidism has also been observed in relation to decreased red blood cell zinc (Pangaro *et al.*, 1974; Swaminathan *et al.*, 1976).
3.1.3.8 Role of zinc in reproduction processes

Zinc has a major role in growth and reproductive functions of an organism. Several studies established that zinc deficiency may lead to decreased testicular activity. Deviations from the normal ratio of plasma zinc to zinc present in testicular tissue may be a cause of testes malfunction (Ribatto et al., 1994). Low reproduction in cattle has been attributed to scarcity of zinc and the condition was successfully treated with orally administered zinc (Singh and Balaine, 1994). Ballady rabbits fed a diet deficient in zinc display a significant reduction in the levels of hyaluronidase, alkaline phosphatase, acid phosphatase, lactic dehydrogenase, sialic acid, protein, and Zn in testicular tissue and the epididymis. The significant reduction of the level of glycerol-phosphoryl choline in the epididymis is a strong evidence of inhibited epidymial secretions. Additionally, cholesterol and glycogen levels were found elevated. These alterations in testicular biochemistry suggest an inhibition of the testes function (Eltohamy and Younis, 1991). Testicular hypoplasia may result from several factors many of which have not been identified yet, however current literature suggests that zinc deficiency is one of the probable causes (Ladds, 1993). Retardation of testicular development and function in ruminants in pasture areas deficient in zinc has also been observed (Ladds, 1993) and deformed seminiferous tubules of the testes were found in goats fed low dietary zinc (Chhabra and Arora, 1993).

3.1.3.9 Influence of zinc level on central nervous system

A sufficient level of zinc is necessary for normal cellular growth and in particular for normal brain development. One role of zinc, which is critical for brain function and development, is stabilization and preservation of cell membranes. Zinc prevents peroxidation of unsaturated fatty acids in the phospholipid fragment of the membrane. Peroxidation may result in cell membrane damage which in turn may alter the cellular transport (Toppel, 1973). Zinc is also present in the nerve growth factor (Patteson and
Dunn, 1975) which is responsible for dorsal root ganglion and sensory neurons (Mobley et al., 1977). Brain is characterized by one of the highest concentrations of zinc in the organism (Harrison et al., 1968) with significant amounts present in the nerve endings of the hypothalamus and cerebellum (Zelkowitz et al., 1980). With age, the level of zinc in the brain decreases (Harrison et al., 1968).

3.1.3.10 Zinc deficiency

Several reasons for zinc deficiency are described in the literature. Based on the review by Vallee and Falchuk (1993), cases of zinc deficiency may be categorized based on the requirements versus availability in the diet, with genetic factors as a separate category. The first group contains situations of zinc deficiency caused by inadequate intake or absorption with zinc requirements not being altered. A typical example is a low dietary zinc concentration or the presence of agents in the diet that bind zinc and prevent its absorption (phytases). Competition with other metals present in the diet (Ca, Cu, Fe, Pb) may also prevent proper zinc absorption from the feed. Alteration of the digestive system (such as intestinal surgery) may disrupt normal dietary absorption even when excess zinc is present in the feed. Chronic diarrhea diseases and those diseases associated with malabsorption, such as enteritis, cystic fibrosis, or disaccharide malabsorption result in decreased absorption of zinc as well as other nutrients.

Another class of zinc deficiency includes cases when the organism demands more than the standard requirements. Some drugs such as penicillamine, or skin injuries (burns) increase the organism’s zinc requirement and if no additional zinc is provided, may lead to rapid development of zinc deficiency symptoms.

A separate group of zinc deficiency cases is induced by genetic disorders. Acrodermatitis enterophatica, adema disease, lethal acrodermatitis, and lethal milk syndrome are widely known examples and are described in section 3.1.2.3.

Another classification of zinc inadequacy is into primary and secondary zinc deficiencies (Cunnane, 1988). Primary zinc deficiencies are of dietary origin and are
caused by low zinc content in the food or by inadequate food consumption even if the dietary zinc concentration not lowered. Often low food or feed consumption is associated with low zinc content in the foodstuff.

A secondary zinc deficiency may be induced by malabsorption of zinc available in the diet. Inadequate zinc absorption is a leading cause of secondary zinc depletion. The possible causes include presence of an abundance of competitors or failure of zinc transport. Other factors inhibiting absorption of zinc such as other metal ions, arginine, histidine or phytic acid may also adversely affect zinc levels in the organism. The blood transport system is a major component in zinc metabolism. In the blood, zinc ions are transported by albumin and α2 macroglobulin. If the concentrations of these two major proteins responsible for zinc transport are low (hypoalbuminemia), symptoms of zinc deficiency develop (Foote and Delves, 1983; Giroux et al., 1976). Histidine and cysteine in the blood may bind the zinc not already bound to the transport proteins and may lower the level of zinc available (Cunnane, 1988). Increased zinc excretion may be another source for secondary zinc depletion. Muscle catabolism associated with starvation (Henry and Elmes, 1976), trauma (Larson et al., 1970), alcohol consumption (Mills et al., 1983), renal diseases (Cunnane, 1988), muscular dystrophy (Jackson and Edwards, 1982) and several others may lead to increased excretion of zinc ions and provoke zinc deficiency.

The foundations of primary or secondary zinc depletion may be similar: disease, genetics, injuries and drugs, but in the case of a primary zinc depletion, the symptoms are much more acute than in secondary zinc deficiency. Signs of zinc depletion are numerous. Tissues that undergo rapid turnover and enhanced proliferation are the most sensitive to zinc deficiency. Therefore, gonadal, dermatological and neural tissues are particularly vulnerable to zinc depletion (Vallee and Falchuk, 1993).

Two of the most obvious dermal lesions in zinc deficiency are hyperkeratosis and parakeratosis in mammals (Anderson et al., 1967; Follis et al., 1941) and hyperkeratosis without or with moderate parakeratosis in birds (O'Dell et al., 1958; Wight and Dewar, 1976). Thinner subcutaneous tissue, alopecia, may be observed in zinc deficient rats (Follis et al., 1941). Parakeratosis of lambs and calves, alopecia, fragile wool, hoof
deformation and modified keratin hardening were observed in zinc depleted animals (Mills et al., 1967). Parakeratosis associated with abundance of phytic acid in the feed has been observed in swine. Lesions were located on the limbs, around eyes, ears, snout, scrotum and tail, and the severely affected animals had the whole skin surface affected (Kernkampp and Ferrin, 1953). In humans, erosion of the skin, hyperkeratosis, or alopecia are common results of zinc deficiency. One of the symptoms is loss of hair and nail atrophy (Vallee and Falchuk, 1993). Experimentally induced zinc deficiency in dogs resulted in emaciation, keratitis, scaling around the eyes, lips and nose, eroded skin in areas exposed to friction or contact with hard surfaces (Yager and Scott, 1993).

Reproductive systems are also affected in cases of zinc deficiency. Retarded secondary sexual development or hypogonadism in juvenile males, and infertility of adult males was associated with low serum zinc level induced by low zinc in the diet. Milder zinc deficiencies may cause oligospermic sterility and impotence in human and rats (Favier, 1992b). Young male sheep respond to low zinc in the diet by decreased masses of epididymal and testicular tissues and retarded seminiferous tubules. The capacity to produce testosterone was reduced in zinc depleted animals (Martin et al., 1994b). Alaskan Malamute males with zinc deficiency exhibit delayed sexual maturity and 45% of their spermatozoa contain acrosomal defects (Palmer, 1993).

In females, some sexual problems were associated with zinc deficiency. Mild zinc inadequacy results in a high number of morphologically and cytogenetically degenerated ovocytes (Vormann et al., 1986). Cessation of ovulation due to anomalies in the estrogen cycle caused by zinc deficiency was observed in rats (Swenerton and Hurley, 1968) and rabbits (Shaw et al., 1974). Prolongation of duration of successive reproductive cycles was exhibited in zinc deficiency together with an increased rate of resorption of follicles in ovary in rats (Bedwal and Bahuguna, 1994). Malfunction of the ovary in hen was also affected by zinc depletion (Breeding et al., 1992). Low zinc in production hens caused an abrupt cessation of eggs laid since zinc is responsible for the synthesis of yolk precursors (Mitchell and Carlisle, 1991).

Zinc deficiency affects the central nervous system. Behavioral disorders including inability to learn and memorize can be manifested and emotional stability is influenced
as well by zinc scarcity (Shagal, 1980; Vallee and Falchuk, 1993). Zinc deficiency can
result in mental depression, lethargy, emotional problems, tremor, and cerebellar ataxia,
which can be reversed by dietary zinc supplementation (Zelkowitz et al., 1980). In the
acute zinc deficiency, the onset of mental changes is very rapid and it involves sleepiness
and lethargy, or euphoria, nervousness and hallucinations. The normal behavior is
usually restored after supplementation of diet with zinc (Zelkowitz et al., 1980).

3.1.3.11 Zinc toxicity

Although zinc is one of the heavy metals, it is not as toxic as lead, mercury or
copper and is characterized by a large margin of safety. The toxic level of zinc in the
diet is much higher than the recommended dozes. Toxic concentration of zinc in the
poultry diet is estimated as 2000 ppm in the chicken and 4000 ppm in the turkey
(National Research Council, 1994). Moderate overdoses of zinc usually are neutralized
by homeostatic mechanisms. In case of large overdoses, renal failure may be the major
cause of death (Brocks et al., 1977). Chronic toxicity symptoms may include fever,
chills, gastroenteritis, and reduced growth if the excess zinc is introduced through
respiratory tracts - inhaled (Papp, 1968). Dietary consumption of excess zinc reduces
growth, causes anemia, and adversely affects reproduction Van Reen, 1966).

3.2 Effect of methionine on feather development in IF turkeys

3.2.1 Introduction

As a first attempt to improve feathering in IF turkeys through dietary intervention,
feed supplementation with methionine was investigated. It was thought that feathering
may be inhibited due to disturbances in the process of keratinization (fundamental for the
feather growth). Keratinization may depend on the level of amino acids supplied in the
diet. Thus, close attention was directed to the biochemistry of keratinization and in particular to amino acids that participate in it.

Keratinization that occurs in epithelial cells may be considered specific form of cell differentiation. During keratinization, the germinal epithelial cells which are metabolically active become filled with a horny substance called keratin (Matoltsy, 1960). Germinal cells that are located in the nail, claw, hoof, beak or tooth give rise to only one specialized type of cell specific for a given skin appendage. On the other hand, cells located in the root of hair, wool, quill and feather differentiate into various specialized cells and as a result form a complex structure consisting of several types of cells that differ in the keratin content and type (Matoltsy, 1962). Keratinization processes that occur in the hair are a good example of diversity within that process. The cuticle of the hair produces only an amorphous (granular) form of keratin whereas cortex cells synthesize the fibrous type of keratin exclusively (α-keratin). The inner sheath cells of the hair follicle root and other specialized epithelial cells are capable of synthesizing both types of keratin. In that situation a fibrous material is produced and the amorphous keratin is deposited between the fibers (Rogers, 1959). Keratins from different parts of the skin appendages differ in their sulphur content. The amorphous keratin of hair cortex is high in sulphur whereas the granular keratin of the hair follicle contains only trace amount of sulphur (Matoltsy, 1962). Content and configuration of "sulphur rich" and "sulphur-poor" components of the epithelium and epithelial appendages determine the physical and chemical properties of the appendages. The high-sulphur α-keratins contain large amounts of sulphur amino acids especially cystine (Gillespie, 1962, 1965). The sulphur content in keratins is not constant, and may depend on the animal species, and the tissue type (Lundgren and Ward, 1963).

Sulphur content of wool may fluctuate seasonally (Gillespie, 1965), and may oscillate with age (Fisher et al., 1981). It can also be changed by infusion of sulphur amino acids into the sheep’s abomasum (Reis and Schnickel, 1963, 1964) or by diet supplementation with sulphur amino acids (Reis, 1965). When sulphur amino acids or protein with high contents of these amino acids are fed to the sheep, a substantial increase in growth rate of wool is observed (Gillespie, 1991). On the other hand, a
deficiency of methionine and lysine may weaken the fleece and inhibit wool growth (Reis, 1986; Yager and Scott, 1993). Increased concentration of sulphur in keratin was highly correlated with the rate of wool growth (Reis, 1965). The only exception for this correlation was observed when wool growth declined in hypophysectomized sheep. The surgically altered animals displayed symptoms of inadequate thyroxin production and retarded wool growth. Unexpectedly, the content of high sulphur proteins increased despite of the halted wool growth. This can be explained by an increased ratio of dietary sulphur amino acid to weight of wool produced. After treatment of hypophysectomized animals with thyroxine the proportion of the high sulphur protein to wool weight returned to the pre-surgery levels (Gillespie, 1965).

The relative distribution of sulphydryl and disulfide groups varies between different fragments of skin appendages. In the hair follicle, the upper portion of the bulb and up to one third of the hair shaft are characterized by high concentrations of sulphydryl groups, while in the distal part of the cortex no free sulphydryl groups are formed (Barnett and Sognnaes, 1962). The distribution of disulfide groups is reversed there, that is the regions that are rich in sulphydryl groups are almost devoid of disulfide groups. On the other hand, abundance of disulfide groups is observed in the distal region of the hair shaft (Barnett and Sognnaes, 1962). The distribution of sulphydryl and disulfate groups can differentiate the keratogenous zone (high in sulphydryl groups) from the keratinized zone (high in disulfide bridges).

The keratinization processes of the avian epidermal tissue and its appendages has not been investigated as thoroughly as that of mammalian tissues. Most of the information provided here is adopted from experiments involving other groups of animals. The available data, however, suggest that the basic processes of keratinization in mammals and birds are similar and differences exist mostly in the arrangement of the keratinized cells within feather versus hair (Harrap and Woods, 1964). The distribution of sulphydryl and disulfate groups in mature feathers was studied and revealed the existence of structures similar to α-keratin in mammal hair. The distribution pattern of different fractions of keratin, however, was more complicated in the feather than in the mammalian hair (Harrap and Woods, 1964). Sulphydryl groups are present in all portions
of the feather shaft in similar amounts, therefore the keratogenous zone cannot be
determined in similar manner as in hair (Barrnett and Sognnaes, 1962). This may indicate
that the keratinization processes in the feather lasts longer than in the hair, and that the
histochemical aspects of keratinization may differ in the time frame among different skin
appendages, such as hair and feather (Van Scott and Flesch, 1954; Barrnett and

It is very well documented that dietary requirements for sulphur amino acids
differ between genetically distinct strains of birds. Lean chicken require higher dietary
concentration of sulphur amino acids than fat birds (LeClercq and Guy, 1991; LeClercq
et al., 1993). Feather-sexed slow feathering lines of chickens differ in their requisites for
methionine and cystine (Moran, 1981). Such differences were also found between
feather-sexed and vent-sexed strains of laying hens. The response of slow feathering
female chickens to elevated levels of methionine in the diet was an increase in egg
production, as opposed to normal hens in which methionine supplementation had no
effect (Tsiagbe, et al., 1992). Sexual dissimilarity in response to the amount of sulphur
amino acids in the diet was also investigated. Slow feathering females require higher
levels of methionine and cystine than slow feathering males (Moran, 1984).

Methionine is one of the most often utilized sulphur amino acid additives. An
avian organism can utilize methionine to synthesize other sulphur amino acids.
Methionine, after transformation to homocysteine can be utilized to synthesize cysteine.
Subsequently, cystine is formed from cysteine after two sulphydryl groups are oxidized
to form a disulfide bond. These reactions cannot be reversed. Therefore, methionine can
replace cystine in the diet but requirements for methionine cannot be met with cystine
(National Research Council, 1994).

The metabolism of methionine is very complex and it has more functions than in
protein synthesis only. It acts as a methyl group donor and it participate in formation of
inorganic sulphur compounds. Methionine functions in bile salt formation and in
synthesis of mucopolysaccharides (Nesheim, 1975). Methionine is also exceptional among
the other amino acids by its role in initiating newly synthesized peptide chains after
conversion to S-adenosylmethionine (Regina et al., 1993).
It is known that proteins are the major components of the feather - 89-97% (Fisher et al., 1981). Despite differences in the protein composition of the feathers between different avian species (Harrap and Woods, 1967), proteins of the epidermal tissues are generally rich in sulphur amino acids especially cystine (Hsu and Anthony, 1971). Therefore, it is reasonable to expect that the sulphur amino acid content of the feed should play an important role in feather growth. Since IF mutant turkey in the current study develop fewer feathers and the quality of the feather produced is extremely poor and the fact that sulphur amino acids may be involved in feather development, it is reasonable to study the effect of dietary d,l-methionine supplementation on the feather development in Inhibited Feathering turkeys. Since genetic variation in nutrient requirements exists between genetically different animals, finding such modification in the IF mutant is anticipated. This variation may serve as an explanation of other physiologic anomalies that distinguish IF turkeys from those with normal plumage. Therefore, the objective of this study was to determine the effect of methionine supplementation in the diet for IF turkeys on the feather growth at various ages of birds.

3.2.2 Materials and methods

Two experiments were conducted to evaluate the influence of dietary methionine supplementation on the feather growth ratio in two stages of the turkey growth.

3.2.2.1 Experiment 1. The effects of dietary methionine in the brooding period

Experiment 1 involved supplementation of dietary methionine for turkey poults from day-old to 8 weeks of age. Poults used in Experiment 1 were offspring from NF hens of the normal feathered line of Wrolstad Medium White Turkey. Hens were artificially inseminated with pooled semen from several IF heterozygous turkey toms. Twenty four IF poults and twenty four NF poults were randomly selected from a group of day-old birds, wingbanded and randomly assigned to the treatment groups. During the
eight weeks of this experiment, poult's were housed in two pens, in a ventilated brooder house under standard OSU brooding conditions for poult's. Each pen contained twelve NF and twelve IF poult's. The poult's were fed either the control (Control) or experimental (Methionine-1) turkey diets ad libitum together with free access to water. The Control diet was the standard turkey starter containing 28% CP, 2804 ME/kg and 0.54% of methionine. The treatment diet (Methionine-1) was formulated by supplementation of the Control diet with an additional 0.55% of DL-methionine and contained 28% CP, 2789 kcal/kg, and 1.09% methionine (Table 3.1).

Two measurements were performed on the poult's used in this study. The first was the classifications of the feather development in the IF poult's. The feather classification on IF poult's were performed at 56 days of age. Four feather classes corresponding to scores 1 to 4 were used in the experiment. A score of 1 was applied to birds with normal feather development. The best feathered of IF poult's had an assigned feather score of 2, Figure 3.1. A score of 3 was given to poult's with an average feather development, Figure 3.2, and a feather score of 4 was applied to IF poult's with poor feather development, Figure 3.3. The influence of the dietary methionine in the diet on the feather development in IF poult's was estimated as the average score of the plumage in the experimental group and compared to the similar value in the control group. The score of the bird's plumage was evaluated by the same person. Prior to the feather evaluation, all poult's were combined in one pen to prevent bias.

The second measurement was the weight gain of the NF and IF poult's from hatch and to 56 days of age. All results were interpreted using analysis of variance - ANOVA (Snedecor and Cochran, 1965).

3.2.2.2 Experiment 2. The effects of dietary methionine in the growing period

Three dietary levels of methionine in the turkey grower diets were fed to growing turkeys from 12 to 20 weeks of age (Experiment 2). Seventy-two twelve-week-old poult's were used. The birds were obtained from the cross of normal feathered Wrolstad
Table 3.1 Composition of Control-1 and Methionine-1 diets fed to IF (Inhibited Feathering) and NF (Normal Feathering) poults from day-old to 8 weeks of age

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control-1</th>
<th>Methionine-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, Yellow (8.9% CP)</td>
<td>49.25</td>
<td>48.70</td>
</tr>
<tr>
<td>Soybean meal (47.5% CP)</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Meat and bone meal (50% CP)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Fish meal (65% CP)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate (16% Ca, 21%P)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.10</td>
<td>0.65</td>
</tr>
<tr>
<td>Trace mineral premix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Coban-60 premix (sodium monesin 132 g/kg)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Calculated analysis:**

<table>
<thead>
<tr>
<th></th>
<th>Control-1</th>
<th>Methionine-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>28.04</td>
<td>28.45</td>
</tr>
<tr>
<td>Met. Energy, kcal/kg</td>
<td>2804</td>
<td>2786</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>0.54</td>
<td>1.09</td>
</tr>
<tr>
<td>Methionine and Cystine, %</td>
<td>0.95</td>
<td>1.49</td>
</tr>
</tbody>
</table>

<sup>1</sup> Supplied per kilogram of diet: vitamin A (retinyl acetate), 4950 IU; vitamin D<sub>3</sub>, 1650 ICU; vitamin E (dl-α-tocopherol acetate), 1.65 IU; vitamin B<sub>2</sub>, 8.25 µg; riboflavin, 4.95 mg; niacin, 33 mg; d-pantothenic acid, 4.95 mg; menadione bisulfite, 0.825 mg; folic acid, 330 µg; choline chloride, 330 mg; and ethoxyquin, 93.6 mg.

<sup>2</sup> Supplied per kilogram of diet: Mn, 60 mg; Zn, 20 mg; Fe, 20 mg; Cu, 2 mg; I, 1.2 mg; Co, 0.2 mg.
Figure 3.1 Comparison of the feather development in an eight week old IF turkey with a feather score of 2 (left) and a NF turkey with a feather score of 1 (right)

Figure 3.2 Comparison of the feather development in two eight week old IF turkeys with a feather score of 2 (left) and with a feather score of 3 (right)
Figure 3.3 Comparison of the feather development in two eight week old IF turkeys with a feather score of 3 (left) and with a feather score of 4 (right)
Medium White females and IF heterozygous males. The three diets were isonitrogenous and isocaloric except for the methionine levels. The Control, Methionine-2, and Methionine-4 diets contained 0.3%, 0.62%, and 1.22% methionine (Table 3.2). Prior to the experiment, poults were fed turkey starter and grower feeds (National Research Council, 1984).

A feather score improvement index (FSII) and weight gain were determined at 12 and at 20 weeks of age. The feather scores were obtained by the same person to whom the identity, that is, wingband numbers and the treatment group membership of the birds was unknown so that the objectivity of the feather grading could be maintained. The values used for determination of the influence of the dietary methionine supplementation on the performance of poults were calculated individually for each poult according to the following formula:

\[
FSII = FC_1 - FC_2
\]

FSII - Feather Score Improvement Index  
FC\(_1\) - Feather score at the beginning of the trial  
FC\(_2\) - Feather score at the end of the trial

Note that to obtain a positive improvement index when the feather score improves, the final score is subtracted from the initial score. This is because a higher score corresponded to poorer feather quality, so that improvement is observed if the score decreases. The FSII obtained were statistically evaluated using analysis of variance - ANOVA (Snedecor and Cochran, 1965). A linear regression model was fit to the experimental data.

3.2.3 Results

3.2.3.1 Results of Experiment 1

The results of the Experiment 1 are summarized in Table 3.3. Supplementing of the Methionine-1 diet with additional methionine (2 x 0.55%), which was twice the
Table 3.2 Composition of Control-2, Methionine-2 and Methionine-4 diets fed to IF poults from 12 to 20 weeks of age

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control-2</th>
<th>Methionine-2</th>
<th>Methionine-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, Yellow (8.9% CP)</td>
<td>77.44</td>
<td>77.14</td>
<td>76.54</td>
</tr>
<tr>
<td>Soybean meal (47.5% CP)</td>
<td>12.25</td>
<td>12.25</td>
<td>12.25</td>
</tr>
<tr>
<td>Fish meal (65% CP)</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>Meat and bone meal (50% CP)</td>
<td>5.08</td>
<td>5.08</td>
<td>5.08</td>
</tr>
<tr>
<td>Fat (blended, animal)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Dicalc. phosph. (16% Ca, 21% P)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.04</td>
<td>0.34</td>
<td>0.94</td>
</tr>
<tr>
<td>Trace mineral premix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Calculated analysis:**

<table>
<thead>
<tr>
<th></th>
<th>Control-2</th>
<th>Methionine-2</th>
<th>Methionine-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>16.84</td>
<td>16.87</td>
<td>16.93</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3132</td>
<td>3132</td>
<td>3133</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>0.32</td>
<td>0.62</td>
<td>1.22</td>
</tr>
<tr>
<td>Methionine and Cystine</td>
<td>0.58</td>
<td>0.88</td>
<td>1.47</td>
</tr>
</tbody>
</table>

<sup>1</sup> Supplied per kilogram of diet: vitamin A (retinyl acetate), 3630 IU; vitamin D<sub>3</sub>, 1210 ICU; vitamin E (dl-α-tocopherol acetate), 0.121 IU; vitamin B<sub>12</sub>, 6.05 μg; riboflavin, 3.63 mg; niacin, 24.2 mg; d-pantothenic acid, 6.05 mg; menadione bisulfite, 0.605 mg; folic acid, 242 μg; choline chloride, 242 mg; and ethoxyquin, 68.6 mg.

<sup>2</sup> Supplied per kilogram of diet: Mn, 48 mg; Zn, 16 mg; Fe, 16 mg; Cu, 1.6 mg; I, 0.96 mg; Co, 0.16 mg.
National Research Council recommendation (National Research Council, 1994) resulted in significantly ($P = 0.03$) reduced feather growth in IF line poults (Figure 3.4) during the first 8 weeks of growth when compared to the IF poults fed the Control diet (Figure 3.5). The average feather score of the IF poults fed control diet was 2.9 and was 0.6 lower than the average feather score for IF poults fed diet supplemented with methionine.

The elevated dietary methionine fed to the IF turkeys significantly reduced the weight gain ($P = 0.04$) when compared to IF poults fed the Control diet. The weight gain of poults fed the Control diet was 1.74 kg per bird and was 0.25 kg higher than those birds fed the Methionine-1 diet. Similar effect of Methionine-1 diet on the growth of NF poults were also observed. Turkeys fed the Control diet gained 0.22 kg more per bird ($P = 0.05$) than those fed Methionine-1.

### 3.2.3.2 Results of Experiment 2

Table 3.4 presents results for Experiment 2. Supplementation of the Control diet with 2 and 4 times the methionine level (0.62% and 1.22% respectively) did not affect feather development in IF line turkeys during the 8 weeks of experiment (from 12 to 20 weeks of age). FSII values in the control group of IF birds were 0.35 and did not differ ($P > 0.05$) from FSII of birds fed Methionine-2 (FSII = 0.10), or from FSII of birds fed Methionine-4 (FSII = 0.36).

Increasing dietary methionine fed to IF birds did not affect weight gain in the same period of development ($P > 0.05$). The average weight gain in the IF line turkeys fed the Control-2 diet was 8.47 kg per bird. The mean values for the weight gain in a line of IF poults fed Methionine-2 and Methionine-4 were 9.02 kg per bird and 8.01 kg per bird, respectively (Table 3.4). For both FSII and weight gain attempts to fit nonlinear regression models did not result in statistically significant models.
Figure 3.4 Feather development in two IF turkeys at 8 weeks of age fed the Control diet containing 0.54% methionine

Figure 3.5 Feather development in two IF turkeys at 8 weeks of age fed the Methionine-1 diet containing 1.09% methionine
Table 3.3 Feather score and body weight gain in IF (Inhibited Feathering) and NF (Normal Feathering) poultcs fed diets containing 0.54% and 1.09% methionine from day-old to 8 weeks of age (Experiment 1)

<table>
<thead>
<tr>
<th>Phenotype / Dietary methionine</th>
<th>Feather Score</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF / 0.54%</td>
<td>2.9&lt;sup&gt;A&lt;/sup&gt; 0.2</td>
<td>1.74&lt;sup&gt;C&lt;/sup&gt; 0.86</td>
</tr>
<tr>
<td>IF / 1.09%</td>
<td>3.5&lt;sup&gt;B&lt;/sup&gt; 0.2</td>
<td>1.49&lt;sup&gt;D&lt;/sup&gt; 0.32</td>
</tr>
<tr>
<td>NF / 0.54%</td>
<td>2.07&lt;sup&gt;E&lt;/sup&gt;</td>
<td>0.67</td>
</tr>
<tr>
<td>NF / 1.09%</td>
<td>1.85&lt;sup&gt;F&lt;/sup&gt;</td>
<td>0.75</td>
</tr>
</tbody>
</table>

<sup>A B</sup> differences significant only for IF turkeys (P = 0.03)
<sup>C D</sup> differences significant only for IF turkeys (P = 0.04)
<sup>E F</sup> differences significant only for NF turkeys (P = 0.05)

Table 3.4 Feather score improvement index (FSII) and weight gain in IF (Inhibited Feathering) poultcs fed diets containing 0.32% (control), 0.62% and 1.22% methionine from 12 to 20 weeks of age (Experiment 2)

<table>
<thead>
<tr>
<th>Dietary methionine</th>
<th>FSII</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEM</td>
<td>kg</td>
</tr>
<tr>
<td>0.32%</td>
<td>0.35</td>
<td>8.47</td>
</tr>
<tr>
<td>0.62%</td>
<td>0.10</td>
<td>9.02</td>
</tr>
<tr>
<td>1.22%</td>
<td>0.36</td>
<td>8.01</td>
</tr>
</tbody>
</table>
3.2.4 Discussion

Dietary deficiencies of several amino acids may not cause specific symptoms and may be easily misinterpreted for other dietary problems. Depigmentation of the plumage caused by inadequate amounts of lysine, tyrosine or phenylalanine may also be an indicator of scarcity of folic acid, copper or iron in the feed. Similar symptoms may be present in surgically or chemically thyroidectomized birds (Grau et al., 1989). Complex metabolism of methionine which is involved in synthesis of proteins implies that various possible symptoms of methionine deficiency may be also be nonspecific (Nesheim, 1975). In addition to this, dissimilar responses to certain levels of methionine observed in several studies involving genetically different birds make the interpretation difficult (McDonald, 1958; Abbott et al., 1962; Leclercq et al., 1993). Aggravated feather growth and reduction of weight gain observed in Experiment 1 of this study of IF poults fed the Methionine-1 diet suggest that the methionine was not the first limiting amino acid in the poult’s diet from day-old to 8 weeks of age. Similar results were observed in studies involving dietary methionine utilization by scaleless chickens (Abbott et al., 1962). These chickens with affected plumage were not sensitive to a low dietary methionine level. The same birds, however, gained less weight when fed 1.09% dietary methionine. The reduced weight gain in that study was considered a symptom of methionine toxicity (Abbott et al., 1962). Since methionine is toxic if consumed in excess (Ueda et al., 1981), it is reasonable to assume that reduced weight gain attained by scaleless birds was caused by an excess of methionine which in turn was an indirect result of excessive consumption of feed to maintain the body temperature by featherless birds (Nesheim, 1975). It was also suggested that a reduced level of methionine in the diet for slow feathering chicken may be more appropriate than methionine supplementation. Comparison of the effects of methionine supplementation Single Comb White Leghorn and Australorp diets indicated that in slow feathering lines (Australorps) less methionine was converted to cystine than in normal feathering chickens (Miller et al., 1960). These implications seem to be legitimate if the difference in the feather production is taken into consideration. IF poults used in experiments as well as scaleless
and slow feathering chickens developed less feathers, therefore they probably need to convert less methionine to cystine. A surplus of methionine consumed but not utilized by the bird may undergo chemical transformations to other compounds which may be detrimental to the bird (Regina et al., 1993). Consequently, lower levels of methionine seem to be more appropriate for birds with impaired feather development. These results are contrary to conclusions of a study by Harms and Buresh (1987) that should increase methionine in poults' diets from day-old to 3 weeks of age resulted in improved body gain or where addition of methionine improved feather growth and alleviated toxic results of copper (Christmas and Harms, 1979). It is difficult to infer the reason for such differences between results of different experiments. Nevertheless, one has to take into consideration that the availability of methionine in feeds may not be similar. In addition, age and strain of the birds may also play an important role in the process of methionine utilization (Hess et al., 1962).

In this study, amino acids in the Control diet were balanced according to National Research Council requirements (National Research Council, 1994) unlike amino acids in the Methionine-1 diet containing 1.14% of methionine. The results of supplementing the diet of IF poults with methionine were similar with the results obtained for the NF turkeys, both exhibited reduced weight gain. Therefore, it may be concluded that the mutation responsible for inhibited feather development in IF poults did not significantly affect methionine metabolism. The reduced growth ratio with supplemental methionine (Experiment 1) is probably the result of an imbalanced diet due to supplemental d,l-methionine. Other studies involving diets with imbalanced amino acids provided similar results to those described herein. Abnormal structure of proximal parts of the remiges was observed in chickens fed excess leucine, isoleucine, glycine, phenylalanine, and tyrosine (Robel, 1977). Decreased protein content of feathers and lowered cysteine level in feathers resulted from feeding chickens a diet deficient in valine (Farran and Thomas, 1992). Similar effects were achieved by feeding excess levels of leucine. In addition to the altered cysteine content of feathers, feather morphology was affected and feathers were more fragile (Pentz and Kratzer, 1984).
The negative effects of the methionine supplementation for IF and NF poults (0 to 8 weeks of age) were probably a result of methionine toxicity. Methionine, despite being nutritionally essential for the bird can be poisonous if fed in excess and in an imbalanced diet. Usually, chickens regulate the consumption of methionine and of other critical substances by reducing feed consumption. Chickens fed low protein diet (10% CP) and with high amounts of methionine can regulate feed consumption drastically to prevent overdosing of the methionine. If feed consumption remains unchanged (such as in force feeding), deaths increase significantly (Ueda et al., 1981). In such a situation, a high level of methionine may result in accumulation of toxic catabolites, mostly S-adenosyl-L-methionine, S-adenosyl-L-homocysteine, and decarboxylated S-adenosyl-L-methionine in the liver of rats fed high methionine diets (Regina et al., 1993)

Older turkeys with inhibited feathering were less sensitive to 0.62% and 1.22% methionine supplementation (Experiment 2) than younger poults in Experiment 1. Young, fast growing poults with rapid metabolism are more vulnerable to an inferior feed ration than the older poults from 12 to 20 weeks of age.

The results of these experiments demonstrated that elevated methionine levels in diets for turkeys with IF gene did not influence feather development as was expected. The proper amount of methionine in the diet for IF poults from day-old to 8 weeks of age is significant for feather development as well as for the weight gain. Since the supplementation of increased levels of methionine for IF poults did not improve feather development, other dietary factors that may enhance feather growth of IF turkeys will be considered in the following chapters.

3.3 Thyroid gland and its function in feather development of IF turkeys

3.3.1 Introduction

Thyroid hormone participation in the regulation of the metabolism and especially in the protein synthesis plays a major role in growth and maturation of animal or human
organism. Reproduction, feather or hair growth and development, neural transmission, cerebration and neuronal development in young animals depend greatly on the proper level of thyroid hormones (Capen, 1993). Any deviations of the thyroid activity from the level required in a given stage of development may lead to gross abnormalities in the affected organism. For example, reproductive performance of birds depends on the thyroid gland and its products. The thyroid’s activities are very complex and vary between species, sexes and age. Proper thyroid gland function is crucial for inducing and sustaining egg production in turkey hens, however the effects of thyroidectomy may vary between hens in distinct phases of egg production (Lien and Siopes, 1989a). Proper thyroid function is also necessary for testes development and semen production in some birds (Snapir et al., 1982) however it is not required in water fowl (Jalleageas and Assenmacher, 1979).

The effects of the thyroid hormones on the growth and differentiation of the embryonic tissue as well as in postnatal life have been thoroughly investigated (Lissitzky, 1990). Although the early development of the embryo is independent of thyroid hormones and regulated only by genes, the later stages of prenatal as well as postnatal growth of an organism depends greatly on proper functioning of the thyroid gland. Thyroid hormones affect mitotic activity and cell population, and influence growth and differentiation of the skeleton, lungs, and endocrine glands (Cody, 1980).

Proper development of the central nervous system requires the presence of thyroid hormones as well. Thyroid deficiency may adversely affect the number of neurons in the cortex, myelinization processes, and vascularization of the brain. In extreme cases of thyroid hormones deficiency, growth of neurites and formation of microtubules is imperfect which may trigger development of cretinism (Fellous et al., 1979).

The role that thyroid gland products play in feather formation through regulation of the metabolism and in particular protein synthesis was studied. The thyroid gland has a major role in feather formation by the regulation of the metabolism and especially by regulation of protein synthesis.
The regulatory influence of the thyroid gland on the development of hair has been long investigated. The first supporting facts were strictly empirical. The Papyrus Ebers, the most famous and the longest of the ancient medical documents describes the methods that ancient Egyptians used to prevent baldness and to restore the lost hair. This is what one of the interpreters of the Papyrus Ebers, Dr. H. Joachim, comments on the fragments relating to the restoration of the hair:

Not quite so revolting are the several remedies which aim at once at preserving the hair and removing grey hairs. The Blood-of-a-Black-Calf, cooked in Oil, figures in one such; the same of a Black Cow in another; while the third is composed of Tortoise-shell and the Neck-of-the-gabgu-bird, cooked in Oil. Whether these Ancient Egyptians were in any way cognizant of the selective action of the Thyroid Gland on the hair, or whether indeed the gabgu-bird possessed a Thyroid at all, the fact remains that they must have imagined they had found some specific for hair-growth in the neck of this unknown bird; for in another transcription we find 'Blood-from-the-Neck-of-the-gabgu-bird’ not only recommended, but backed up by a word-picture of the fortunate user...

(Papyrus Ebers, 1931).

Early experiments with hormonal preparations revealed that large doses of dried thyroid glands fed to birds resulted in faster molting and the development of replacement feathers (Voitkevitch, 1966). Presently, it has been proven that the thyroid gland plays an important role in feather development and some of the thyroid disorders are known to cause feather abnormalities in birds. Oral supplementation of thyroxine is used to induce molt in captive birds (Blackmore and Cooper, 1982).

It is a well established fact that the thyroid gland affects the basic metabolic rate by mobilizing glucose for the increased metabolism. As a result, glycolytic reactions are accelerated, the rate of the gluconeogenesis is increased, and the absorption of the glucose from intestines is enhanced. The other domain of influence of thyroid gland hormones on metabolic processes is in protein and lipid metabolism (Leak, 1970; Pant and Chandola-Saklami, 1993). When the thyroid becomes more active, protein synthesis
becomes accelerated and the rate of lipolysis increases (Capen, 1993). During feather development or feather growth in the phase associated with rapid protein synthesis, the levels of oxidative processes rise. Oxygen consumption increases with the level of thyroid hormones but is influenced to a higher degree by triiodothyronine (T₃) than thyroxine (T₄). The correlation coefficient between the level of T₃ and oxygen consumption is estimated to be between +0.19 and +0.78, while in the case of T₄ the correlation is between -0.63 and +0.19 (Bobek et al., 1977). The highest level of oxygen consumption is observed during first two weeks after hatching - a period of rapid growth and feather development. The level of T₃ raises slowly until the second week of life, when it reaches its peak. The level of T₄ rises slowly from the day of hatch until four weeks of age and then may fluctuate greatly (Bobek et al., 1977). With this information in mind it is possible to use measurements of T₃ and T₄ as indicators of oxygen consumption which in turn may be useful in interpretation of ratio of metabolism in organism.

The first symptoms of the thyroid malfunction can be noticed in the development and growth of hair or feather follicles (Ebling et al., 1991). Thyroid hormones are necessary for the proper development of secondary wool follicles in neonate lambs (Ferguson et al., 1956) and their higher levels may result in an increase in hair length and density in adult sheep (Ferguson et al., 1965). Similar results were obtained in rats where the growth rate was increased following thyroxine supplementation (Hale and Ebling, 1975). Thyroid function during feather development was thoroughly investigated by many researchers. It was determined that major differences may be observed in activity of the thyroid between species of birds that hatch with down fully developed (chicken) and those with sparse feathers at hatching (pigeon) (Voitkevich, 1966). Surgically or chemically thyroidectomized young birds do not develop feathers, however after administration of thyroid hormones the feathers develop in affected birds although they are not perfect (Williams, 1946; Mellen, 1958; Voitkevich, 1966; Cherry and Savage, 1974) which proved the involvement of the thyroid gland in feather development. In birds with poor feather development the tissues of the thyroid gland are barely differentiated with no signs of thyroid activity in the microstructure of the gland. All the gland morphology changes may be associated with the level of feather follicle activity.
including the metamorphosis of the follicle microstructure. Changes in the activity of the thyroid gland are also reflected in the morphology of the organ (Voitkevich, 1966, Pietras et al., 1983). The thyroid tissue that initiates production of hormones and becomes active, undergoes major changes. The follicular cells, surrounding a follicle are high cuboidal or even columnar and the follicle itself is rather small. The inactive gland tissue consists mainly of large follicles surrounded by follicular cells that are of squamous type (Voitkevich, 1966).

Based on the preceding information, a research hypothesis was formed regarding IF turkeys and their thyroid development. Thyroid activity may be lower in IF turkeys and dietary thyroxine supplementation may offset this effect and increase feather growth. To verify these hypotheses two experiments were performed involving IF turkeys. The changes of the thyroid activity at select ages were examined and the effect of dietary supplementation of the thyroxine on the feather growth of IF turkeys was analyzed.

3.3.2 Materials and methods

Correlation between the level of the thyroid gland hormonal production and feather growth and development can be assessed in several ways. These may be anatomical and microscopic observations of the thyroid gland morphology, analysis of the activity of the thyroid hormones, or observation for the effects of supplemental amounts of thyroid hormones introduced into the bird. Several difficulties were encountered when attempting to measure T₃ and T₄ hormone activities. First, the total T₄ concentration is lower in birds than in mammals. As a consequence the measurements are below the limits of sensitivity of commercial assays, which are designed for humans. Special validation procedures and techniques are required to determine the T₄ concentration in birds (Harms et al., 1994). Moreover, T₃ concentrations measured in birds were found inconsistent and difficult to interpret (Lothrop, et al., 1985; Zenoble et al., 1985). The activity of T₄ is sensitive to various environmental factors such as stress associated with parasite infections such as Eimeria maxima (Davidson et al., 1985),
or Laminitis (Hood et al., 1987). Nutritional factors, such as essential amino acids (Elkin et al., 1980, and 1981), or alkaloids (Schone et al., 1993) in the diet may also affect the levels of thyroid hormones. Age may influence the thyroid hormone concentrations as well (Bobek et al., 1977). An attempt to perform such measurements in two laboratories yielded totally inconsistent and contradictory results and funding constraints did not allow to perform more detailed and precise measurements. Consequently, only histological observations regarding thyroid were performed on experimental birds.

In this section two experiments are described that were performed to assess relationships between thyroid activity and feather development.

3.3.2.1 Experiment 1. Comparative evaluation of thyroid microanatomy of IF and NF turkeys

This experiment was performed to investigate the relationships between the activity of the thyroid gland and the ratio of the feather development in various stages of the turkey growth.

3.3.2.1.1 Birds and management

Poults used in Experiment 1 were offspring of artificially inseminated NF hens from the Wrolstad Medium White line of the turkeys. Pooled semen from several IF heterozygous turkey toms was used for insemination. The birds were housed in two pens, in a ventilated brooder house under standard Oregon State University brooding conditions. Each pen contained twelve NF and twelve IF poults. The birds received standard turkey diet ad libitum according to the feeding program (Table 2.4) and free access to water.
3.3.2.1.2 Thyroid collection

Eight IF turkey poults and four NF poults were randomly selected from a group of 4 week old birds. Three IF and three NF poults were selected randomly from the remaining group of 9 week old poults. All the selected poults were euthanatized. Sternum and pectoral muscles were removed and the lower cervical region of the thoracic inlet was exposed. The thyroid gland was located between the jugular vein and the trachea (King and McLelland, 1975; McLelland, 1990) and both lobes of the gland were removed. The general appearance of the thyroid lobes, their color and shape were preliminarily evaluated. The removed thyroid glands were placed in a solution of 10% neutral buffered formalin, and subsequently were embedded in paraffin for histological analysis. Standard H&E staining of the tissue sections was performed. During histologic examination the emphasis was focused on size and the shape of the follicular cells, and on the size of the follicles. The colloid was examined for color and presence of vacuoles.

3.3.2.2 Experiment 2. The effects of thyroxine in the diet on the ratio of feather development of 12 to 20 weeks old turkeys

This experiment involved dietary thyroxine supplementation for IF turkeys from 12 to 20 weeks of age and was performed to evaluate the effects of the dietary thyroxine on IF turkey feather development.

3.3.2.2.1 Management of the birds

Forty eight IF turkey poults at 12 weeks of age were obtained from a cross of NF Wrolstad Medium White females and IF heterozygous males. Prior to the experiment the birds were fed a standard turkey starter and developer according to National Research

---

2Oregon State University Veterinary Diagnostic Laboratory
Council guidelines (National Research Council, 1984). At the beginning of the experiment when the birds were twelve weeks old, the first feather classification was performed. As a result, all birds were divided into three separate groups with the same feather score. A feather score of 1 was reserved for birds with normal feather development, and feather scores of 2, 3, and 4 were assigned to IF poults with different quality of the plumage. Then, the classification was performed similarly to that described in section 3.2. Birds (both sexes) from each group were randomly assigned to one of the four pens which in turn were randomly assigned to two treatment groups (two pens per group). Additionally, two NF turkeys of the same age were placed in each of the experimental pens for the purpose of evaluating the level of the thyroxine in the diet. During the term of the study, poults were fed either a Control diet or a diet containing thyroxine. The Control diet contained 16.84% CP, 3132 kcal/kg ME, and no thyroxine. The experimental Thyroxine diet was prepared by supplementation of the Control diet with 1 ppm of thyroxine (in the form of L-thyroxine free acid obtained from SIGMA Chemical Co., St. Louis, MO). The composition of the feeds used in the Experiment 2 are summarized in Table 3.5.

3.3.2.2 Evaluation of the thyroxine level in the diet

To verify whether the quantity of the thyroxine supplemented in the experimental diet was sufficient to alter the metabolic rate of the birds, two NF poults of the same age were placed in each pen to serve as a control for examination of thyroid gland response to dietary thyroxine. Because of difficulties in measuring thyroxin concentration in a diet with such low levels (1ppm) it was decided that the level of thyroxine in the diet would not be evaluated on a quantitative basis but rather qualitatively. That is, it was assumed that the level of the hormone could be judged adequate for the purpose of the experiment if effects of thyroxine supplementation were apparent in the control NF turkeys. To evaluate the influence of the thyroxine on the feather growth and to observe the thyroid gland response one of the two NF poults from each pen had feathers removed from one
Table 3.5 Composition of diets without (Control) and with supplemental thyroxine at 1 ppm (Thyroxine) fed to IF poults from 12 to 20 weeks of age

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>Thyroxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, Yellow (47.5% CP)</td>
<td>77.44</td>
<td>77.44</td>
</tr>
<tr>
<td>Soybean meal (8.9% CP)</td>
<td>12.25</td>
<td>12.25</td>
</tr>
<tr>
<td>Meat and bone meal (50% CP)</td>
<td>5.08</td>
<td>5.08</td>
</tr>
<tr>
<td>Fish meal (65% CP)</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>Fat (blended, animal)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate (16% Ca, 21% P)</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Trace mineral premix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Thyroxine&lt;sup&gt;3, 4&lt;/sup&gt;</td>
<td>-</td>
<td>0.000001</td>
</tr>
</tbody>
</table>

**Calculated analysis:**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Thyroxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>16.8</td>
<td>16.8</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>3132</td>
<td>3132</td>
</tr>
</tbody>
</table>

<sup>1</sup> Supplied per kilogram of diet: vitamin A (retinyl acetate), 3630 IU; vitamin D<sub>3</sub>, 1210 ICU; vitamin E (dl-α-tocopherol acetate), 0.121 IU; vitamin B<sub>6</sub>, 6.05 µg; riboflavin, 3.63 mg; niacin, 24.2 mg; d-pantothenic acid, 6.05 mg; menadione bisulfite, 0.605 mg; folic acid, 242 µg; choline chloride, 242 mg; and ethoxyquin, 68.6 mg.

<sup>2</sup> Supplied per kilogram of diet: Mn, 48 mg; Zn, 16 mg; Fe, 16 mg; Cu, 1.6 mg; I, 0.96 mg; Co, 0.16 mg.

<sup>3</sup> L- thyroxine free acid, Sigma Chem. Co.

<sup>4</sup> Premixed with soybean meal.
half of the body. All covert feathers were removed while the primary and secondary feathers were left undisturbed to prevent excessive bleeding and stress. The reduction of the insulation layer of feathers results in an increase of the heat loss from the organism and elevates the metabolic rate. Moreover, removing feathers from the feather follicles stimulates the cell proliferation in the feather follicles and increases the metabolic rate as well (Pietras, 1981). By removing the feathers from the NF turkeys the processes of the feather growth in IF turkeys were imitated giving the certainty that thyroid stimulation was associated with feather growth induced by plucking. The NF birds with removed feathers but fed the control diet served as a control for the processes occurring in the bird during rapid feather growth.

3.3.2.2.3 Measurements

Two measurements were taken for each bird to assess the effect of thyroxine supplementation - the feather score improvement index (FSII) and the weight gain. Feather score and weight were obtained in 12 weeks old turkeys and repeated at the end of the study in 20 weeks old turkeys. The feather scores were obtained as described in section 3.2.2.2.

The FSII obtained were evaluated using analysis of variance - ANOVA (Snedecor and Cochran, 1965).

3.3.2.2.4 Thyroid collection and examination

Thyroid glands were collected from 2 birds from each pen. Additionally, glands were obtained from all control NF pouls in the study to demonstrate the effect of dietary thyroxine on the thyroid activity during active feather growth in IF turkeys. The collection and histological preparation of thyroids was performed similarly as described in Experiment 1.
3.3.3 Results and discussion

3.3.3.1 Experiment 1

The thyroid glands of four week old IF and NF poults (Figures 3.6, and 3.7) are similar in appearance. The follicles of the glands are filled with small amounts of colloid with attributes characteristic of active thyroid glands. Only a few vacuoles are present in the fluid. The follicular cells which secrete thyroglobulin into the lumen of the follicles were of the cuboidal type. Some of the cells are tall cuboidal or even columnar. The similar appearance of the thyroids of IF and NF poults at four weeks of age suggests no major differences in the activity of the gland.

There may be several reasons to explain the lack of pronounced differences between thyroid tissues of IF and NF turkeys at four weeks of age. The down of IF poults is very well developed and no differences in its density or length can be observed in day old IF poults when compared to NF birds. Differences in the appearance of the thyroid glands of very young birds were observed only between the birds that differ in the ratio of down development at hatch such as between chickens and pigeons (Voitkievich, 1966). It is then probable that the anatomy of the thyroid gland observed in IF turkeys was not affected in very young birds and the similar density of the down in both groups of birds was reflected by an absence of differences in the appearance of the thyroid sections. Inactive thyroid gland, reflected in the histological observations of the tissues (squamous epithelium, large follicles) with regard to the time of the rapid feather growth was described previously (Voitkevich, 1966). Changes in thyroid tissue can be observed only after a few days of delay after the feather growth has begun (Voitkevich, 1966). The same investigator suggests that the development and differentiation of the thyroid tissue is not completed at the time of hatching and that the gland is still developing in the first weeks of life. Then, the lack of differences could be associated with the stage of maturation process of the thyroid gland rather than with the phase of the activity (Voitkevich, 1966). Finally, since at four weeks of age birds were
Figure 3.6 A cross section of the thyroid gland from an IF turkey at 4 weeks of age. H&E. The arrows identify the squamous epithelium. Bar represents 100 μm

Figure 3.7 A cross section of the thyroid gland from a NF turkey at 4 weeks of age. H&E. Arrows identify cuboidal epithelium. Bar represents 100 μm
naked, the active stage of the thyroid at this age could reflect the reaction of the thermoregulation system and increased metabolic activity of the body trying to maintain the required constant body temperature (Pietras et al., 1983). Rapid growth of young birds increases levels of metabolism in the organism which in turn is regulated by higher levels of thyroid hormone in the blood stream (Elkin et al., 1980, and 1981).

Histology of the thyroid of the IF and NF poults at 9 weeks of age is presented in Figures 3.8 and 3.9, respectively. The thyroid glands of the IF birds are clearly less active than those of the NF birds. The following differences are easily recognized. The epithelium of the thyroid tissue from IF poult is of the squamous type with the cell height being smaller than the cell width where the cells’ height is understood as the distance between the basal and the apical borders of the cell, and the width is the inner distance between side walls of the cells. The apical surface of the epithelium is flat and not developed. In the thyroid gland of the NF poults, the epithelium is of the cuboidal type. The apical surface of the epithelium is well developed providing a large active surface necessary for large hormonal production. The colloid is homogenous and only a few vacuoles are present in both tissue preparations. There are considerable differences in the size of the thyroid follicles. These findings correspond to the observations of the time of the feather growth in the IF turkeys. At approximately 9 weeks of age, the IF birds start to develop some feathers but most of the birds are still lacking feathers. At that age, it is possible to differentiate the IF turkeys with better or poorer feather development (section 2.2). It must be noted that whenever possible, IF birds with the poorest feather development were selected for thyroid examinations to enhance possible differences between thyroid tissues of NF and IF poults. It is then rather obvious that the thyroid tissue should be less active in those IF turkeys which did not develop any feathers till 9 weeks of age.

Figures 3.10 and 3.11 illustrate section of a typical 16 week old thyroid gland of an IF and NF poult, respectively. At this age substantial differences are observed in the appearance of the gland. In the IF turkey, the thyroid appears to be active whereas the NF turkeys are characterized by glandular inactivity. The follicular cells of the IF mutants are cuboidal. The size of the follicles do not differ when compared with those
Figure 3.8 A cross section of the thyroid gland from an IF turkey at 9 weeks of age. H&E. Arrows identify cuboidal epithelium. Bar represents 100 μm

Figure 3.9 A cross section of the thyroid gland from a NF turkey at 9 weeks of age. H&E. Arrows identify cuboidal epithelium. Bar represents 100 μm
Figure 3.10 A cross section of the thyroid gland from an IF turkey at 16 weeks of age. H&E. Arrows identify cuboidal epithelium. Bar represents 100 μm

Figure 3.11 A cross section of the thyroid gland from a NF turkey at 16 weeks of age. H&E. Arrows identify squamous epithelium. Bar represents 100 μm
in thyroid gland from normal birds. The thyroid of NF turkeys at 16 weeks of age is characterized by squamous epithelium suggesting that the gland has become inactive. The development of the feathers in NF turkeys is completed at 16 weeks of age. Since that time, the thyroid tissue is inactive unless activated by factors requiring enhanced ratio of metabolism (Russel, 1977; Elkin et al., 1980 and 1981; Davidson et al., 1985; Hood et al., 1987; Schone et al., 1993). An essential differences in the appearance of the thyroids were described in ducks that differed in the feather quality. The results suggested that the thyroid becomes inactive in well feathered birds after plumage development has been completed. In poor feathered ducks however, thyroids were activated later when feathers began to grow (Pietras, et al., 1983). Also, seasonal variation in the activity of the thyroid gland is well documented in birds (Payne, 1972; Cherrel et al., 1988; Pant and Chandola-Saklami, 1993; Harms, et al., 1994). These variations are present also if constant light and temperature environment is maintained which indicate presence of an endogenous hormonal mechanism that continues to function even in the lack of external stimuli (Harms, et al., 1994).

3.3.3.2 Experiment 2

Dietary supplementation of 1 ppm of thyroxine was sufficient to observe changes in thyroid gland microanatomy of the NF birds. The observed glandular response is illustrated by changes in the thyroid gland’s histology of those NF poults which had their feathers removed and those with intact feathers. Normally, at 20 weeks of age the turkey’s thyroid gland is in an inactive stage. Figure 3.12 presents an NF turkey with intact feathers fed the Control diet (without thyroxine). Figure 3.13 illustrates the result of thyroxine supplementation on thyroid gland in an NF bird with intact feathers. The epithelium of the thyroid gland is extremely flat indicating that the additional thyroxine in the diet halted the synthesis of the thyroxine in the thyroid completely. Figure 3.14 presents the thyroid gland of NF birds after removing feathers from half of the body. The production of the thyroxine was reestablished which is seen from the presence of the
Figure 3.12 A cross section of the thyroid gland from a NF turkey at 20 weeks of age fed the Control diet. H&E. Bar represents 100 µm

Figure 3.13 A cross section of the thyroid gland from a NF turkey at 20 weeks of age fed a diet containing 1 ppm thyroxine. Note the squamous epithelium (arrows) inactivated by exogenous thyroxine. H&E. Bar represents 100 µm
Figure 3.14 A cross section of the thyroid gland from a NF turkey at 20 weeks of age with feathers removed at 12 weeks of age fed the Control diet. Active, cuboidal epithelium indicated by arrows. H&E. Bar represents 100 µm

Figure 3.15 A cross section of the thyroid gland from a NF turkey at 20 weeks of age, with feathers removed at 12 weeks of age fed a diet with 1 ppm thyroxine. Note the squamous epithelium (arrows). H&E. Bar represents 100 µm
cuboidal epithelium and from smaller size of the follicles. Figure 3.15 presents the thyroid gland after the supplementation of thyroxine to a bird with removed feathers. First, it appears that the thyroid gland has been activated by feather removal. The follicles start to release the collected colloid. Consequently, the size of the thyroid follicle is diminished. By dietary thyroxine supplementation, the level of the hormone in the bird increased which inactivated the epithelium of the thyroid gland. Therefore, the epithelium became squamous. The above results suggests that the amount of the thyroxine in the experimental diet was high enough to generate meaningful results.

The average FSII of IF turkeys fed the control diet was 0.75, and the average FSII of turkeys fed diet containing 1 ppm of thyroxine in the form of L-Thyroxine free acid was 0.63 (Table 3.6) which was not statistically different (P > 0.05). There were also no differences in the weight gain of the IF turkeys fed standard diet and diet supplemented with 1 ppm of thyroxine (P > 0.05). Histology of the thyroid gland did not appear altered by 1 ppm of thyroxine supplemented in the diet. Figures 3.16 and 3.17 illustrate the thyroid gland of the IF turkey fed the Control and Thyroxine diets, respectively. These results suggest that in the mutant birds, the lack of proper feathering is not due to insufficient levels of the thyroxine in the organism. Rather, the observed insufficient hormonal production of the thyroid gland is probably a consequence of the more complex effects of the IF gene. It is probable that one result of the mutation maybe altered TSH synthesis whose results are visible in the thyroid gland. The histologic results of the thyroids collected from birds fed thyroxine and compared to those fed the control diet support this conclusion. The only visible effect of the thyroxine supplemented diet on thyroid anatomy was a flattening of the already inactive epithelium of the thyroid gland. Lack of thyroid hormones usually prevents feather growth. In thyrectomized birds no feather growth occurs due to lack of thyroxine (Lien, and Siopes, 1989a). However, if the birds’ diet is supplemented by an exogenous source of thyroxine, feather growth is possible (Lien, and Siopes, 1993a). In this experiment the amount of the thyroxine supplemented in the diet was adequate to trigger a response from NF birds, yet did not
Table 3.6  Feather score improvement index (FSII) and weight gain (kg) of the IF (Inhibited Feathering) turkeys fed unsupplemented (Control) and supplemented 1ppm of L-thyroxine free acid (Thyroxine) diets from 12 to 20 weeks of age

<table>
<thead>
<tr>
<th>Diet</th>
<th>FSII</th>
<th>SEM</th>
<th>Weight gain (kg)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.75</td>
<td>0.29</td>
<td>3.57</td>
<td>0.37</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>0.625</td>
<td>0.20</td>
<td>3.72</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Figure 3.16 A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet with no thyroxine supplementation. H&E. Bar represents 100 μm

Figure 3.17 A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 1 ppm thyroxine. H&E. Arrows identify squamous epithelium. Bar represents 100 μm
did not induce feather growth in IF turkeys. Therefore it is concluded that the altered gene affects feather growth and thyroid gland activity independently.

**3.4 Role of Zinc in Feather Growth and Development in the Inhibited Feathering Turkey**

**3.4.1 Introduction**

Zinc is a metal that is necessary for the proper function of the organism and its supplementation is usually required in the diet of all animals. The concentration of zinc in various tissues differ significantly. The skin contains about 20% of the total body zinc. Approximately 75% of this amount is located in the epidermis (Molokhia and Portnoy, 1980). The highest concentration of ions in the animal tissue may be found in the granular cell layer of the epidermis (Reaven and Cox, 1962). This suggests that zinc is associated with the intense enzyme activity exhibited by the granular layer (Molokhia and Portnoy, 1980). Zinc plays an important role in the keratinization processes. One of the enzymes actively involved in these processes is alkaline phosphatase which is known as a Zn-containing metalloenzyme and whose activity is severely affected by zinc deficiency (Jarret et al., 1959).

Zinc levels in the human or animal hair vary from 8 to 700 ppm, and its concentration in feathers is between 8 and 40 ppm (Strain and Pories, 1966). Despite a relatively large amount of zinc in the skin and its appendages, as well as in the bone, zinc supplementation must be continuous because only small amounts of readily available zinc can be stored. Usually the effects of zinc deficiency develop rapidly following the decrease in dietary zinc intake and include growth cessation and skin lesions. Most of the symptoms of zinc deficiency are common in the majority of animal species and involve skin anomalies. The probable reason for this is that skin and its appendages in their healthy state are relatively rich in zinc (Berglund, 1984). Dermal lesions were reported in human (Buerk et al., 1973), alopecia and parakeratosis in the rat (Diamond et al., 1971), mouse (National Research Council, 1972b), dog (National Research Council,
1972a), cattle (Miller, 1969), the monkey (Barney et al., 1967). Sparse and matted hair was observed in rabbit (Apgar, 1971) and the guinea pig (McBean et al., 1972). Poor feather conditions, described as fried or frizzled feathers were reported in chickens (Strain and Pories, 1966; Sunde, 1972; National Research Council, 1994) and in Japanese quail (Vohra, 1971). Zinc levels in hair and in feathers are considered as valuable and sensitive indicators of zinc metabolism by some of investigators (Strain and Pories, 1966) but are negated by others as not correlated with plasma zinc levels (Cunnane, 1988). Another rather common symptom of an deficiency of dietary zinc intake is impairment of the reproductive functions reported in rats (Underwood, 1971) and rabbits (Shaw et al., 1974). In the chicken reduced egg production was associated with a zinc deficiency (National Research Council, 1994).

Requirements for the zinc ion and the reaction animals to insufficient amounts of zinc in the diet vary greatly and depend on several factors. Age is an important factor in developing zinc deficiency symptoms. Young, fast growing animals require higher doses of zinc in the diet. Moreover, more severe lesions are usually observed in young animals that are in an active growth stage. Immediate growth termination was observed in rats subjected to zinc deprivation (Williams and Mills, 1970). Older animals can develop signs of the deficiency after being placed on a zinc-deficient diet but they are milder and not always obvious. Studies with mature rats indicate that a diet inadequate in zinc may result in slower wound healing in adult males (Oberleas et al., 1971; Sandstead et al., 1970) and affects reproduction in rat females (Underwood, 1971). Genetic differences in the dietary zinc requirements were also reported in swine (Łapińska and Łapiński, 1972) and cattle (Brummerstedt et al., 1971). Stress conditions such as severe injury, starvation, pregnancy or lactation are also factors that may affect the requirements for zinc. Under those conditions, zinc stored in the skin or bones may became available to the rest of the body. However, failure to replace the utilized zinc eventually results in even more severe symptoms of zinc deficiency (Tao and Hurley, 1975).

Different diets are characterized by the differences in the availability of zinc. Zinc originating from plants is believed to be less available to monogastric animals and
chickens than when it is derived from animal protein due to the presence of phytic acid (O’Dell, 1969). A diet can be sufficiently supplemented in zinc ions when zinc oxide, an inorganic form of zinc is used (Leeson and Summers, 1991).

In the following experiments the effect of higher than National Research Council recommended levels of zinc in the diet for IF turkeys were investigated to determine if supplementation of zinc will improve feather development in mutant turkeys (National Research Council, 1984; National Research Council, 1994).

3.4.2 Materials and methods

Three experiments were conducted to investigate the influence of dietary zinc supplementation on the feather growth ratio in IF turkeys. Two studies involved poults from day old to 8 weeks of age and the third experiment was conducted on growing turkeys from 12 to 20 weeks of age.

3.3.2.1 Experiment 1. The effects of dietary zinc in the brooding period (0-8 wks)

In Experiment 1, offsprings of artificially inseminated NF hens (Wrolstad Medium White) and IF heterozygous toms were used. Twenty four IF poults and twenty four NF poults were randomly assigned to two treatment groups and wingbanded. During the eight week duration of this experiment, poults were housed in two pens, in a ventilated brooder house under standard OSU brooding conditions required for turkey poults. Each pen contained twelve NF and twelve IF poults. The birds received either the control (Control) or the experimental (Zinc-I) diet ad libitum with free access to water. The Control diet was a standard starter for turkey poults containing 28% CP, 2804 kcal/kg ME and 65 ppm of zinc (Table 3.7). The treatment diet (Zinc-I) was formulated by supplementation of the (Control) diet with zinc (in the form of ZnO - zinc oxide), and contained 130 ppm of zinc. Two measurements were performed on the birds used in this
Table 3.7 Composition of Control-1 and Zinc-1 diets fed to IF poults from day-old to 8 weeks of age (Experiments 1 and 2)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control-1</th>
<th>Zinc-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, Yellow (8.9% CP)</td>
<td>49.25</td>
<td>49.25</td>
</tr>
<tr>
<td>Soybean meal (47.5 % CP)</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Meat and bone meal (50% CP)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Fish meal (65% CP)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate (16% Ca, 21% P)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin premix1</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>ZnO (80.2% Zn)3</td>
<td>-</td>
<td>0.00081</td>
</tr>
<tr>
<td>Trace mineral premix2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Coban-60 premix (sodium monesin, 132 g/kg)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Calculated analysis**

<table>
<thead>
<tr>
<th></th>
<th>Control-1</th>
<th>Zinc-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>28.04</td>
<td>28.04</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>2804</td>
<td>2804</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>65</td>
<td>130</td>
</tr>
</tbody>
</table>

1 Supplied per kilogram of diet: vitamin A (retinyl acetate), 4950 IU; vitamin D₃, 1650 ICU; vitamin E (dl-α-tocopheryl acetate), 1.65 IU; vitamin B₁₂, 8.25 μg; riboflavin, 4.95 mg; niacin, 33 mg; d-pantothenic acid, 4.95 mg; menadione bisulfite, 0.825 mg; folic acid, 330 μg; choline chloride, 330 mg; and ethoxyquin, 93.6 mg.

2 Supplied per kilogram of diet: Mn, 60 mg; Zn, 20 mg; Fe, 20 mg; Cu, 2 mg; I, 1.2 mg; Co, 0.2 mg.

3 Premixed with soybean meal.
study. The first measurement was classification of the feather development on IF poults performed at 56 days of age. Four feather classes corresponding to scores from 1 to 4 were used in the experiment. A score of 1 was given to birds with normal feather development. The best feathered of the IF poults were given a feather score of 2. A score of 3 was given to poults with average feather development, and a feather score of 4 was given to IF poults with the poorest feather condition. The typical poults assigned scores 2, 3 and 4 are shown in Figures 3.1, 3.2, and 3.3, respectively. The influence of zinc in the diet on the feather development in IF poults was estimated as the average score of the plumage in the experimental group and compared to the control group value. The birds’ plumage score were evaluated by the same person to prevent evaluation bias. Prior to the evaluation, all birds were combined in one pen and the evaluator was not aware which birds belonged to which treatment group.

Weight gain was determined between the first day of the study and its termination. Body weight in NF and IF poults were measured at hatch and at 56 days of age. All results were interpreted using analysis of variance - ANOVA (Snedecor and Cochran, 1965).

The first experiment was performed on a small number of the poults and served as preliminary study. In order to confirm the results of the Experiment 1, the second experiment was performed, involving additional birds. By including replicate pens in each of the dietary treatments the possible influence of the pen rather than the diet was eliminated.

3.4.2.2 Experiment 2. The effects of dietary zinc in the brooding period (0 - 10 wks)

The animals used in this experiment were offspring of IF hens artificially inseminated with semen of several IF toms homozygous for the K' gene. Forty four one-day-old poults were randomly assigned into four groups of 11 birds, and then each group was randomly assigned to one of four experimental pens (two pens per diet, so that each dietary group had two replicates). Each poult was identified by individual
wingbanding at hatch. The control diet (Control) was formulated as a corn-soybean meal-based feed containing 2804 kcal ME/kg, 28% CP and 64 ppm of Zn. The experimental diet (Zinc-2) was formulated from the (Control) feed by supplementation with ZnO to achieve 130 ppm of Zn. The maintenance of the poult’s was as outlined in Experiment 1. Feather score and weight gain were collected as follows: the feather classification was performed on IF poult’s at 70 days of age, using the method described in Experiment 1. The influence of the zinc in the diet on the feather development in IF poult’s was estimated based on feather scores in the control and treatment groups. Body weight of the IF poult’s was measured at hatch and at 70 days of age. As in the previous experiment, the weight gain during the experimental period was a value utilized to evaluate the influence of zinc supplementation on poult’s performance. All numerical results were interpreted using analysis of variance ANOVA (Snedecor and Cochran 1967).

In order to establish whether the dietary zinc supplementation will result in similar feather growth improvement for IF poult’s older than those of Experiment 1 and Experiment 2, another experiment was performed.

3.4.2.3 Experiment 3. The effect of dietary zinc in the growing period

Experiment 3 involved dietary zinc supplementation for IF turkeys from 12 to 20 weeks of age. In this experiment, 64 twelve week old poult’s were used. The birds were obtained from a cross of NF Wrolstad Medium White females and IF heterozygous males. Prior to the experiment, the birds were fed the standard turkey starter and developer according to National Research Council guidelines (National Research Council, 1984). The first feather classification was performed at the beginning of the experiment, when the birds were twelve weeks old. All birds were then separated into three groups with the same feather score. A feather score of 1 was reserved for birds with normal feather development, and feather scores of 2, 3, and 4 were assigned to IF poult’s with different qualities of the plumage. Birds from each group were randomly assigned to one
Table 3.8 Composition of Control-2, Zinc-3, Zinc-6, and Zinc-9 diets fed to IF poults from 12 to 20 weeks of age (Experiment 3)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control-2</th>
<th>Zinc-3</th>
<th>Zinc-6</th>
<th>Zinc-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, yellow (8.9% CP)</td>
<td>77.44</td>
<td>77.44</td>
<td>77.44</td>
<td>77.44</td>
</tr>
<tr>
<td>Soybean meal (47.5% CP)</td>
<td>12.25</td>
<td>12.25</td>
<td>12.25</td>
<td>12.25</td>
</tr>
<tr>
<td>Meat and bone meal (50% CP)</td>
<td>5.08</td>
<td>5.08</td>
<td>5.08</td>
<td>5.08</td>
</tr>
<tr>
<td>Fish meal (65% CP)</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>Fat (blended, animal)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Limestone flour (37% Ca)</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Dicalc. phosphate (16% Ca, 21% P)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix(^1)</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Trace mineral premix(^2)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>ZnO (80.2% Zn)(^3)</td>
<td>-</td>
<td>0.0001</td>
<td>0.00025</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Calculated analysis

<table>
<thead>
<tr>
<th>CP, %</th>
<th>Control-2</th>
<th>Zinc-3</th>
<th>Zinc-6</th>
<th>Zinc-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>3132</td>
<td>3132</td>
<td>3132</td>
<td>3132</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>40</td>
<td>120</td>
<td>240</td>
<td>360</td>
</tr>
</tbody>
</table>

\(^1\) Supplied per kilogram of diet: vitamin A (retinyl acetate), 3630 IU; vitamin D3, 1210 ICU; vitamin E (\(dl-\alpha\)-tocopherol acetate), 0.121 IU; vitamin B\(_2\), 6.05 \(\mu\)g; riboflavin, 3.63 mg; niacin, 24.2 mg; d-pantothenic acid, 6.05 mg; menadione bisulfite complex, 0.605 mg; folic acid, 242 \(\mu\)g; choline chloride, 242 mg; and ethoxyquin, 68.6 mg.

\(^2\) Supplied per kilogram of diet: Mn, 48 mg; Zn, 16 mg; Fe, 16 mg; Cu, 1.6 mg; I, 0.96 mg; Co, 0.16 mg.

\(^3\) Premixed with soybean meal.
of the eight pens. Then, the pens were randomly assigned to four treatment groups (two pens per group). During this study, poult's were fed either the Control diet or one of three experimental treatment diets containing three different elevated levels of zinc. The Control diet contained 16.84% CP, 3132 kcal/kg ME, and 40 ppm of Zn. The experimental diets used in the experiment were identified as Zinc-3, Zinc-6, and Zinc-9, and were prepared by supplementation of the Control diet with zinc (in the form of zinc oxide - ZnO) so that they contained 120 ppm of zinc, 240 ppm of zinc, and 360 ppm of zinc, respectively, which corresponded to supplementation at the level of 3 times, 6 times and 9 times of National Research Council recommendation (Table 3.8).

Two measurements were performed for each bird to assess the effect of zinc supplementation: a feather score improvement index (FSII) and weight gain during the eight weeks of the study as described in section 3.2.2.2. The FSII obtained were analyzed using a linear regression model, and means were examined using ANOVA analysis (Snedecor and Cochran, 1965).

In order to investigate the possible effects of the zinc supplementation on the thyroid gland function, thyroid gland samples were collected from turkeys from each of the experimental treatment. All thyroid glands were collected using the method described in section 3.2.

3.4.3 Results

3.4.3.1 Experiment 1

The results of the Experiment 1 are summarized in Table 3.10. Supplementation of the diet with 130 ppm of zinc resulted in significantly (P = 0.019) better feather development in the IF turkeys during the first 8 weeks of development. The average feather score in the Control treatment was 2.9 and in the Zinc-1 treatment was 2.27. The estimated improvement of the feather score was 0.63. The increased amount of dietary zinc available to this group of birds did not significantly affect (P = 0.777) the weight
gain in the same period of development. The average weight gain in the IF turkeys fed the Control diet was 1.74 kg per bird while the weight gain of the IF poults fed Zinc-1 was 1.78 kg per bird. Figures 3.18 and 3.19 illustrate the effects of dietary zinc supplementation for IF poults from day-old to eight weeks of age (Table 3.9).

Inclusion of supplementary zinc in the diet for NF poults was detrimental ($P = 0.004$) for weight gain. Birds from the NF line fed Control diet gained 2.07 kg per bird, whereas those fed Zinc-1 diet gained only 1.74 kg of weight per bird during the experiment.

3.4.3.2. Experiment 2

The results of the Experiment 2 are summarized in Table 3.11. Fortification of the diet with 130 ppm of zinc resulted in better ($P < 0.0063$) feather growth in the IF poults during the 8 weeks of growth. The average feather score of the IF poults fed the control diet was 3.19 and was 0.67 higher (which indicates poorer feathering) than the average feather score calculated for IF poults fed zinc supplemented diet. Thus zinc supplementation improved the feather growth in IF birds. The increased dietary zinc available to this line of birds did not affect ($P = 0.98$) weight gain. The average weight gain in the birds fed the control diet was 3.81 kg per bird and not different to that of birds fed the zinc supplemented diet (3.80 kg per bird).

3.4.3.3 Experiment 3

The results of Experiment 3 are summarized in Table 3.12. No pen differences within diets were detected and the results of FSII and weight gain were pooled. The average improvement in the feather score of IF turkeys fed Zinc-3 was 0.65 and was different ($P = 0.026$) from the average feather score improvement calculated for IF turkeys fed the 360 ppm Zn supplemented diet (Zinc-9). The average improvement in feather score of the IF turkeys fed 120 ppm of Zn (Zinc-3) diet, was 1.21 and the turkeys
Figure 3.18 Feather development in two IF turkeys at 8 weeks of age fed the Control diet containing 65 ppm zinc

Figure 3.19 Feather development in two IF turkeys at 8 weeks of age fed the Zinc-1 diet containing 130 ppm zinc
Table 3.9 Feather score and weight gain in IF (Inhibited Feathering) and NF (Normal Feathering) poults fed a starter containing 65 ppm and 130 ppm of zinc from day-old to 8 weeks of age (Experiment 1)

<table>
<thead>
<tr>
<th>Feather type/Dietary zinc</th>
<th>Feather Score</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF / 65 ppm</td>
<td>2.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.74</td>
</tr>
<tr>
<td>IF / 130 ppm</td>
<td>2.3&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.78</td>
</tr>
<tr>
<td>NF / 65 ppm</td>
<td>2.07&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.07</td>
</tr>
<tr>
<td>NF / 130 ppm</td>
<td>1.74&lt;sup&gt;D&lt;/sup&gt;</td>
<td>1.74</td>
</tr>
</tbody>
</table>

<sup>A</sup> <sup>B</sup> differences significant only for IF turkeys (P = 0.019)

<sup>C</sup> <sup>D</sup> differences significant only for NF turkeys (P = 0.004)

Table 3.10 Results of dietary zinc supplementation on feather score of IF (Inhibited Feathering) poults fed a starter containing 65 ppm of zinc (Control-1) or 130 of zinc (Zinc-1) from day-old to 10 weeks of age (Experiment 2).

<table>
<thead>
<tr>
<th>Dietary zinc</th>
<th>Feather Score</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>65 ppm</td>
<td>3.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.81</td>
</tr>
<tr>
<td>130 ppm</td>
<td>2.52&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.80</td>
</tr>
</tbody>
</table>

<sup>A</sup> <sup>B</sup> P = 0.0063
turkeys fed the 360 ppm Zn supplemented diet (Zinc-9). The average improvement in feather score of the IF turkeys fed 120 ppm of Zn (Zinc-3) diet, was 1.21 and the turkeys fed 240 ppm of Zn (Zinc-6) diet had a feather score improved by 1.33.

The results of this experiment (Table 3.12) indicate that no significant pairwise differences in feather score improvement occured between Control and Zinc-3, Zinc-3 and Zinc-6, Zinc-6 and Zinc-9 diets. The only significant difference between any two groups was found between Control and Zinc-9 diets. Yet, there is a clear increasing tendency in FSII as the zinc level increased. In this situation, pairwise group comparisons are clearly not a recommended statistical procedure. Instead, a regression model is fitted to evaluate relationship between dietary zinc level and FSII. Let X denote the dietary zinc level expressed as multiplicity of the National Research Council suggested level, and Y be the resulting average feather improvement score. Then a linear regression model is

\[ Y = 0.718 + 0.09 \, X \]

\[ \text{SEM (.22)} \quad \text{SEM (0.04)} \]

which is significant at \( P = 0.0019 \). Average weight gains in all treatment groups in this experiment did not differ significantly for any pairwise group comparisons.

The results of zinc supplementation on the thyroid gland microanatomy are illustrated in Figures 3.20 and 3.21. Figure 3.20 represents a thyroid gland typical for the IF turkey fed diet with a standard level of zinc - 40 ppm (National Research Council, 1994). The thyroid appears to be in an inactive state, the follicles are large and the epithelium is flat (squamous). Figure 3.21 represents a thyroid gland typical for the IF turkey fed diet with 360 ppm of zinc, which constitutes nine times the recommended National Research Council level of dietary zinc for turkeys from 12 to 20 weeks of age (National Research Council, 1994). The thyroid gland appears to be active and the follicles are smaller than those in the IF poults on the control diet. The epithelium surrounding the follicles is cuboidal which suggests that the thyroid is producing hormones.
Table 3.11 Feather score improvement index (FSII) and weight gain in IF (Inhibited Feathering) poults fed diets containing 40 ppm (control), 120 ppm, 240 ppm and 360 ppm of zinc from 12 to 20 weeks of age (Experiment 3)

<table>
<thead>
<tr>
<th>Dietary zinc</th>
<th>FSII</th>
<th>SEM</th>
<th>Weight gain</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ppm</td>
<td>0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22</td>
<td>3.72</td>
<td>0.41</td>
</tr>
<tr>
<td>120 ppm</td>
<td>1.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.22</td>
<td>3.14</td>
<td>0.40</td>
</tr>
<tr>
<td>240 ppm</td>
<td>1.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.22</td>
<td>2.73</td>
<td>0.31</td>
</tr>
<tr>
<td>360 ppm</td>
<td>1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33</td>
<td>3.48</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<sup>a b</sup> P=0.026
Figure 3.20 A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 40 ppm zinc. H&E. Arrows identify squamous epithelium. Bar represents 100 \( \mu m \)

Figure 3.21 A cross section of the thyroid gland from an IF turkey at 20 weeks of age fed the diet containing 360 ppm of zinc. H&E. Arrows identify cuboidal epithelium. Bar represents 100 \( \mu m \)
3.4.4 Discussion

Zinc has an extremely important role in amino acid and protein synthesis (Fujioka and Lieberman, 1964; O'Neal et al., 1970; Sandstead, and Rinaldi, 1969; Swenerton et al., 1969; Prasad, 1985). By its presence in DNA and RNA polymerases, it regulates the normal activity of DNA (Scrutton et al., 1971). Polymerase activity is impaired in states of zinc deficiency and can be restored upon zinc supplementation (Auld et al., 1974; Slater et al., 1971). Biochemical observations of organisms deficient in zinc indicate the importance of zinc in processes of growth and development since all of the critical processes require high levels of DNA and protein syntheses. Zinc is an integral part of many enzyme systems and zinc deficiency is often related to alterations in those enzymes (Hubber and Gershoff, 1973). Very strong evidence exists that zinc plays a critical role in the control of the cell cycle and a deficiency of this element may result in halting of normal cell synthetic processes. It is suspected that zinc regulation of the cell cycle may occur directly prior or immediate after DNA synthesis and the entry of the cell to the S phase may be affected by abnormal chromatin decondensation process prior to mitosis or by failure to assembly of the mitotic spindle (Clegg et al., 1986; Clegg et al., 1989). Rapid hair or feather growth, processes of wound healing or any other processes requiring rapid cellular divisions may be affected by zinc inadequacy (Prasad, 1985). The beneficial role of zinc in processes of growth and development is well established (Vallee and Falchuk, 1993).

Several reports indicate that oral zinc supplementation for animals and humans with various signs of zinc deficiency or zinc malabsorption improve the health of affected organisms regardless whether the primary reason of zinc inadequacy is hereditary or environmental (Prasad, 1985). Skin lesions, such as hyperkeratosis and parakeratosis, accompanied by loss of wool and wool breaking were found in two flocks of sheep and one herd of Pygmy goats. The animals were treated by supplementation of inorganic zinc compounds (zinc carbonate and zinc sulphate) orally. The improvement of the condition, such as new wool growth and the alleviation of the skin lesions was evident after 3-4 weeks of treatment in sheep while a longer time (12 to 14 weeks) was required for
improvement of skin condition and hair growth in the goat (Nelson et al., 1984). In the human and other mammals, prolonged zinc deficiency may result in hyperkeratotic lesions on the various areas of the skin which may be followed by the hair damage (Prasad, 1985; Aggett, 1989). In patients displaying Acrodermatitis enterophatica, in whom the intestinal uptake and transfer of the zinc seems to be impaired, dietary supplementation of zinc reversed the lesions (Garrets and Molokhia, 1977; Krieger and Evans, 1980). This effect may be attributed to creation of a new mechanism of the zinc absorption from the intestines. In a normal, healthy organism the intestinal absorption is mediated by a saturable carrier that binds zinc to specific sites at a concentration of 1-50 ug Zn/ml as well as by nonspecific binding (Berglund, 1984). High doses of zinc in the diet may create a sufficient concentration of zinc in the lumen of the intestine. This might result in establishing of the nonspecific mechanism of zinc transportation through the intestinal wall (Milla et al., 1979). The mechanism of action of the increased zinc in the diet of IF turkeys may be similar to that in patients with Acrodermatitis enterophatica. It is possible that the usual mechanism of intestinal zinc absorption is severely impaired in IF turkeys. Low zinc concentration in the organism may activate the non-specific form of zinc transportation, so that additional amounts of zinc may be transported through the intestinal wall. This concept helps to explain why additional amounts of the zinc in the feed increased feather growth of mutant birds significantly but at the same time were harmful for NF birds in which the usual mechanism of the zinc absorption was not affected.

A zinc deficiency does not always result in obvious lesions as hyperkeratosis and parakeratosis. In an experimentally induced zinc deficiency in Merino lambs fed approximately 50% of recommended zinc levels, the only observed symptom of zinc deficiency was slower wool growth. The histological examination, however, revealed more complex effects due to a lack of zinc. The low zinc diet adversely affected the process of keratinization of the wool fibers (White et al., 1994). Zinc deficient rats had low vitamin A levels in plasma although dietary supplementation of the vitamin A was at a normal level. Poor condition of the skin and appendages caused by zinc deficiency is thus probably aggravated by a low plasma vitamin A level which is also responsible
for poor condition of the ectodermal tissue (Brown et al., 1976). Hsu and Anthony (1971) established that the incorporation of cystine into skin proteins was severely affected by zinc deficiency. Since sulphur amino acids are required for normal processes of keratinization, a dietary zinc inadequacy may influence keratinization not only by affecting enzymes activity but also by influencing one of the substrates (Hsu and Anthony, 1971; Vallee and Falchuk, 1993). Improper keratinization can be reason for slower hair or feather growth and one of its symptoms may be retained cell nuclei which were observed in the wool fiber. Moreover, high numbers of immature follicles can be found in the skin of zinc deficient animals. Cell width and cell volume in the follicles are affected as well (White et al., 1994). In IF poults, the number of actively functioning feather follicles was definitely lower than their normal counterparts since large areas of the skin of IF poults were featherless. Whether the retardation of the feather follicles activity resulted from zinc deficiency as described by White et al. (1994) or whether zinc inadequacy slowed keratinization cannot be determined from this study, since no histological examinations of the skin of IF poults were performed. The obtained results however, would not be surprising in the light of evidence that zinc is required for proper functioning of several enzymes which control gene expression and cell division events (Clegg et al., 1989; Vallee and Falchuk, 1981). Similar effects of inactive follicles, inadequate feather growth, and damage of existing feathers suggest similar reasons of feather growth retardation that is insufficient zinc levels in the feed when compared to increased requirement. An apparent improvement of the feather condition after relatively short (8 to 10 weeks) treatment with zinc supplementation supports the above statement. Since zinc supplementation of the poult's diet significantly improved feather growth very significantly, it seems that the IF gene alters zinc requirements by negatively influencing either the absorption or the metabolism of the zinc in IF turkeys.

The advantageous results of zinc supplementation in the diet for IF turkey poults could be observed during studies of zinc effects in the thyroid. The positive response of this gland demonstrates a complex role of zinc in the bird. A zinc deficiency contributes to an impaired metabolism of thyroid hormones. These effects may be by supplementation of zinc in the diet of afflicted children (Favier, 1992a). Zinc deficiency
in rats is associated with decreased T\textsubscript{3} levels and may be responsible for decreased levels of hypothalamic thyrotropin-releasing hormone (Morley et al., 1980, Berglund, 1984). Thyroid hormone levels have been found to be lower and the basic metabolism rate was affected in zinc deficient adult men (Wada and Kong, 1986). In Experiment 3 of this study involving 12 to 20 week old IF poults, zinc supplementation seems to have restored the activity of the thyroid gland. Although the activity of the thyroid hormones was not investigated herein, the histological examination of the thyroid gland suggests notable differences in the microanatomy of thyroid gland in birds fed different levels of zinc. These observations are supported by the results of dietary zinc supplementation for normal humans whose serum zinc and thyroxine levels were positively correlated (Hartoma et al., 1979). Positive correlations between circulating triiodothyronine and thyroxine and the level of zinc supplemented in the diet were found in another experiment (Gordon et al., 1979). This same group of investigators verified that low dietary zinc was accompanied by hypothalamic thyroid-releasing hormone but contrary to the outcome of the Experiment 3 in this study, zinc supplementation in their experiment failed to return the thyroid hormone levels to normal (Morley et al., 1980). Those results suggest that dietary zinc intake may influence feather development in the IF turkeys in a very profound way. Not only is the feather growth impaired but also activity of thyroid gland appears to be impaired. Additional studies, involving biochemical analyses of thyroid activity and investigation of intestinal transportation of zinc would allow to clarify the mechanism by which zinc allows to improve feather growth in IF turkeys.
Chapter 4
Final conclusions

4.1 Is the Inhibited Feathering a new mutation?

Among the mutations that are known to affect feather development and structure in the turkey, only one, late feathering investigated by Asmundson and Abbott (1961) was sex-linked and dominant. Therefore, the IF gene described in this thesis is compared only to that mutation. The limited description of the late feathering phenotype mutant does not allow for a thorough comparisons of both genes. However, from the available data it is possible to conclude that the inhibited feathering in the turkey investigated herein is a new mutation, which was never reported elsewhere. This conclusion is based on the following facts.

The IF gene was first observed in 1990, in a single female within a flock of 5000 commercial line hens. It is highly unlikely that the dominant mutation would survive in a commercial line for about 30 years without being noticed by turkey breeders. It is also extremely improbable that exactly the same fragment of DNA was altered in both cases. If however, it were assumed that the two mutations occurred in the same region of nucleic acid possibly due to the fragility of the particular fragment of DNA, it would be possible that both inhibited feathering and late feathering might be the alleles at the same locus. The deliberations on this can be only theoretical since the gene for the late feathering in the turkey is no longer available and experimental determination of allelism is not possible.

Several differences between the two mutations support the belief that late feathering and inhibited feathering are not the same mutation. Asmundson and Abbott (1961) reported delayed down formation, and shorter down in 16 day-old embryos. In this study no differences were detected in down length between IF and NF embryos either on the 16th day of the incubation or at the day of hatching. This observation allows the conclusion that down development in IF turkeys during embryonic development is not affected. The appearance of the rectrices makes another contrast
between late feathering and IF turkeys. In late feathering turkeys, rectrices were not
affected and birds developed full size tail feathers. The rectrices of IF turkeys do not
develop or are significantly altered.

The reproductive performance of the IF turkeys is adversely affected. Egg
production and male ability to produce semen are significantly lower in IF birds
compared to NF. Asmundson and Abbott (1961) reported no differences in the number
of eggs produced of late feathering turkey. Therefore, it is justified to conclude that the
gene described herein is a new mutation.

4.2 The effects of the thyroid gland on the reproductive performance of
the IF turkey

The ratio of males that failed to produce semen was significantly higher in IF
turkeys when compared with NF males of same age. Several previous reports have
indicated the significance of the thyroid gland and its products in the proper functioning
for the reproductive system. Thyroidectomy of turkey males during semen production
period result in termination of semen production and severe testicular regression (Lien
and Siopes, 1991). Those results support earlier observations of thyroidectomy results
in cockerels (Snapir et al., 1982). Interestingly, thyroidectomy performed on turkey
males prior to the onset of semen production resulted in increased semen production and
prevented a molt. Results of studies performed in thyroidectomized turkeys indicate that
thyroid gland activity might have significant effects on maintaining testicular activity
In the present study, it was observed that those IF turkey males which responded to
photostimulation and began to produce semen, maintained semen production for more
than 6 months, even though regular semen collections ceased after two months and were
reestablished after 5 months from the first day of semen collection. It is then probable
that the thyroid gland status had no negative effect on the continuation of the semen
production.
Egg production of the IF females was low compared to NF females. Assuming that the thyroid gland malfunctions observed in younger IF turkeys, existed in mature IF turkey hens, reduced egg production was not unexpected. The thyroid gland is necessary for the initiation and maintenance of egg production (Lien and Siopes, 1989a,b). It determines the rate of metabolism and is one of the major factors affecting protein synthesis necessary for proper egg production. Any deviations in thyroxine production will therefore affect any process requiring high protein synthesis such as egg production.

Differences in reproductive performances were observed in females and males with inhibited feather mutation. Variability in thyroid involvement associated with reproduction was observed and discussed previously (Lien and Siopes, 1991, 1993a,b), but was never rationalized. Lien and Siopes (1989a,b) hypothesized that turkey females require thyroid hormones during the onset of egg production whereas in males the thyroid plays a more important role in the cessation of semen production and the beginning of the molt. More data should be obtained with regard to the status of the thyroid gland of IF turkeys during egg and semen production. Until then, only speculations on the effects of the thyroids on the reproductive performance in IF turkeys can be offered. It appears highly probable that at least a portion of the reproductive maladies in the IF mutants are related to the gene's effects on thyroid activity.

4.3 The effects of zinc on the reproduction of IF turkeys

High concentration of zinc in tissues and fluids of the male reproductive system in various animals attracted considerable interest (Saito et al., 1967; Arver and Eliason, 1980; Aonuma et al., 1981). Zinc is an important factor in the function and the development of the male reproductive system. The results of the studies on correlations between seminal plasma zinc concentration and functional properties of spermatozoa are contradictory but low zinc levels are usually associated with reproductive dysfunctions (Berglund, 1984). Zinc deficiency in animals and humans can be associated with atrophy of the seminiferous epithelium of the testes. The testicular tissues in zinc deficient males
may be described as immature, the number of layers of the seminiferous epithelium is reduced and fusion of spermatocytes may be noticed (Mason et al., 1982). Failure to produce semen in several of the IF males could be due to a zinc deficiency inducing complications in the development of testicular tissues. This theory can be substantiated by reported observations of testes aberrations in zinc deficient animals. Large numbers of malformed spermatozoa were observed in zinc deficient rats (Dinsdale and Williams, 1980) and low testosterone production due to the degeneration of the Leydig cells was also reported (Abbasi et al., 1980; Berglund, 1984). Also, the conversion of testosterone to dihydrotestosterone is higher at low zinc concentrations in the diet. Unfortunately, no measurements of the testosterone levels with regard to male infertility were performed on IF turkeys and it can only be speculated whether IF male infertility was due to low concentration of this hormone.

Results from several reported studies indicate that dietary zinc plays a role in the female reproductive processes. High dietary concentrations of zinc cause the cessation of egg production by depressing feed intake (Breeding et al., 1992) and by its inhibitory action on the granulosa cells responsible for production of progesterone (Johnson and Brake, 1992). Zinc deficiency is responsible for the degeneration of ovocytes in rats, anomalies in the estrogen cycle and halting ovulation in rabbits and monkeys. A few developing follicles and no atresic follicles were observed in zinc deficient animals (Favier, 1992b). The level of the zinc in serum of the mature female chicken is highly correlated with the presence of vitellogenin, the egg yolk precursor which indicates an associations between zinc metabolism and egg production (Mitchell and Carlisle, 1991). Moreover, zinc deficiency in females may cause difficulties in the synthesis and secretion of FSH and LH and may disrupt the estrous cycle (Bedwal and Bahuguna, 1994). It is probable that the low egg production by IF hens may be due to an altered zinc metabolism. The infertile females were probably unable to ovulate either due to malformations of the follicles, degeneration of the ovary, or inadequate levels of FSH
and LH. Extremely low egg production observed in other mutant hens could be due to low level of vitellogenin in the blood preventing yolk formation.

4.4 Zinc and thyroxine interactions

It is not clear whether the physiological alterations observed in the IF turkeys may be due to the impaired zinc metabolism or is associated with thyroid malfunctions. Several reports suggest that there are interactions between zinc deficiency and thyroid dysfunctions. Low zinc levels were positively correlated with hypothyroidism in several animals and humans. (Tiran et al., 1993; Nishiyama et al., 1994; Sustrova and Strbak, 1994). The mechanism of these interactions has not been explained so far and hypotheses have been posted that the thyroxine metabolism may be affected due to malfunctions of one of the enzymes involved in the deiodonization processes of T₄ to T₃ (Oliver et al., 1987). It was also found that zinc deficiency affected the serum T₄ level but did not change T₄ level in the thyroid gland. It is possible that T₄ release from the thyroid gland is associated with zinc deficiency (Smit et al., 1993).

The findings of the present study imply that the feathering in IF turkeys is closely related to an altered metabolism of zinc (section 3.4) and to changes in thyroid activity (section 3.3). From the above discussion it is probable, that inferior reproductive performance in IF turkey reported in section 2.3 is also due to zinc and thyroid aberrations. Moreover, it is justified to suspect that the thyroid activity and zinc metabolism are themselves closely related. What it means in the context of this thesis is that dietary zinc supplementation could not only improve the feathering in IF turkeys but at the same time also could enhance semen and egg production in mutant birds. This in turn may mean that the commercial utilization of the IF line may in fact be much closer than it could be expected from reviewing the list of disadvantageous characteristics of the mutant birds.
Chapter 5
Future research and final remarks

The research described herein if continued has a potential to revolutionize the commercial turkey breeding. An easy and inexpensive method of separating males from females at hatch by feather sexing would result in improvement of profitability of turkey production. In today’s competitive food market, even minute price changes can lead to financial success or bankruptcy in food production. Less expensive than cloacal sexing, feather sexing at hatch holds a promise of such savings and therefore should be recognized as one of the strategic objectives to the turkey research community. Obviously, the current state of the IF line discussed here does not allow for immediate commercial utilization of the inhibited feathering mutation. The main practical disadvantage is that IF birds which remain partially devoid of feathers for long portions of their lives utilize much more energy for thermal regulation than the NF birds and therefore incur much higher feeding costs. Also, lack of the natural protective layer of feathers the IF bird is very vulnerable to various injuries. Finally, reproductive performance of IF turkeys is rather unimpressive. It is this author’s belief however that all these shortcomings may be eliminated through proper nutrition, management and genetic selection for improved feathering.

The results of the zinc study indicate much promise in the exploration of trace mineral requirements. Any future research on nutrition of IF birds should probably start with a more detailed and thorough study of zinc supplementation. What was determined here is only the existence of a relationship between dietary zinc intake and feather growth rate. The supplementation levels used, were "an educated guess" procedure and are probably far from the optimal. Thus, it would be necessary to investigate different supplementation levels to arrive at an approximately optimal amount, as discussed in section 4.4. Given the sex-linked character of the IF gene, it is possible that the optimal doses may be different for both sexes. It is believed that proper zinc supplementation alone may dramatically improve commercial applicability of the IF birds.
Further studies regarding the investigation of the thyroid gland and zinc metabolism should be performed in order to learn more about the physiology of the IF turkey. The emphasis should be placed not only on the problems of the poor feathering but also some examinations of the association of the zinc and thyroid gland with respect to reproduction and behavior should be performed. It would be very interesting to find the relationships in the formation and development of gonads in both sexes and the levels of zinc and thyroid hormones in serum and various tissues of the mutants. It would be essential to try dietary zinc supplementation for breeder males and females to determine if the supplementation would help to improve the reproductive characteristics. More information is needed with regard to the actual cause of infertility in several of males and females. Histological analysis of the reproductive organs could reveal the possible causes of reproductive inefficiency but also would provide more information on the general physiology of the bird.

Another avenue to ameliorate production characteristics of the IF turkey could be through genetic intervention. From the observations of the IF turkeys, it is suspected that both the degree of nakedness (lack of feathers) and susceptibility of the feather condition to dietary supplements, may be at least partially inherited. Therefore, an intensive selection program might lead to development of a sub-line of IF turkeys with fast post-hatch feathering and a strong response to zinc supplementation (or other additives that might be determined beneficial from the point of view of feathering). Other characteristics of the IF turkeys that might be used as selection indices include reproductive performance (number of eggs laid, successful hatch ratio, etc.), weight gain, and feed conversion ratio. It is also possible that some of the detrimental attributes of the IF birds do not result from the IF mutation itself but may be independently inherited from the few birds involved in establishing the line, including the original IF hen. That is, the linkage between inhibited feathering and for example low number of eggs might be not through the same gene, but rather through an ancestor carrying two independent genes. If this is the case, appropriately designed line crosses would be able to remove the unwanted characteristics while maintaining the sex-linked pleiotropic difference at hatch (lack of primaries and secondaries).
Another way to improve the feathering and reproductive characteristics of the IF turkeys is through molecular manipulation within the genetic material of birds. Although far from being practical today, the incredibly rapid progress in molecular genetics observed in recent years make it possible that such an approach could be realistic in the near future. If it were true that the IF gene is linked to some other deleterious hereditary traits through proximity on the chromosome, it would be possible to isolate and remove the unwanted elements of the genome. As futuristic as it may sound, such a scenario is becoming more and more feasible as production of transgenic birds is already a reality.

Whether any of the suggested courses of action will actually be taken to improve characteristics of the IF line remain unknown at this moment. Unfortunately, studies on IF turkeys at Oregon State University have been terminated and at the time of this writing no IF birds are present in Corvallis. It is perhaps slightly ironic that the study, which held such a high promise of restoration of turkey industry in Pacific Northwest was canceled precisely because that industry was defunct and seemingly no commercial interest and support could be found locally for such research. Fortunately, Hybrid Inc., a large commercial breeder became interested in the IF line and saved it from an untimely termination. In May and June 1995, several shipments of IF turkey semen were provided by Oregon State University to Hybrid Turkey’s Inc. of Kitchner, Ontario, Canada and a successful hatch of over a thousand of IF birds followed. Thus the IF line managed to escape, at least temporarily, the fate of the line of Asmundson and Abbott (1961). At this time (December 1995), Hybrid Turkey’s Inc. is in possession of a larger number of IF birds than the cumulative sum of all IF turkeys ever maintained at Oregon State University in the whole duration of the studies reported herein. Financial means of a large commercial establishment like Hybrid allow the hope that the progress they will make with the line may be much faster than that obtained so far. In particular, studies involving larger groups of birds should give more definitive results. Also, as major facts about the IF line have already been established and the most promising directions of the line development are discussed in this dissertation, it should be easier for Hybrid to move rapidly towards full commercial utilization of the line.
Bibliography


Cline, L. E., 1933. Turkey Production. Orange Judd Publishing Comp., Inc. New York, N. Y.


Degussa, 1989. DL-Methionine - the amino acid for animal nutrition. Degussa Publ. Frankfurt, Germany


Fergusson, T. M., H. P. Vanhgt, L. D. Matterson, B. L. Reid, and J. R. Couch, 1957. The effect of different levels of productive energy, protein and methionine uptake upon the growth of Broad breasted Bronze turkey poults. Poultry Sci. 36:124-128.


Appendix
Two sample permutation test for binary measurements

The permutation test used in section 2.3 is described here. The test is designed to be used with binary response data, that is when the interesting characteristic to be analyzed statistically can be described by two values, zero and one. For example in case of male fertility zero and one may correspond to an infertile and fertile male, respectively. It is inessential which of the two possibilities is assigned which of the binary values. The test is concerned with incidence of the investigated characteristic in two sample groups. The null hypothesis is that the ratio of specimens characterized by value one (or zero) is the same in both samples, and any observed differences are due only to chance. The test evaluates all possible random assignments (permutations) of all investigated specimens into two groups of given sizes. For each analyzed permutation the observed ratios of the interesting characteristic are calculated and their difference is compared to the corresponding difference observed in the actual experimental data. The 2-sided p-value for the null hypothesis is obtained as a percentage of cases in which random assignment into two groups resulted in the difference of ratios larger than that observed in the experiment. In other words the test uses the permutation distribution of the absolute difference of the sample means.

The number of permutations that has to be evaluated in a permutation test is usually astronomically large even for modest sample sizes, which precludes practical application of such tests. An exception is a case of binary measurements, when it is not necessary to generate all possible permutations. Instead, it is easy to calculate the number of permutations that would result in larger than observed difference of ratios.

It is important to note that a permutation test assumes that the specimens from both samples could be independently rearranged into both groups. This independence assumption is crucial for validity of the permutation test. If, for some reason, certain specimens cannot be treated independently and must always appear in one group (such as eggs from one female), then the results of the test should be analyzed with caution, because the number of permutations taken into account will be too high, and the
calculated p-value will not be accurate.

The test was implemented in Matlab programming environment. The listing of the Matlab script file is given below. When executed, the program requires input of four numbers fully characterizing the experimental data - the sizes of both experimental groups and the numbers of specimens characterized as zeros (or ones). The output of the program is the 2-sided p-value.

% This routine performs a permutation test for the hypothesis that two groups do not differ with respect to a certain trait which may be characterized in binary terms - that is each element of a group either displays its or not. The null hypothesis is that the ratios of elements characterized by the trait are the same in both groups, and that the observed difference is due purely to chance. The permutation test calculates all possible random assignments of all elements into two groups of \( N_1 \) and \( N_2 \) elements, where \( N_1 \) and \( N_2 \) are numbers of elements in the two investigated groups. Then, out of all possible random assignments, those are selected, which result in difference larger or equal than actually observed. The ratio of selected assignments to all total assignments is the probability that the difference as large or larger than observed could have resulted from a random assignment of \( N_1+N_2 \) elements from the same population (with respect to the investigated trait) into two groups of \( N_1 \) and \( N_2 \) elements.

\[ N_1=\text{input('How many specimens in the first group? ')}; \]
\[ K_1=\text{input('How many characterized by the investigated feature? ')}; \]
\[ N_2=\text{input('How many specimens in the second group? ')}; \]
\[ K_2=\text{input('How many characterized by the investigated feature? ')}; \]

\[ N=N_1+N_2; \quad \text{% Total number of specimens} \]
\[ K=K_1+K_2; \quad \text{% Total number of ones} \]
\[ L=N-K; \quad \text{% Total number of zeros} \]

\[ N_{MX}=\text{max}(N_1,N_2); \]
\[ N_{MN}=\text{min}(N_1,N_2); \]

\[ \text{% Total number of all random assignments } = (N_1+N_2)!/(N_1! \cdot N_2!) \]
\[ \text{totnum=prod([NMX+1:1:N])/prod([1:1:NMN])}; \]
\[ \text{% To avoid numerical overflow, multiplications and divisions performed simultaneously} \]
\[ \text{totnum=1}; \]
\[ \text{for (i=1:1:NMN)} \]
\[ \quad \text{totnum=totnum*((NMX+i)/i);} \]
\[ \text{end}; \]
\[ \text{num1=0}; \]
\[ \text{num2=0}; \]
\[ \text{if (N1<=L)} \]
\[ \quad \text{minK1=0}; \]
\[ \text{else} \]
\[ \quad \text{minK1=N1-L}; \]
\[ \text{end}; \]
if (N1>=K)
    \text{maxK1} = K;
else
    \text{maxK1} = \text{N1};
end;

m1=K1/N1;
\% The ratio of elements displaying the investigated trait in group 1
m2=K2/N2;
\% The ratio of elements displaying the investigated trait in group 2
crd=abs(m1-m2);
\% Critical difference of the ratios

\% For the difference in ratios of elements displaying
\% the investigated trait
\% to be larger than the critical value crd,
\% the number of elements which display
\% the trait in group 1 must be either
\% greater or equal to \text{N1}*(\text{crd}*\text{N2}+\text{K1}+\text{K2})/(\text{N1}+\text{N2}) or
\% less or equal to \text{N1}*(-\text{crd}*\text{N2}+\text{K1}+\text{K2})/(\text{N1}+\text{N2})
\% [the p-value will be two-sided]

if (m1>=m2)
    \text{kup} = \text{K1};
    \text{klo} = \text{floor}((\text{N1}*(-\text{crd})*\text{N2}+\text{K1}))/\text{N});
else
    \text{kup} = \text{ceil}((\text{N1}*(\text{crd})*\text{N2}+\text{K1}))/\text{N};
    \text{klo} = \text{K1};
end;

\text{kup} = \text{max}(\text{klo}+1, \text{kup});

for (k=\text{minK1}:1:\text{klo})
    \text{kk} = \text{max}(k, K-k);
    \text{kn} = \text{min}(k, K-k);
    \text{c1} = 1;
    for (i=1:1:\text{kn})
        \text{c1} = \text{c1}*((\text{kk}+i)/i);
        end;

    \text{kk} = \text{max}(\text{N1}-k, \text{L}-\text{N1}+k);
    \text{kn} = \text{min}(\text{N1}-k, \text{L}-\text{N1}+k);
    \text{c2} = 1;
    for (i=1:1:\text{kn})
        \text{c2} = \text{c2}*((\text{kk}+i)/i);
        end;

    \text{num1} = \text{num1} + \text{c1}*\text{c2};
    end;

for (k=\text{kup}:1:\text{maxK1})
    \text{kk} = \text{max}(k, K-k);
    \text{kn} = \text{min}(k, K-k);
    \text{c1} = 1;
    for (i=1:1:\text{kn})
        \text{c1} = \text{c1}*((\text{kk}+i)/i);
        end;
kx=max(N1-k,L-N1+k);
kn=min(N1-k,L-N1+k);
c2=1;
for (i=1:1:kn)
    c2=c2*((kx+i)/i);
end;

num2=num2+c1*c2;
end;

% This is the number of random assignments
% for which the difference in ratios
% of elements that display the investigated trait
% is as large or larger than the observed one.

num=num1+num2;

[s1]=sprintf('Total number of possible random assignments to the two groups is %d',
totnum);
[s2]=sprintf('Number of random assignments resulting in a larger difference is %d',
num);
[s3]=sprintf('The (two-sided) p-value for the no-difference hypothesis is %f',
num/totnum);

disp([1]);
disp([1]);
disp(s1);
disp(s2);
disp(s3);