ABSTRACT

ROMERO-SANCHEZ, HUGO. Effect of Male Broiler Breeder Feeding Programs on Growth, Reproductive Performance, and Broiler Progeny.

(Under the direction of Dr. John T. Brake).

Four studies were conducted to evaluate the effects of different feeding programs during rearing and production on broiler breeder male growth, fertility, and progeny performance. In Experiment I, the effects of two levels (High and Low) of cumulative nutrient intake during the rearing period to photostimulation at 21 wk of age through different dietary formulations or feeding programs were evaluated. The High cumulative nutrition program supplied 33.5 Mcal ME and 1,730 g CP, while the Low cumulative nutrition program supplied 29.6 Mcal ME and 1,470 g CP. At 21 wk of age in Experiment I-2, males were classified into Heavy or Light BW groups. A cumulative nutrient intake during the rearing period of 29.6 Mcal ME and 1,470 g CP, regardless of diet or feeding program resulted in a male of adequate BW that was able to maintain good fertility throughout the production period and produce broilers with increased 42-d BW. In Experiment III, two levels of dietary CP (12% and 17%) and two feeding programs (Concave and Sigmoid) were evaluated during the rearing period to 26 wk of age. The Concave or 17% CP treatments were unable to sustain fertility after 40 wk of age without an increased allocation of feed. In Experiments II, two antioxidant levels and different source of selenium were evaluated during the production period. In Experiment IV different feeding programs from 16 to 26 wk of age and during the production period were evaluated. The data showed that feed allocation relative to BW affected fertility more than did antioxidant inclusion. Furthermore, slow and consistently increasing feed
increments from 16 to 26 wk of age and during the production period improved fertility and favorably impacted progeny performance. In conclusion, when males failed in accumulate adequate nutrients during the rearing period or gain adequate BW, as an indication of ME allocation, after photostimulation fertility declined and significant negative effects were observed in progeny performance.

*Key words:* antioxidant, broiler performance, fertility, photostimulation
EFFECT OF MALE BROILER BREEDER FEEDING PROGRAMS ON GROWTH, REPRODUCTIVE PERFORMANCE, AND BROILER PROGENY

by

HUGO ROMERO-SANCHEZ

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

ANIMAL SCIENCE and POULTRY SCIENCE

Raleigh

2005

APPROVED BY:

S. L. Pardue                                             J. W. Spears

P. Arroway                                           J. T. Brake, Chair
BIOGRAPHY

Hugo Romero Sanchez was born in Garagoa, Colombia. After his studies were completed at the primary school in his hometown, he moved to Bogotá for his secondary education at Liceo de Cervantes. He received his Bachelor of Science degree in Zootecnia from La Salle University in April, 1993. After finishing at La Salle University he worked for an agriculture extension program in his hometown for two years. He then began working on his Master of Science in Animal Health and Production with a concentration in Poultry Nutrition at the National University of Colombia in Bogotá. Before finishing his program he accepted a part-time position as a lecturer at La Salle University, where he taught Poultry Production and Animal Nutrition. He led different research projects in poultry production and nutrition with local companies that involved his undergraduate students. After graduation he accepted a scholarship to pursue a course in Poultry Management at the Hebrew University of Jerusalem, and then Hugo accepted a full-time position as a faculty member of the University of Antioquia, in Medellin, Colombia. He received a grant to study the nutritional value of cassava in poultry diets and then accepted a Fulbright scholarship to pursue his Ph.D. degree in Poultry Science at North Carolina State University. He currently holds a Research Assistant position at the laboratory of Dr. John T. Brake where he has studied different nutritional strategies to improve broiler breeder male fertility, including the use of different feeding programs, antioxidants, and phytase. Hugo has been frequently involved with community projects and was elected twice to the city council of his hometown. He also was Resident Advisor at NCSU during 3 years of his doctoral studies. Hugo was married to Veronica Benavente in 2004 and they will settle in Medellin after completion of his doctoral program, and continue working for the University of Antioquia.
ACKNOWLEDGEMENTS

The author wishes to express his sincere acknowledge to all the people and the institutions that made possible this research. Special gratitude is extended to Dr. John T. Brake, for his guidance, advice, support, and dedication during his residency at NCSU. His support has been beyond the scope of the graduate program. Appreciation is also expressed to the several institutions that sponsored his studies: University of Antioquia, Colciencias, Fulbright, and LASPAU, whose support was essential to the successfully completion of his Ph.D. program. Also the author would like to express his sincere appreciation to Drs. Jerry Spears, Sam Pardue, and Pam Arroway for serving on his graduate committee and for providing helpful criticism and insight. Appreciation is also extended to those who provided technical assistance. The help, patience and friendship of Susan Creech were invaluable. The company, criticism and friendship of his fellow graduate students, Peter W. Plumstead, Nirada Leksrisompong, Bridget Lenfestey, and Kelly Brannan were crucial. The author would also like to acknowledge the help, assistance, and hard work of the staff at the NCSU Chicken Educational Unit and the Piedmont Research Station. A special thanks to Quinton Jones, Terry Reynolds, and Lisa Wilson who assured that all data was reliable and accurate. Finally, the author would like to acknowledge the personal support and encouragement provided by his family. Sincere gratitude is expressed to his father Hugo Romero Rojas for supporting his education over these many years, and to his lovely wife Veronica Benavente for her support and for making this experience more valuable.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xiv</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>Nutrient Balance, Feed Restriction, and Reproduction in Broiler Breeders</td>
<td>4</td>
</tr>
<tr>
<td>Excess Body Weight and Obesity in Broiler Breeders</td>
<td>6</td>
</tr>
<tr>
<td>The Effect of Nutrient Deficiency on Reproduction</td>
<td>7</td>
</tr>
<tr>
<td>Hormonal Control of Male Reproduction</td>
<td>9</td>
</tr>
<tr>
<td>Testes Development and Male Fertility</td>
<td>10</td>
</tr>
<tr>
<td>Effect of Nutrition on Male Fertility</td>
<td>13</td>
</tr>
<tr>
<td>Effect of Metabolizable Energy Intake (ME) on Reproduction</td>
<td>13</td>
</tr>
<tr>
<td>Effect of Crude Protein (CP) Intake on Reproduction</td>
<td>15</td>
</tr>
<tr>
<td>Feed Programs and Growth Curves for Broiler Breeder Males</td>
<td>17</td>
</tr>
<tr>
<td>Photoperiodic Responses in Chickens</td>
<td>19</td>
</tr>
<tr>
<td>Photoperiod and Broiler Breeder Reproductive Performance</td>
<td>19</td>
</tr>
<tr>
<td>Interaction of Nutrition, Genetics, and Photoperiod</td>
<td>21</td>
</tr>
<tr>
<td>The Role of Antioxidants in Reproduction</td>
<td>22</td>
</tr>
<tr>
<td>Vitamin E and Vitamin C in Male Fertility</td>
<td>24</td>
</tr>
<tr>
<td>Metabolic Role of Selenium</td>
<td>26</td>
</tr>
<tr>
<td>Selenium in the Male Reproductive Tract</td>
<td>30</td>
</tr>
<tr>
<td>Applications of Selenium in Male Fertility</td>
<td>33</td>
</tr>
<tr>
<td>References</td>
<td>35</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

## MANUSCRIPT I

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>Composition of starter (0 to 2 wk), grower (2 to 21 wk), and breeder (22 to 64) diets in Experiments I-1 and I-2</td>
<td>55</td>
</tr>
<tr>
<td>I-2</td>
<td>Male broiler breeder carcass composition as affected by plane of nutrition during the rearing period in Experiment I-1 and Experiment I-2</td>
<td>65</td>
</tr>
<tr>
<td>I-3</td>
<td>Broiler breeder fertility during the laying period as affected by plane of nutrition during the rearing period in Experiment I-1</td>
<td>66</td>
</tr>
<tr>
<td>I-4</td>
<td>Broiler breeder fertility during the laying period as affected by male feeding program during the rearing period and male body weight classification in Experiment I-2</td>
<td>67</td>
</tr>
<tr>
<td>I-5</td>
<td>Effect of the plane of nutrition of male broiler breeders during the rearing period through two diets (HiDiet and LoDiet) or two feeding programs (HiFeed and HiLow) on broiler male performance as indicated by body weight (BW), adjusted feed conversion (AdjFCR), and feed intake. In Experiment I-2 broiler breeder males were classified by BW at 21 wk of age.</td>
<td>68</td>
</tr>
</tbody>
</table>

## MANUSCRIPT II

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>Composition of diets in Experiment II-1</td>
<td>87</td>
</tr>
<tr>
<td>II-2</td>
<td>Composition of diets in Experiment II-2</td>
<td>89</td>
</tr>
<tr>
<td>II-3</td>
<td>Broiler breeder fertility as affected by level of antioxidant in Experiment II-1</td>
<td>94</td>
</tr>
<tr>
<td>II-4</td>
<td>Broiler breeder fertility as affected by male comb height classification at 21 wk of age and selenium source during the laying period in Experiment II-2. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC).</td>
<td>100</td>
</tr>
</tbody>
</table>
MANUSCRIPT III

Table III-1. Composition of rearing and breeder diets ....................... 122

Table III-2. Broiler breeder fertility during the production period as affected by crude protein (CP) level and male feed allocation program during the rearing period (2 to 26 wk of age) 1 ......................................................... 127

MANUSCRIPT IV

Table IV-1. Composition of broiler breeder and broiler diets ................. 140

Table IV-2. Broiler breeder fertility as affected by male feeding program from 16 to 26 weeks of age in Experiment IV-1 1 ......................................................... 148

Table IV-3. Broiler breeder fertility as affected by male feeding program from 16 to 26 weeks of age and during the production period in Experiment IV-2 1 ........... 153

Table IV-4. Effect of the broiler breeder male feeding program from 16 to 26 wk of age and during the production period in Experiment IV-2 on broiler performance as measured by body weight (BW), adjusted feed conversion (AdjFCR), and feed intake ................................................................. 155
LIST OF FIGURES

MANUSCRIPT I

Figure I-1. Male feeding programs during rearing (A) and production period (B) in Experiment I-1 and Experiment I-2. In Experiment I-1, Ross 344 males were fed a single feed program using two diets containing either High or Low nutrient density. In Experiment I-2 a single diet was used with either High or Low plane of nutrition. Both Experiments supplied the same cumulative nutrition to 21 wk of age. 56

Figure I-2. Male BW as affected by feeding program during the rearing period in Experiment I-1 (Panel A) and Experiment I-II (Panels B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (HiDiet and LoDiet, in Experiment I-1) or two feeding programs (HiFeed and LoFeed, in Experiment I-2). Males were classified as being in the 50% Heavy or 50% Light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Analyses of slopes during the rearing period (0 to 21 wk of age) is shown in the right box. Transition period in Figure I-2A indicates when the HiDiet and LoDiet were blended in various proportions. 60

Figure I-3. Male shank length as affected by feeding program during the rearing period in Experiment I-1 (A) and Experiment I-II (B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (Experiment I-1) or two feeding programs (Experiment I-2). Males were classified as being in the 50% heavy and 50% light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Analyses of slopes during the rearing period (0 to 21 wk of age) is shown in the right box. Transition period in Figure I-3A indicates when the HiDiet and LoDiet were blended in various proportions. 61

Figure I-4. Male comb height as affected by feeding program during the rearing period in Experiment I-1 (A) and Experiment I-II (B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (Experiment I-1) or two feeding programs (Experiment I-2). Males were classified as being in the 50% heavy and 50% light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Transition period in Figure I-4A indicates when the HiDiet and LoDiet were blended in various proportions. 62
Figure I-5. Male body weight (BW) during the production period as affected by rearing diet in Experiment I-1. Ross 344 males were fed diets containing either High (HiDiet) or Low (LoDiet) nutrient density. Males received the same diet after 24 wk of age and were divided in the 50% heavy and 50% light within experimental groups to determine growth pattern. Transition period indicates when the HiDiet and LoDiet were blended in various proportions.

Figure I-6. Energy balance for males in HiDiet in Experiment I-1. Ross 344 males were fed a single diet providing 340 kcal/d of ME during the early production period. Males received the same diet after 24 wk of age and were divided in the 50% Heavy and 50% Light within experimental groups to determine growth pattern. The metabolizable energy requirement for maintenance (MEm) at experimental conditions for the Heavy and Light group within the HiDiet treatment was estimated as function of the BW as $\text{ME}_m = 1.45\times BW^{0.65}\times (1.78 - 0.012\times T)$ where $T$ is average temperature in °F (Combs, 1968).

Figure I-7. Effect of the rearing broiler breeder male diet on fertility during the early production period and its effect on broiler male body weight (BW) at 42 d of age in Experiment I-1. Ross 344 males were fed diets containing either High (HiDiet) or Low (LoDiet) nutrient density.

MANUSCRIPT II

Figure II-1. Broiler breeder male feeding programs during the production period in Experiment II-1 and Experiment II-2. A typical commercial feeding program is shown for comparison.

Figure II-2. Male BW as affected by antioxidant level (Control or High) during the production period in Experiment II-1. Asterisk (*) represents a significant difference ($P < 0.05$), determined by GLM procedure at each age.

Figure II-3. Fertility during the production period as affected by antioxidant level (Control or High) in Experiment II-1. The arrow indicates the age when the male feed allocation was increased by 5 g/male/d. Asterisk (*) represents a significant difference ($P < 0.05$) determined by GLM procedure at each age.

Figure II-4. Egg production as affected by antioxidant level in Experiment II-1. Asterisk (*) represents a significant difference ($P < 0.05$) determined by GLM procedure at each age.
Figure II-5. Egg shell weight during the production period as affected by antioxidant level during the production period in Experiment II-1. Plus symbol (+) indicates a numeric difference that approached significance ($P < 0.1$) as determined by GLM procedure at each age.

Figure II-6. Male body weight (BW) during the rearing and production period as affected by male comb height classification at 21 wk of age and selenium source during the production period in Experiment II-2. Selenium was supplied as organic selenium (HiSe Yeast), or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC).

Figure II-7. Male shank length (Panel A) and comb height (Panel B) as affected by selenium source and male comb height classification at 21 wk in Experiment II-2. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC).

Figure II-8. Total embryo mortality (Panel A) and percentage fertile hatchability (Panel B) as affected by selenium source and male comb height classification at 21 wk (Experiment II-2). Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC). Means with different superscript are significantly different ($P < 0.05$). Time by treatment (comb height and selenium source) interaction was significant ($P < 0.01$).

Figure II-9. Egg production and female body weight (BW) at 48 wk of age, as affected by male comb height classification at 21 wk of age and selenium source during the production period in Experiment II-2. Asterisk (*) represents a significant difference ($P < 0.05$) determined by GLM procedure at each age. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe).

Figure II-10. Energy balance in males from 22 to 32 wk of age. Males were classified by comb height at 21 wk of age as either large comb (LC) or small comb (SC) males. This figure compares ME intake with ME required for maintenance ($ME_m$) at experimental temperature as function of the BW as $ME_m = 1.45*BW^{0.65} * (1.78 – 0.012*T)$ where $T$ is average temperature in °F (Combs, 1968).
MANUSCRIPT III

Figure III-1. Male feeding program during the rearing period to 26 wk of age. After 26 wk of age both feeding programs supplied the same amount of feed (110 g/d) until 49 wk when the allocation was increased by 5 g/male/d.……………………………………121

Figure III-2. Male BW as affected by feeding program and crude protein (CP) level during the rearing period to 26 wk of age. Ross 344 males received two different feed programs, either Concave or Sigmoid, as shown in Figure III-1, and two different CP levels, either 12 or 17% as shown in Table III-1. Asterisk (*) represents significant difference ($P < 0.05$) as determined by GLM procedure at each age………………………………………………………………………………125

Figure III-3. Fertility during the production period as affected by feeding program and crude protein (CP) level during the rearing period to 26 wk of age. Ross 344 males received two different feed programs, either Concave or Sigmoid, as shown in Figure III-1, and two different CP levels, either 12 or 17% as shown in Table III-1. The arrow indicates the age when the feed male allocation was increased by 5 g/male/d. An asterisk (*) represents significant differences ($P < 0.05$) determined by GLM procedure at each age………………126

MANUSCRIPT IV

Figure IV-1. Male feeding programs from 16 to 26 wk of age and during the production period. Panel A shows Experiment IV-1 including three feeding programs (Fast, Medium and Slow) while panel B shows Experiment IV-2 using only the Fast and Slow programs from 16 to 26 wk of age and the Constant and Increasing programs during the production period……………………………………142

Figure IV-2. Male BW as affected by feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different feed programs, either Fast, Medium, or Slow. $a,b$ Means with different superscript are significantly different ($P < 0.05$) as determined by GLM procedure at each age………………………………………………………………………………146

Figure IV-3. Fertility during the production period as affected by male feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different feed programs, either Fast, Medium, or Slow. The arrow indicates the age when the feed male allocation was increased by 5g/bird/d. $a,b$ Means with different superscripts are significantly different ($P < 0.05$) as determined by GLM procedure at each age……………………………………147
Figure IV-4. Male mortality as affected by feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different feed programs, either Fast, Medium, or Slow. \textsuperscript{a,b} Means with different superscripts are significantly different ($P < 0.05$) as determined by GLM procedure at each age.

Figure IV-5. Male BW as affected by feeding program from 16 to 26 wk of age (Panel A) and during the subsequent production period (Panel B) in Experiment IV-2. Ross 344 males received a single growing diet using two different feed programs, either Fast or Slow as shown in Figure IV-1, followed by either a Constant or Increasing feed allocation during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.

Figure IV-6. Fertility as affected by male feeding program from 16 to 26 wk of age (Panel A) and during the production period (Panel B) in Experiment IV-2. Ross 344 males received a single growing diet using two different feed programs from 16 to 16 wk of age (Fast or Slow), and then a group received either the Constant or Increasing feed program during the production period.

Figure IV-7. Male mortality as affected by male feeding program from 16 to 26 wk of age and during production period in Experiment IV-2. Ross 344 males received a single growing diet using two different feed programs from 16 to 16 wk of age (Fast or Slow), and then a group received either the Constant or Increasing feed program during the production period. \textsuperscript{a,b} Means with different superscripts are significantly different ($P < 0.05$) as determined by GLM procedure at each age.

Figure IV-8. Effect of the male feeding program during the production period on broiler breeder fertility and its effect on progeny BW at 42 d in Experiment IV-2. Ross 344 broiler breeder males were fed a single diet following an Increasing or a Constant program during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.

Figure IV-9. Effect of the male feeding program during the production period on broiler breeder fertility and its effect on the progeny adjusted feed conversion (AdjFCR) at 42 d in Experiment IV-2. Ross 344 broiler breeder males were fed a single diet following an Increasing or a Constant program during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdjFCR</td>
<td>Adjusted feed conversion ratio, corrected for mortality</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CP</td>
<td>Crude Protein</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>FCR</td>
<td>Feed conversion ratio</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>kcal</td>
<td>Kilocalories (1,000 calories)</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>Mcal</td>
<td>Mega calories (1,000 kilocalories)</td>
</tr>
<tr>
<td>ME</td>
<td>Metabolizable energy</td>
</tr>
<tr>
<td>NaSe</td>
<td>Sodium selenite (Na$_2$SeO$_3$)</td>
</tr>
<tr>
<td>PHGPx</td>
<td>Phospholipid hydroperoxide glutathione peroxidase</td>
</tr>
<tr>
<td>SeMe</td>
<td>Selenomethionine</td>
</tr>
<tr>
<td>T$_3$</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T$_4$</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Alpha tocopherol</td>
</tr>
<tr>
<td>wk</td>
<td>Week</td>
</tr>
</tbody>
</table>
INTRODUCTION

Meat-type broiler breeders, i.e. parent stock of domestic fowl kept for the production of broiler chicks for meat production, have often been characterized as having poor reproductive performance, especially with respect to fertility. Fertility problems occur frequently in both male (Hocking, 1990; Mauldin, 1992) and female broiler breeders (McDaniel et al., 1981; Lopez and Leeson, 1995). Selection for increased broiler growth-rate has been associated in females with lower levels of fertility due to shorter duration of spermatozoal storage (Vankrey and Siegel, 1974), and fewer completed matings (Chambers, 1990); while in males it has been associated with reduced semen motility (Siegel, 1963), fewer courts, and fewer completed matings (Chambers, 1990). Furthermore, the remarkable genetic improvement in feed conversion efficiency during the last 20 years has decreased the nutrient intake necessary to reach an “adequate” body weight (BW) for reproduction. With this scenario broiler breeder males can be photostimulated without sufficient cumulative nutrient intake during rearing to sustain adequate fertility during the subsequent reproductive cycle. During the last decade, it was clearly demonstrated that broiler breeder females, irrespective of BW, should consume more than 1,200 g of crude protein (CP) and 23 Mcal of metabolizable energy (ME) prior to photostimulation (Walsh and Brake, 1997; 1999) in order to sustain fertility throughout the reproductive cycle. Although a cumulative nutrient intake target during the rearing period for broiler breeder males had been suggested when the present studies were initiated (Peak, 2001), additional information was required to adequately define the target.

The rearing period has always been given attention by parent stock breeding (genetic) companies since important anatomic and physiological events occur during this period of
life. During the early rearing period skeletal development has been shown to be completed and males without an adequate frame size have been thought to develop excess breast meat and have poor conformation in later life that would contribute to problems with balance during mating. This has been thought to limit the ability of the male to mate successfully. Additionally, during the late rearing period around photostimulation, generally between 16 and 26 wk of age, birds have been typically changed from an alternate day to an every day feeding program while rapid development of the reproductive system occurred and photostimulation was used to trigger sexual maturity. A deficient nutrient intake has been shown to retard sexual maturity but an excessive nutrient intake has been shown to result in excessive BW and breast meat development with an accompanying increased maintenance requirement that adversely affected mating activity. Therefore, it was known when the present studies began that nutrient intake must be carefully monitored and programmed such that the proper balance between BW, nutrient requirement, and nutrient allocation would be maintained. Conversely, the feeding program during the rearing period had been shown to affect the growth curve and the deposition of nutrients in the body (Leeson and Summers, 1997). Although photostimulation had been suggested to play a major role in the overall process of nutrient accumulation, data from broiler breeder females had shown that the nutrient intake during the latter part of the rearing period and after photostimulation could also play an important role in subsequent fertility (Walsh and Brake, 1997;1999). Furthermore, different feeding approaches had been shown to achieve similar cumulative nutrient intake but with different reproductive performance during the production period. Therefore, the first objective of the present research was to evaluate different rearing planes
of cumulative nutrition in male broiler breeders that were photostimulated at 21 wk of age, as well as to evaluate different feeding programs from 16 to 26 wk of age.

Similarly, most research during the production period had given priority to female broiler breeders although the male represented 50% of the physical component of reproduction. Furthermore, many of the investigations with males had been conducted in cages using artificial insemination in conditions that were not particularly representative of the problems found under commercial conditions. For many years, the primary breeding (genetic) companies have strongly recommended feed restriction throughout the production period to avoid wastage and excessively fat birds (Cerolini et al., 1995). Although obesity markedly reduced fertility in males (Nir et al., 1975), there had been several reports that suggested that consistently increasing increments in feed intake improved semen quality and fertility (Harris et al., 1984; Cerolini et al., 1995).

In an attempt to overcome fertility problems due to poor male performance, several studies have suggested that certain antioxidants could protect the spermatozoal membrane and increase spermatozoal viability. However, when males of the same advanced age were interchanged (spiking) an increase in fertility has often been observed, suggesting that older males had semen capable of sustaining fertility, and in a “new environment” they simply increased their mating activity, which was all that was required to increase fertility. This observation suggested that antioxidant supplementation may interact with sexual maturity or feed allocation during the production period so one objective was to investigate this idea further. Furthermore, Attia et al. (1993; 1995) suggested that fertility problems due to low ME intake may be reflected in progeny performance, since the males with the highest genetic potential for broiler growth rate were more adversely affected. Therefore, an objective was
to evaluate the effect of different male feeding programs during the production period on fertility, hatchability, and broiler performance.

**LITERATURE REVIEW**

**Nutrient Balance, Feed Restriction, and Reproduction in Broiler Breeders.** The onset of sexual maturity and the number of eggs that were produced as well as the fertility, have been reported to be profoundly affected by the intake of nutrients accumulated during the rearing period prior to photostimulation (Walsh and Brake, 1997; 1999; Peak, 2001). Further, the form in which these nutrients were deposited appeared to impact reproductive performance. It has been recognized that a minimal accumulation of fat was necessary for both commencement and maintenance of ovulatory cycles (Waddington and Hocking, 1993; Hocking and Robertson, 2000; Spicer, 2001). Excessive breast meat deposition will apparently increase the daily maintenance requirement and decrease the metabolizable energy (ME) available to sustain reproduction. In the same manner, excessive crude protein (CP) has been reported to increase uric acid production as well as increase the ME required for formation and excretion of this waste product. Numerous studies have revealed an association between nutritional status, adiposity, and reproductive performance, but the mechanisms have not been clearly defined (Nolan *et al.*, 1990; Bruggeman *et al.*, 1998; Cassy *et al.*, 2004).

Since broiler breeders have a voracious appetite, they will consume a quantity of feed that will exceed their nutrient requirements when allowed to consume feed on an *ad libitum* basis. Excess consumption of ME has been shown to result in excess abdominal fat and hepatic lipid deposition as well as abnormal ovarian development (Etches, 1996). Reproductive
performance has been long known to decrease in both males and females that become obese. To avoid these problems, broiler breeders have long been subject to quantitative feed (caloric) restriction (Leeson and Summers, 2000). However, this traditional strategy may lead to an excessive feed restriction in some case that paradoxically results in fertility problems (Bruggeman et al., 1998).

Feed restriction may start at an early age as de Reviers and Seigneurin (1990) reported greater spermatozoal production, albeit delayed, from males restricted from 2 wk of age versus 6 wk of age. Some studies have shown the importance of maintaining high a CP intake in conjunction with feed restriction in order to maximize fertility (Schumaier and McGinnis, 1969; Blair et al., 1976; Yu et al., 1992a; Yu et al., 1992b; Lopez and Leeson, 1995). In a previous series of experiments (Walsh and Brake, 1997) broiler breeder females were reared to similar 20-wk BW with diets containing levels of CP ranging from 11% to 20%. Irrespective of cumulative ME intake or 20-wk BW, pullets that consumed less than 1,180 g of CP exhibited reduced fertility. There may also be a specific requirement for adequate CP during the prelay period when growth and development of the reproductive tract occurs (Schjeide et al., 1963; Yu and Marquardt, 1974). In males, feeding low levels of CP during the growing period delayed sexual maturity (Wilson et al., 1971; Vaughters et al., 1987), impaired testicular growth (Jones et al., 1967), and decreased spermatozoal volume and concentration at the onset of semen production (Wilson et al., 1971). Therefore, it appeared that during the growing period there was some minimum CP requirement for male broiler breeders to obtain proper sexual maturity by approximately 20 wk of age. If this requirement was not met, delayed male sexual maturity and poor initial fertility resulted. Sorneson (1980; 1985) selected meat-type chickens for increased growth-rate on high and
low CP diets. When subsequently tested on a low CP diet, the low CP selected line grew substantially faster than the high CP selected line. This demonstrated a direct relationship between nutrition during genetic selection and the subsequent nutritional needs of progeny. Presently, most elite broiler breeding stocks have been selected on a high CP diet while parent stocks (broiler breeders) have been typically grown on lower CP diets. It may be possible; therefore, that the reported decrease in broiler breeder reproductive performance may actually be the result of increased nutrient requirements due to genetic selection for broiler growth-rate in the presence of high CP diets. It has been postulated that a restricted feeding program designed to achieve a given BW standard during rearing, without attention to the details of specific nutrient intake, may not provide sufficient nutrients for optimal reproductive development (Brake and Peak, 1999). Indeed, it was found that the delayed sexual maturity of a parent stock from a Japanese quail line selected for high 4-wk BW on a high CP diet was ameliorated by feeding a dietary CP level during rearing that was commensurate to that fed during the genetic selection process (Lilburn et al., 1992). However, certain reasonable dietary limits must be set to avoid excessive breast development and achieve economic targets under practical conditions.

**Excess Body Weight and Obesity in Broiler Breeders.** Broiler breeder hens that were *ad libitum* fed reached onset of puberty earlier than restricted birds, but produced fewer settable eggs (lower egg numbers, more double yolks, and more soft eggshells) (Hocking *et al.*, 1987; Hocking *et al.*, 1989; Yu *et al.*, 1992b; Cassy *et al.*, 2004). The physiological interactions that hasten the onset of puberty and diminish reproductive performance have not been well defined and only a pragmatic and empirical understanding of this relationship allowed the establishment of the existing commercial feed restriction programs.
A large portion of the fertility problems related to obesity have been explained due to polycystic ovary syndrome in females (Leeson and Summers, 2000) male leg problems, or atrophied testes (Sexton and Renden, 1988; Leeson and Summers, 2000). Male leg problems and excess BW have been reported to reduce the success, more than the frequency, of mating (McGary et al., 2002; McGary et al., 2003). Some reports have mentioned an increase in adiposity in the eggshell gland decreasing spermatozoal storage capacity (Breque et al., 2003). Although many problems remain without explanation, the recent development of genetically obese (ob/ob) mice (Zhang et al., 1994), which lack leptin and have been found to be infertile provide some clues. It has been proposed that leptin can stimulate the activity of the reproductive endocrine system inducing fertility in both sexes (Spicer, 2001). Also, the elegant work by Cassy et al. (2004) showed that ad libitum feeding of broiler breeder hens dramatically up-regulated expression of the leptin receptor in the granulosa cells of yellow follicles while feed restriction reduced the level of expression of the leptin receptor and maintained the expression of the receptor in proportion to follicular maturation. Since ad libitum feeding has been shown to affect the hierarchical endocrine order of the follicles, leptin could help explain the reported disruptions of follicular hierarchy (Cassy et al., 2004)

**The Effect of Nutrient Deficiency on Reproduction.** By definition, excessive feed restriction reduces the supply of amino acids required for synthesis of proteins. Therefore, feed restriction may reduce synthesis of glycoprotein hormones that control reproduction such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid stimulating hormone (TSH). In a similar manner, extreme limitations of nutrient intake produced leanness that has been correlated with delayed onset of puberty in humans (Weimann, 2002). Excessive feed restriction in poultry directly suppressed the release of
gonadotropin releasing hormones (GnRH) and indirectly LH (Tanabe et al., 1982; Bruggeman et al., 1998). Therefore, it was postulated that the hypothalamus may be sensitive to a puberty-triggering signal related to metabolic rate or food (feed) intake (Barb et al., 2004; Barb and Kraeling, 2004). Other studies have shown that the attainment of a critical percentage body fat was necessary for attainment of puberty (Frisch, 1984; Weimann, 2002). It has been observed that males consistently gaining BW exhibited better reproductive performance than those that were more erratic in their BW gain (Cerolini et al., 1995; Brake and Peak, 1999; Romero-Sanchez et al., 2004). This may be consistent with the attainment of a minimum percentage body fat required for liver and ovary development, as well as for steroid hormone production.

Although the hypothesis of an accumulation of a critical quantity of fat has been challenged and the postulated metabolic signal has remained elusive, some recent studies have suggested that leptin, a hormone secreted by adipose tissue, may be that signal (Barb et al., 2004; Barb and Kraeling, 2004). Recent studies documenting delayed puberty in lean female ballet dancers and accelerated puberty in obese females support the concept that a metabolic signal produced by adipose tissue, or at least associated with the development of such tissue, may control the onset of reproductive function (Weimann, 2002). Leptin could act as a permissive signal triggering puberty, thus supporting the hypothesis that fat accumulation may enhance maturation of the reproductive tract (Barb, 1999; Barb and Kraeling, 2004). Leptin restored fertility in obese mice that were genetically deficient in leptin (Chehab et al., 1996). Leptin may act as a signal for puberty, as evidenced by its ability to stimulate GnRH secretion, vaginal opening, onset of the first estrous cycle, and maturation of reproductive functions concomitant with changes in LH levels (Spicer, 2001; Zhang et al., 2005b).
The activity of the reproductive axis has been shown to be sensitive to the adequacy of nutrition and the stores of metabolic reserves (Nolan et al., 1990; Zhang et al., 2005a). Severely food-restricted animals exhibit reduced circulating levels of leptin (Richards, 2003), which has been associated with markedly reduced secretion of the gonadotropins, LH and FSH, and inhibition of the activity of the neuroendocrine reproductive axis in both sexes (Barb et al., 2004; Barb and Kraeling, 2004). Treatment of food-restricted mice (Ahima et al., 1997), rats (Cheung et al., 1997), and chickens (Paczoska-Eliasiewicz et al., 2003) with exogenous leptin reversed the diet-induced inhibition of gonadotropin secretion. Leptin has also been suggested to have a role in timing the onset of puberty in several species (Barb and Kraeling, 2004), although evidence that leptin was the primary metabolic signal for initiating the onset of puberty in any species remains controversial. However, additional research must be conducted to determine the mechanisms that control the interaction between nutrient balance, leptin, and reproduction. On the other hand, broiler breeder males deposit significantly lower quantities of fat than do females, suggesting that the leptin theory was more suitable for females and that perhaps other hormonal mechanisms could play important roles in males.

**Hormonal Control of Male Reproduction.** Male sexual development, function, and behavior have been clearly demonstrated to be under endocrine and environmental control (Johnson, 1986; Duncan et al., 1990). The hypothalamic-hypophysal-gonadal axis controls all physiological and metabolic events that occur during sexual development and mating. The release of GnRH has been shown to control the subsequent release of FSH and LH from the hypophysis (Meyer, 1986). These dimeric glycoprotein hormones bind to particular receptors located in the Sertoli cells and Leydig cells (Johnson, 1986) with their effects mediated by
cyclic adenosine monophosphate (cAMP) dependent kinases that regulate a multitude of intracellular cascade (second messenger) reactions.

Steroidogenic activity of the Leydig cells was reported to be controlled by LH in the testis while FSH stimulated Sertoli cells located in the seminiferous tubules to induce aromatase activity (Johnson, 1986). Sertoli cells produce inhibin and activin to regulate FSH release at a central level (long loop) (Thurston and Korn, 2000). Inhibin acts primarily to inhibit FSH and secondarily GnRH, while activin stimulates FSH release. Sertoli cells also produce small amounts of estrogens that inhibit FSH in males, and the second messenger cAMP results in the production of transferrin necessary to provide iron for spermatogenesis (Suire et al., 1996). The availability of activin or inhibin was reported to be regulated at the hypophyseal level by follistatin, a monomeric glycoprotein, the messenger ribonucleic acid (mRNA) of which has been found in the testes of roosters (Davis and Johnson, 1998).

**Testes Development and Male Fertility.** During the first weeks of life the male seminiferous tubules become organized, primary spermatocytes appear, and subsequent multiplication of the spermatogonia occurs. At about 10 wk of age, as a result of the reduction-division (meiosis) of the primary spermatocytes, the secondary spermatocytes begin to appear. However, only after 12 wk of age can spermatids be observed in the seminiferous tubules, and by 20 wk of age these cells have usually been found to be present in all of the tubules (Johnson, 1986). A phase of rapid testicular growth and the completion of spermatogenesis (from 16 to 24 wk of age) has been reported to characterize the onset of puberty in the cockerel (Johnson, 1986).

The growth and development of the testes was found to be largely influenced by both internal and external factors that trigger the hypothalamic releasing factors that induce the secretion
of gonadotropic hormones. Although age and photoperiod influence hypothalamic
neurosecretory events, this activity was shown to be facilitated by the permissive effect of
thyroid hormones and the endocrine-paracrine action of growth hormone (GH) (Hull and
Harvey, 2000a).

FSH has been found to stimulate growth, differentiation, and spermatogenesis in the
seminiferous tubules; thus determining spermatozoal production following sexual maturity.
Developing testes exhibited hyperplasia of the Sertoli cells sufficient to block formation of
the Mullerian tissues (female oviduct). On the other hand, LH promoted production of
androgens required for development of the male ductal system, as well as male external
genitalia. Following these initial events, the testes has been found to grow to a certain size
and become quiescent, awaiting the time of sexual maturity (Thurston and Korn, 2000).

Thurston and Korn (2000) defined three basic components for a neural system that regulated
puberty in birds: “1) encephalic receptors located in the septal and mediobasal hypothalamic
regions that can respond to light; 2) a GnRH pulse generator and a primary neural system
comprising the hypothalamic-pituitary-gonadal axis that was coordinated and controlled by
hypothalamic nuclei, intracellular calcium, and an intrinsic GnRH pulse generator; and 3)
nearl loci containing elements that activated the HPG axis.”

At the onset of puberty, the increase in plasma LH level can be accelerated in birds reared on
short days by increasing the day length (Sharp and Gow, 1983). This LH release was
reported to be controlled by the negative feedback effect of testicular steroids. Testosterone
and other androgens have been shown to be potent inhibitors of LH secretion (Johnson,
1986). However, estrogens, present in small quantities in the male, were even more potent
inhibitors of LH secretion and played a crucial role in the negative feedback control (Sharp and Gow, 1983).

On the other hand, puberty has a behavioral component regulated by gonadal hormones (Ottinger, 1983). This behavior has been shown to include courtship, mating, and dominance structure (Duncan et al., 1990). Testosterone stimulated growth of the comb and wattles in the rooster (Nakamura and Tanabe, 1973) and testicular development in male quail (Follett and Maung, 1978). Additionally, testosterone has been reported to act at the level of the brain to regulate gonadotropin secretion and sexual behavior (Balthazart et al., 1981). Injected testosterone elicited precocial strutting and crowing, and males receiving testosterone restored their dominant position lost following castration. Stress conditions, including aggressive encounters, nutritional deprivation, and handling can reduce plasma concentration of LH, affecting testosterone production, mating behavior, and fertility (Johnson, 1986). The pineal gland has also been postulated to affect the development of sexual maturation through hypothalamic centers that contain releasing factors that subsequently act on the hypophysis (Meyer, 1986).

As was previously mentioned, thyroid hormones have important effects on spermatogenesis and testes development. This effect has been studied using the reversible goitrogen 6-N-propyl-2-thiouracil (PTU). Administration of PTU in the prepuberal male (6-12 wk of age) resulted in increased adult testis size, spermatogenic efficiency (spermatozoa/g testis), and early spermatozoal production (Kirby et al., 1996). GH has been thought to play an important role in testicular growth and development, since human GH was associated with abnormally small testes, as well as reduced testes functions such as steroidogenesis and gametogenesis. GH may alter gametogenesis by affecting testosterone synthesis, since testosterone was
shown to be necessary for spermatozoal production and a mRNA coding growth receptor was found to be present in Leydig cells (Hull and Harvey, 2000a; 2000b).

Effect of Nutrition on Male Fertility. Detailed studies of the nutritional requirements of adult male birds have been conducted in an attempt to optimize reproductive potential. Restricted feeding regimes have been routinely used in the broiler breeder industry to control BW and lengthen the reproductive lifespan. However, if the diet was insufficient, reproductive performance was found to decline (Ottinger, 1982). Nutritional status has been shown to affect semen volume and quality, fertility, hatchability, and BW of hatched chicks (Maslieu and Davtyan, 1969). Nutrition can exert its effects through activation or inhibition of hormonal response, the supply of essential components or cofactors for reproductive function (Scanes and Harvey, 1982), or supply of contaminants such as mycotoxins (Ortatattli et al., 2002). Circulating nutrients derived from dietary sources may exert direct effects on LH secretion at the pituitary level or indirect effects by decreasing LHRH release from the median eminence (Buonomo et al., 1982).

Effect of Metabolizable Energy (ME) Intake on Reproduction. Specific ME requirements have been established for maintenance, growth, and egg production. However, ME requirements for optimum reproductive performance in males have not been established; although it has been shown that excess or deficient intake of ME can have negative effects. It has also been suggested that a minimum cumulative ME requirement was needed during rearing for optimal reproduction (Brake and Peak, 1999). Furthermore, the ME intake of broiler breeder males can impact the BW of the progeny (Attia et al., 1995). Several studies have demonstrated the deleterious effects of overfeeding on leghorn breeders, turkey breeders, and broiler breeders. These negative effects were generally attributed to
excess BW, rather than a deficit ME intake, as the major factor negatively affecting fertility (Lopez and Leeson, 1994). However, many data suggest that excessive ME intake can directly affect spermatogenesis irrespective of BW (Robinson et al., 1982). Overfeeding of male Leghorn breeders resulted in a dramatic decline in total spermatozoal production with an associated increased incidence of dead spermatozoa. The introduction of separate male feeding systems has resulted in better fertility in broiler breeders, apparently simply because of more precise control of the feed intake of the roosters.

Conversely, a number of studies have reported that limiting nutrient intake, and therefore BW gain, delayed onset of puberty in many species (Nolan et al., 1990). The effects of decreased nutrient intake, as related to puberty, have been examined primarily at the testicular level. As an example, when bulls were found to gain BW at a higher than normal rate, there was an increased size of the Leydig cells and subsequently increased testosterone production. Increasing dietary ME intake also enhanced Sertoli cell function, resulting in expanded seminiferous tubules (Nolan et al., 1990).

When broiler breeder males received low ME diets, they were able to maintain their BW with little or no surplus ME remaining for BW gain. In this scenario, the spermatozoal maturation process might be altered and motility affected so that fewer spermatozoa would reach the uterovaginal spermatozoal storage sites and a lower degree of polyspermy would result (Attia et al., 1995). Although testes weights could be adversely affected by a low ME diet, there was not always a clear relationship between testes weight and semen production (Brown and McCartney, 1986; Wilson et al., 1987). Further, mating frequency was reduced in response to low ME intake, which reduced polyspermy.
It has been suggested that a cumulative ME requirement prior to photostimulation exists for male broiler breeders. This requirement has been suggested to be between 29.5 and 32 Mcal per male (Brake and Peak, 1999; Romero-Sanchez et al., 2004). During the mating period various maintenance ME requirement values have been estimated. Attia (1993) suggested 81-95 kcal ME/kg BW/d. This value was in agreement with traditional values obtained using maintenance equations (Combs, 1968). These traditional maintenance values must have included activity as they were higher than those suggested by Sexton (1989), who estimated the requirement for males in cages to be only 56 kcal ME/kg BW/d. Although no requirements for sexual activity have been established, Peak (2001) with a prediction model suggested that 7 kcal/kg BW/d was sufficient to account for sexual activity during the period of maximum fertility.

**Effect of Crude Protein (CP) Intake on Reproduction.** Although the same general principles of ME intake may be applied to CP intake, there have been specific differences described that must be considered. Recently, it has been commonly accepted that broiler breeder males could be fed low CP diets and maintain fertility at levels equal to or better than those obtained with conventional dietary CP concentrations in the range of 15 to 18% (Wilson et al., 1987). The reported adverse effect of high CP diets could be explained as a consequence of the elevated plasma uric acid concentration associated with the excretion of the nitrogen from excess protein catabolism (Wilson et al., 1987). Although separate sex feeding during the laying period seems to ameliorate the problem of excess caloric intake, there was an apparent advantage when low CP diets were used (McDaniel, 1986). However, excessive restriction of CP intake can delay sexual development and impair fertility. In fact, sexual maturity of White Leghorn cockerels was delayed, and spermatogenesis arrested, by
feeding diets with 8.0% CP or less from 7 to 21 wk of age (Jones et al., 1967). Protein restriction (with isocaloric diets) decreased the circulating concentration of LH in young growing birds (Bruggeman et al., 1998). This effect was more severe in males than females and accounted for decreased gonadal weight. A similar condition has also been reported in mammals (Dong et al., 1994).

Male BW was not affected by dietary CP when different levels of CP were fed during the rearing period and up to 52 wk of age (Wilson et al., 1987). More recently, with new genetic strains, it was found that males fed a 17% CP diet during the rearing period were heavier than those receiving 14% CP diets, but these differences tended to disappear after sexual maturity when all males had received the same 16% CP diet for a period of time (Brake, 2002).

Dietary CP had a significant linear effect on the percentage males producing semen during an early evaluation. When diets between 12% and 14% CP were fed on a restricted basis during the rearing of broiler breeder males, BW, sexual maturation, duration of semen production, and semen quality were not affected (Wilson et al., 1987). However, with artificial ejaculation, a higher percentage of males fed the 12% and 14% CP diets produced semen from 27 to 30 wk of age than males fed higher CP diets. No significant differences in testis weight or number of spermatozoa per ejaculate were noted. This effect could have been an artifact of having a leaner animal that was simply easier to manipulate during the ejaculation process. Moreover, in recent years, continued intensive genetic selection could have changed dietary requirements. This can be observed in quail when, after several generations of selection for heavy BW on high CP diets, there was an obvious delay in sexual maturity. However, when parent stock birds from this selected line were fed higher CP diets during rearing the delay in sexual maturity was noticeably reduced (Lilburn et al., 1992). These data
made the strong suggestion that the nutrient requirements of the male could have been altered by genetic selection within a particular nutritional regime, since most of the genetic selection has been focused on enhanced broiler traits.

Most of the results dealing with fertility have been attributed to either individual performance and/or increased mortality. It was important, however, to point out that the level of CP can affect individuals differently, and thus the observed effects may have been due to an altered proportion of males producing semen rather than an effect on semen production and quality per se (Hocking and Duff, 1989).

**Feeding Programs and Growth Curves for Broiler Breeder Males.** While small differences in dietary CP intake have been found to produce only slight differences in BW, small differences in ME intake have been shown to affect BW more profoundly (Vaughters et al., 1987). Additionally, the weekly increments of the feed program may affect BW gain and body composition during the rearing period, but especially during the pre-lay and early production periods.

Growth can be described mathematically using various linear or non-linear models (Rogers et al., 1987). These models have been useful for predicting nutritional requirements and deposition of nutrients by the body (Peak, 2001; Sterling et al., 2005). Although most of these growth curves have been sigmoidal, the growth curve can also be simplistically described as linear, concave, or convex (Leeson and Summers, 1997). Following this approach we can deduce that birds growing on a concave pattern have more efficient feed utilization. Sigmoid curves can have different interpretations depending upon how body composition was affected. Although most of this type of research has been conducted in broilers that were consuming feed on an *ad libitum* basis, there exists some data that showed
male breeders growing in a concave pattern to have a more predictable BW, due to greater cumulative nutrition and lower mortality. Broiler breeder males growing on a linear or sigmoid curve presented greater BW at an early age, with subsequently higher maintenance requirements and subsequently lower BW gain (Peak, 2001). The form of the growth curve may determine how the bird deposits lipids and protein and how the different tissues contribute to heat production, especially in conformation strains (Spratt et al., 1990). It has been found that tissues of the liver, gut, and reproductive tract, which together make up 6% of the BW, account for 30% of the total ME expenditure (Spratt et al., 1990). The poultry breast muscle is high in white-type fibers that have higher glycolytic potential, higher amounts of glycogen, and lower oxidative metabolism when compared to red fibers (Forrest et al., 1975). Since the breast muscle can represent a high proportion of the broiler breeder BW, it may contribute an important proportion of the overall heat production of the bird.

Body composition and BW play a fundamental role in male fertility and have also been shown to be critical for maximum production of chicks with the best potential for growth from broiler breeder hens. It has been assumed that if the hen was producing an egg that the fertility depended on the presence of spermatozoa in the oviduct. Although the female can contribute to spermatozoal storage in the oviduct (Breque et al., 2003), the male continues to play a major role in fertility through mating frequency and mating success. A limited daily feed restriction program during the rearing period has been shown to decrease testes weight, semen volume, and spermatozoal concentration compared with an every-other-day program (Sexton and Renden, 1988), probably because with the daily program the efficiency of ME utilization was higher (Leeson and Summers, 1997) and therefore these birds received less cumulative nutrition during the rearing period if the treatments were grown to a similar BW.
**Photoperiodic Responses in Chickens.** Once the thresholds for minimum BW and age have been attained, entry into lay ensues promptly in chickens provided with an adequate photoperiod. Entry into lay may be delayed, but ensues eventually, in birds kept in total darkness or on short day lengths (Wilson and Woodward, 1958; King, 1961). Upon adequate photostimulation, entry into lay takes place under the control of the pituitary gonadotropins, which stimulate gonadal development. Of these, LH has been studied most extensively. In particular, upon photostimulation, an increase in LH has typically been found (Follett and Pearce-Kelley, 1990; Dunn and Sharp, 1992; Juss et al., 1995). However, there has been a great deal of baseline fluctuation observed prior to photostimulation (de Reviers and Williams, 1984). Photoperiodic information controlling LH release has been suggested to be transduced via extra-retinal photoreceptors, located in or near the hypothalamus, which provide input to hypothalamic neurons synthesizing and secreting GNRH-I and GNRH-II (Sharp et al., 1990). Following attainment of photosensitivity, the magnitude of the response to photostimulation, as measured by the time from photostimulation to first egg, has varied. Presumably, differences in the time to first egg were a consequence of differences in hormonal responsiveness to photostimulation by the hypothalamic-hypophyseal-gonadal axis. This suggestion was supported by a study showing that higher prepuberal LH levels were associated with more rapid comb growth and earlier onset of lay (Sharp, 1975) and by the observation that baseline LH levels were higher in commercial layers than in a dwarf broiler stock (Dunn and Sharp, 1990).

**Photoperiod and Broiler Breeder Reproductive Performance.** The response of a chicken to a specific photoperiod during lay has been shown to be strongly dependent upon the photoperiod during the growing period (Morris, 1967; Ernst et al., 1987; Etches, 1996).
Information with respect to light intensity has been less clear but the general picture has been of a need to have a equivalent or slightly increased intensity in the breeding quarters relative to that in the growing quarters (Harrison et al., 1970; Brake and Baughman, 1989; Brake et al., 1989). Effects of photoperiod in the male have not been as well studied as in the female (Lake, 1969). Turkey toms exposed to increased light intensity at sexual maturity reached peak spermatozoal concentration earlier (Jones et al., 1977) and exhibited greater semen volume (Leighton and Jones, 1984). The pubertal phase of testis development can be hastened by photostimulation but this has generally led to reduced testis weights and poor persistency of testis size (de Reviers, 1977). It was also found that a large increase in photoperiod did not advance sexual maturity but did produce larger testes weights (de Reviers, 1980). However, larger peak testes weights have often been associated with more rapid testicular regression in heavy BW line males (de Reviers, 1980).

The males of wild avian species exhibit an earlier onset of sexual maturity than do the females (Meier and MacGregor, 1972). This may be due to a reduced threshold sensitivity to day length and/or reduced nutrient requirement in the male. However, this may not be true for sire-line males and dam-line females used as broiler parent stock as these animals come from quite different genetic selection programs. Photostimulation of parent stock males in advance of their female counterparts has been recommended (Etches, 1996) but has not been generally practiced due to the intermingling of sexes in many commercial facilities during the rearing period. Brake (1990), however, showed that when broiler breeder males were reared together with females, an increase in photoperiod from 8L:16D to 10L:14D at 18 wk of age increased fertility when compared with an 8L:16D control group with all birds shifted to 14L:10D at 20 wk. Some aspect of mating per se may be involved in these results, as
these males did not differ in BW between treatments. Turkey hens exhibited an intense desire to mate preceding the onset of egg production (Margolf et al., 1947; Carte and Leighton, 1969). When turkey hens were inseminated during this period of prelay receptivity there was a significant increase in life-of-flock fertility in the presence of marginal spermatozoal numbers (McIntyre et al., 1982). This early mating presumably led to enhanced spermatozoal storage and this mechanism may also be present in chickens.

**Interaction of Nutrition, Genetics, and Photoperiod.** As stated previously, selection of quail on a 28% CP diet for heavy BW at 28 d of age resulted in increased BW but a delayed onset of egg production when the breeding stock was reared on the NRC (1994) recommended 24% CP diet. However, rearing on a 30% CP diet significantly reduced the delayed onset of lay without a change in BW at first egg (Lilburn et al., 1992). Studies have reported a decline in broiler breeder male fertility associated with selection for rapid juvenile growth-rate in broilers (Soller and Rappaport, 1971; Vankrey and Siegel, 1974). However, it should be noted that the decline in fertility was observed as the rearing plane of pullet/cockerel nutrition prior to reproduction was not altered as genetic selection for broiler growth-rate on a high CP broiler feed progressed, similar to what was discussed above for quail. Selection for broiler feed conversation has also made significant advances, implying altered nutritional requirements for parent stock. Calculations from published data (Walsh and Brake, 1997) showed that a female broiler breeder grown to a 2.0 kg BW at 20 wk could be accomplished on 22,000 kcal ME whereas 28,000 kcal were required ten years earlier (Brake and Peak, 1999). As there has been no basic change in the ME/CP ratio of typical diets during this time period, this calculated improvement in feed conversion represented a 12% reduction in ME required to achieve a similar female BW. This suggested that males
could also be grown to a recommended BW on insufficient nutrients if a similar minimum
existed for males (Brake and Peak, 1999). Such a minimum for CP does indeed appear to
exist for the female broiler breeder, as well as for males. Irrespective of BW, any females
that consumed less than 1,180 g CP at photostimulation exhibited problems with persistency
of fertility (Walsh and Brake, 1997;1999). This suggested that photostimulation could be
viewed as some sort of “switch” that changed the bird from a nutrient accumulation mode to
a nutrient utilization mode. The extensive French work, led by de Reviers (1977; de Reviers,
1980; de Reviers and Williams, 1981; de Reviers and Wiliams, 1984; de Reviers and
Seigneurin, 1990) showed that heavy BW male lines exhibited greater problems with
persistency of testes size and semen production than did medium BW male lines.
Photostimulation of heavy BW line males typically resulted in a robust, but short, response in
testicular weight and semen production while males from medium BW lines exhibited better
persistency. It was presumed by the present author, as no nutritional data were given, that
both lines were being fed a typical diet that may have been marginal for the heavy BW line
males (Walsh and Brake, 1997) in a manner similar to quail as discussed above (Lilburn et
al., 1992). This problem, from an artificial insemination view, could be solved by simply not
photostimulating the heavy BW line males (i.e., never throwing the “switch”) and allowing
these birds to come into semen production late, presumably after accumulating sufficient
nutrition (Brake and Peak, 1999).

The Role of Antioxidants in Reproduction. The high proportion of polyunsaturated fatty
acids in the lipid fraction of spermatozoa reflects the need to maintain high membrane
fluidity and flexibility for spermatozoal motility and fusion with the egg (Blesbois et al.,
1997). Natural antioxidants (Vitamin E, Vitamin C, and glutathione) together with
antioxidant enzymes combine to give avian semen an integrated antioxidant system capable of protecting the cells from damage from free radicals and toxic products of metabolism (Surai et al., 1998a; Surai et al., 1998b; Breque et al., 2003).

After ejaculation, spermatozoa have been shown to be protected from environmental stress by the antioxidants contained in the seminal plasma (Kobayashi et al., 1991). In general, seminal plasma has been regarded as an excellent nutritive and protective medium for spermatozoa on the basis that it possessed higher concentrations of antioxidants than other biological fluids such as blood serum (Lenzi et al., 2000). The observation that seminal plasma can protect spermatozoa against lipid peroxidation has been reported for mammalian semen (Chen et al., 2003). For example, human seminal plasma effectively prevented, but did not reverse, the toxic effects on spermatozoa of either endogenous or exogenous lipid peroxides (Jones et al., 1979). In avian species, there has also been evidence that seminal plasma can protect spermatozoa against peroxidation (Breque et al., 2003). Firstly, it has been shown that in seminal plasma, calcium, and citrate reduced peroxide production in the spermatozoa by more than three-fold but bovine serum albumin and cholesterol had no such effect (Fujihara and Koga, 1984). In the same experiment, fertilized eggs were obtained from birds inseminated with spermatozoa diluted with seminal plasma extended with phosphate buffer, calcium, or citrate. A similar protective effect was observed with turkey semen (Cecil and Bakst, 1993).

It has been shown that the free radical trapping activity of chicken seminal plasma was dose dependent (Surai et al., 1998a) and that blood plasma free radical trapping activity was only half that of seminal plasma. It was interesting that incubation of seminal plasma for 24 h at 20 C, or boiling the seminal plasma for 10 min, did not reduce its free radical trapping
capacity (Surai et al., 1998a). Therefore, proteins were unlikely to play a major role in such a defense as they have been shown to be heat labile. The total antioxidant activity of chicken seminal plasma was found to be comparable to that in human seminal plasma (Gavella et al., 1996). In fact, human seminal plasma possessed a high antioxidant buffering capacity that protected the spermatozoa from oxidative stress (Rhemrev et al., 2000). Decreased seminal plasma antioxidant activity and increased reactive oxygen species production have been considered to be responsible for idiopathic male infertility (Alkan et al., 1997). Some of the antioxidants such as Vitamin C, Vitamin E, and uric acid exert immediate free radical trapping, whereas hypotaurine and tyrosine exhibit the same slow free radical trapping curve as seminal plasma (van Overveld et al., 2000). In general, in human seminal plasma, ascorbate, urates, and thiols have been considered to be the major antioxidants (Lewis et al., 1997). The rate of oxidative metabolism in chicken spermatozoa was very similar to that in turkey spermatozoa although turkey spermatozoa were very dependent on oxidative metabolism to maintain optimal adenosine triphosphate (ATP) levels (Wishart, 1982). However, the compounds of chicken seminal plasma responsible for this activity remain to be elucidated (Surai, 2002).

**Vitamin E and Vitamin C in Male Fertility.** Vitamin E deficiency has been shown repeatedly to affect mammalian (Thiessen et al., 1975) and avian male fertility (Surai, 1999;2000;2002). Low Vitamin E levels decreased hormone synthesis in the Leydig cells, thus increasing the feedback mechanism control of LH secretion (Akazawa et al., 1987). Vitamin E has been considered to be the main antioxidant of biological membranes (Gotoh et al., 1996). It reacts with free radicals producing ROOH groups. Due to its location inside the membrane, Vitamin E effectively scavenges free radicals. There has also been recently
discussed a mechanism to recycle Vitamin E from its oxidized form. Therefore, even a low concentration of Vitamin E may be able to maintain a high level of antioxidant protection in physiological conditions (Leeson and Summers, 1997).

Briefly, the presence of high levels of polyunsaturated fatty acids in avian spermatozoa provide conditions for incorporation of Vitamin E into the membrane (Surai et al., 1999). It has been suggested that Vitamin E played a role as a biological stabilizer of the spermatozoal plasma membrane (Surai, 1999; Surai et al., 2001; Surai, 2002), making spermatozoa more resistant to the stresses incurred during artificial insemination, short term storage, and cryopreservation (Surai, 2002). It was notable that in experiments with turkeys, the best results were obtained when dietary Vitamin E supplementation was increased from 20 to 80 mg/kg, although under commercial conditions, dietary supplementation of breeding toms with 90 mg/kg diet has been observed (Surai, 1999).

The dietary induced increase in the Vitamin E content of semen was found to result in a significant reduction in the susceptibility of the semen to lipid peroxidation. In fact, the susceptibility of semen to peroxidation displayed a highly negative correlation ($r = -0.998$) with the Vitamin E content of the semen. The susceptibility of testes homogenates to in vitro peroxidation was also reduced by dietary supplementation with Vitamin E (Surai et al., 1998c). Regarding Vitamin C, it has been shown that supplementation with 500 mg/kg of diet in cockerels housed under hot and humid tropical conditions improved semen volume, motile spermatozoa per ejaculate, and spermatozoal numbers per ejaculate (Monsi and Onitchi, 1991). A protective role of Vitamin C in the maintenance of the spermatozoal quality of fish has been suggested as well (Ciereszko et al., 1996). In fact, the supplementation of rainbow trout diets with ascorbyl monophosphate was associated with
reduced lipid peroxidation while Vitamin C concentration increased in seminal plasma (Liu et al., 1997). Another study in trout showed that Vitamin C supplementation improved motility and fertilizing ability after storage (Ciereszko and Dabrowski, 2000). A significant decrease in fertilization rate and hatching rate of embryos resulted when the seminal plasma ascorbate concentration in rainbow trout decreased to 7.3 µg/ml (Dabrowski and Ciereszko, 1996). In a similar manner, it has been shown that dietary Vitamin C can protect human spermatozoa from oxidative DNA damage that could affect spermatozoal quality and increase the associated risk of genetic defects (Fraga et al., 1991).

On the other hand, different studies have shown contrasting results regarding the additive effects of Vitamin E and Vitamin C acid on male fertility. For example, dietary supplementation with low doses of Vitamins C and E did not produce any effect on mouse spermatozoal quality; however, high doses of these vitamins decreased the number of spermatozoa/mg of epididymis and increased the percentage of spermatozoa with misshapen heads (Ten et al., 1997). In contrast, in rabbits, it was found that increased levels of Vitamin C and Vitamin E improved spermatozoal viability and fertilizing ability (Castellini et al., 2000).

**Metabolic Role of Selenium.** Selenium (Se) was discovered in 1871 by Berzelius, and its biological significance has remained controversial. It was initially considered the most toxic of the essential trace elements but was then recognized as an essential mineral that must be added to animal feed (Schrauzer, 2000). Chronic selenosis (“alkali disease”) has been characterized by dullness, emaciation, ataxia, neurological symptoms including demyelination, roughness of coat, loss of hair (especially around the mane and tail of horses and the tail switch in cattle), cracking and even sloughing of hooves or complete loss of the
hoof wall, lameness, atrophy of the heart, and cirrhosis of the liver. In chickens, excessive levels of selenium caused reduced growth rate, egg production, and hatchability, and caused embryo abnormalities such as swollen heads due to liquid accumulation and tissue hypertrophy (Ort and Latshaw, 1978).

The chemistry of selenium is similar to sulfur. Inorganic forms of sulfur and selenium include hydrogen sulfide and hydrogen selenide while common corresponding organic sulfides and selenides are cysteine and selenocysteine, methionine, and selenomethionine (Jacques, 2001). Therefore, one of the mechanisms by which selenium exerts its toxic effects in animals appears to be through its competition with sulfur or through its strong affinity for sulfur in the formation of sulfur-selenium complexes (Leeson and Summers, 2001). Recent research has shown that “blind staggers” may be associated with high sulfur intake and not always with selenium toxicity. Selenium can substitute for sulfur in methionine or cysteine to form the analogs, selenomethionine and selenocysteine, and disrupt certain biochemical reactions and cellular enzyme functions (Jacques, 2001). Since animals cannot synthesize selenomethionine or distinguish it from methionine, it becomes incorporated into a wide range of selenium-containing proteins (Spallholz and Hoffman, 2002). Thus, selenium competes with sulfur for sites at which sulfur normally plays a role in cellular respiration (Schellmann et al., 1986; Ahmed et al., 1990). It has been thought possible that the presence of excess selenium analogs of sulfur-containing enzymes and structural proteins have played a role in avian embryo malformations and embryo toxicity readily caused by dietary selenomethionine. In adult mammals and birds, damage to body structures such as hair, nails, hoofs, and feathers, all of which contain keratin (high in sulfur), becomes more
apparent in the presence of excess dietary selenium (Leeson and Summers, 2001; Spallholz and Hoffman, 2002).

Although the metabolic fates of dietary inorganic and organic selenium forms differ, in order to be used in selenoprotein synthesis (including dietary selenocysteine) selenium must first be converted to the inorganic selenide, hydrogen selenide (H₂Se) before the process of forming and incorporating specific selenocysteine into the active site of the proteins can begin (Daniels, 1996). Hydrogen selenide has been shown to be a key intermediate in the selenium methylation of inorganic and organic selenium compounds in animals (Ganther, 1999). Hydrogen selenide has been reported to accumulate in animals receiving excess selenocysteine as a consequence of inhibition of selenium methylation. Also, excess hydrogen selenide has contributed to hepatotoxicity and could contribute to other selenium-related injuries. Hepatotoxicity has been caused by repeated oral administration of selenocysteine (Nakamuro et al., 2000). Selenocysteine may be metabolized by reduced glutathione and/or glutathione reductase to hydrogen selenide via selenocysteine-glutathione selenenyl sulfide (Hasegawa et al., 1995; Hasegawa et al., 1996).

Selenium has been shown to exist in several oxidation states: selenite (SeO₃⁻²) has been considered to be the toxic form of this element, whereas selenate (SeO₄⁻²) has been considered to be relatively unreactive in biological systems, although some plants can take up selenate and incorporate it into selenocysteine and selenomethionine (Wolffram, 1999). Excessive intake of selenium could lead to toxic effects through the binding of selenite and its biological metabolites (selenide and methylselenol) to sulfhydryl-sensitive enzymes, including squalene monooxygenase in neural tissues (Gupta and Porter, 2002).
On the other hand, although selenium has a role as antioxidant, even when toxic levels of selenium were present, pronounced oxidative stress caused oxidation of thiols in glutathione (Chaudiere et al., 1992) increasing superoxide generation (Yan and Spallholz, 1993). Selenite has been shown to be toxic because it forms superoxide by the oxidation of glutathione in a dose-dependent manner (Yan and Spallholz, 1993). However, both organic and inorganic selenium were able to induce apoptosis by triggering the mitochondrial permeability transition, mediated by cross-linking of protein thiols groups and by generation of superoxide (Kim et al., 2003).

It has been shown that flavin monoxygenase produced selenoxides from organoselenium compounds, such as selenomethionine (Spallholz and Hoffman, 2002). In the presence of NADPH these selenoxides may release seleninic acid (Chen and Ziegler, 1994; Rooseboom et al., 2001). L-Selenomethionine can also produce superoxide radicals in the presence of the enzyme methioninase (Spallholz et al., 2004). Therefore, L-Selenomethionine can be toxic in the presence of the enzyme methioninase. (Palace et al., 2004) suggested that methioninase activity may be responsible for the teratogenesis observed in offspring of adult fish that have ingested elevated concentrations of selenomethionine. It was interesting to recall that in this reaction, methylselenol was the enzymatic product generating superoxide radicals in vitro. Methylselenol and other monomethylated forms appeared to be the selenium metabolite most effective in chemoprevention (Ganther, 1999).

Furthermore, several research reports have described the induction of apoptosis by sodium selenite as well as damage to DNA as measured by the demonstration of breaks in single stranded DNA and DNA ladders. Rather than inhibiting oxidative DNA damage, these findings have suggested that high dietary intake of inorganic selenium may promote in vivo
DNA oxidation. In addition, mitosis was inhibited by selenium at metaphase (Wycherly et al., 2004).

The essentiality of selenium for prevention of liver necrosis and exudative diathesis was demonstrated in the 1950s (Schwarz and Foltz, 1957; Leeson and Summers, 2001). However, the biochemical role of selenium only emerged after Rotruck et al. (1973) discovered that glutathione peroxidase (GSH-Px) was a selenoprotein. Thereafter, many other selenoproteins have been discovered and the scope of selenium biochemistry has broadened from antioxidant defense to other aspects including thyroid hormone activation, participation in the thioredoxin system, and fertility (Allan et al., 1999).

Effects of selenium on fertility and reproductive performance have been explained through its antioxidant function as a component of the GSH-Px system. In poultry females selenium deficiency was shown to decrease egg production and hatchability (Cantor and Scott, 1974). The requirement for all commercial poultry types has been determined to be 0.3 ppm (NRC, 1994). On the other hand, selenium supplementation in gilts above 0.1 ppm did not have any beneficial effect on female reproduction or GSH-Px activity (Mahan and Kim, 1996). However, when the criterion of adequacy was selenium content in milk or tissues, the requirement was 0.3 ppm and the organic selenium was more efficient than the inorganic form (Mahan and Kim, 1996).

**Selenium in the Male Reproductive Tract.** Wu et al. (1973) found that selenium had a specific role in spermatogenesis that could not be replaced by Vitamin E or any other antioxidant. They tested the effect of selenium with and without Vitamin E supplementation on spermatozoal morphology and histology. Selenium deficient diets with Vitamin E supplementation did not affect active spermatogenesis, but diminished motility, and caused
abnormal spermatozoal forms. The most important abnormality was the breakage of the spermatozoal tail. It was hypothesized that selenium might be essential to maintain the quality of the membrane that envelops the axial filament. Subsequently, this group (Wu et al., 1979), using electron microscopy, confirmed that the damage caused by selenium deficiency was in the spermatozoal mid-piece and appeared to affect the membrane system, but did not affect the head portion. These authors proposed that selenium played a direct role in spermatozoal structure, and its presence in the seminal plasma appeared to protect spermatozoal membranes from damage by metabolic free radicals.

Several years later, it was found that the selenium content of testes and associated GSH-Px activity increased in the maturing rat (Behne et al., 1986). These authors measured selenium content and GSH-Px activity in rats at six different ages. An increase of 500% in testes selenium content between 20 to 55 d of age was observed. The activity of the seleno-enzyme GSH-Px, which was measured using H$_2$O$_2$ as a substrate, also increased in the testes with increasing age. Since the changes in selenium content were higher than changes reflected in GSH-Px, it was suggested that selenium must be present in the form of other compounds. The increase in the selenium content during maturation served mainly to ensure that sufficient amounts of the element were available for incorporation into the spermatozoa in the form of a specific selenoprotein that would appear to be necessary for the normal development of the spermatozoa. Since the proportion of selenium in GSH-Px versus the total amount of selenium in testis was small, it could be assumed that the enzyme was not a part of the metabolic pathway that led to the incorporation of the element into the spermatozoa. Calvin et al. (1987) then found that essentially all of the selenium in the rat
spermatozoa was bound to a polypeptide confined to the capsule that surrounded the spermatozoal mitochondria.

The male reproductive tract contains, on a cellular basis, some of the highest concentrations of selenium in the body (Smith *et al.*, 1979). Most of the selenium in the spermatozoa was shown to be contained in a selenoprotein in the spermatozoal mitochondrial capsule (Calvin *et al.*, 1981). After selenium injection the highest rate of selenium incorporation into the spermatozoal mitochondrial capsule was observed during mid-spermiogenesis. However, this incorporation was post-transcriptional through selenation of a precursor at the aminoacyl t-RNA level (Calvin *et al.*, 1987). These authors also found that selenium deficiency led to deterioration of spermiogenesis and to decreased spermatozoal motility.

When Calvin *et al.* (1987) found that the structure that defined morphological mid-piece alterations became more evident in selenium deficiency as the likely result of impaired biosynthesis of the selenoprotein, they suggested that mature spermatozoa might depend on GSH-Px as a structural protein. Subsequently, Ursini et al. (Ursini *et al.*, 1999) found that GSH-Px in the spermatozoal mitochondrial capsule was a phospholipid hydroperoxide glutathione peroxidase (PHGPx) that changed its physical characteristics and biological functions during spermatozoal maturation. PHGPx exists as a soluble peroxidase in spermatids but persists in mature spermatozoa as an enzymatically inactive, oxidatively cross-linked, insoluble protein (Ursini *et al.*, 1999). As a consequence, it was not the antioxidant capacity of PHGPx but the ability to use hydroperoxides for the formation of a structural element of the spermatozoa that was pivotal to male fertility. The predominance of the selenoprotein PHGPx in the male reproductive system had been believed to reflect the necessity to shield germ line cells from oxidative damage. This concept was apparently true.
for early phases of spermatogenesis but mature spermatozoa depend on PHGPx as a structural protein as well (Ursini et al., 1999).

In the mid-piece of mature spermatozoa, the PHGPx protein has been reported to comprise at least half of the capsule material that embeds the helix of the mitochondria (Ursini et al., 1999). PHGPx can use protein thiols as alternate substrates to create protein aggregates that will be cross-linked by selenodisulfide or disulfide bonds. This likely occurred when cells were exposed to hydroperoxides in the presence of low concentrations of glutathione as was documented during late stages of spermatogenesis (Ursini et al., 1999).

Since selenium was highly incorporated into testes, the mechanism of distribution has become of interest. Due to its plasma location, the function of selenoprotein P (Se-P) has been postulated to be as part of a selenium transporter system. Hill et al. (2003) have studied the distribution of selenium using mice with the Se-P gene deleted. In this experiment brain and testes were identified as tissues that retained selenium even under conditions of extreme selenium deficiency. These organs removed Se-P from the plasma to acquire its selenium and no evidence was found that the testes were able to utilize a form of selenium other than Se-P (Hill et al., 2003).

**Applications of Selenium in Male Fertility.** As the role of selenium in male fertility has been recognized for many years, attempts to improve fertility have included selenium supplementation. Apparently, humans might be occupationally exposed to pro-oxidative components, which will increase the need for antioxidants. Since a defective incorporation of selenium into spermatozoal cells was a characteristic of some forms of male infertility, and human dietary patterns vary to a great extent, selenium recommendations have been broadened (Hansen and Deguchi, 1996). As a matter of fact, an increase in spermatozoal
motility in human males given 100 µg of selenium during a three-month period has been observed (MacPherson et al., 1995). Further, higher levels of plasma selenium were observed in subjects taking a selenium supplement in contrast to the subnormal selenium levels of the control group, which suggested an inadequate provision of selenium in the normal diet of some populations (Hansen and Deguchi, 1996).

In testes from selenium deficient roosters, there were fewer Sertoli cells and hierarchies of spermatogonia that were committed to spermatid formation as compared with those fed selenium. Furthermore, Leydig cells also appeared to be less developed in the basal fed birds, negatively affecting testosterone production and secretion (Edens, 2002). Although this selenium effect has not been explained, it has been suggested that gonadotropic hormones affect selenium metabolism during puberty (Behne et al., 1986) when testosterone release has been shown to increase.

Additional experiments (Edens, 2002) have shown that roosters fed a selenium deficient diet exhibited reduced ejaculate volumes and decreased spermatozoal concentrations than those given selenium supplementation (0.3 ppm) in their diets. It was also reported that organic selenium, in the form of selenomethionine, could improve fertility compared to its selenite form, because selenomethionine was taken up more efficiently by tissues. However, this response was not consistent, suggesting that the effect of selenium source was dependant upon other factors such as the male feeding program. Since selenomethionine was taken up more efficiently than sodium selenite, a different dietary allocation should be considered for organic sources than for inorganic sources to obtain the same selenium status in the animal.


de Reviers, M., and F. Seigneurin. 1990. Interactions between light regimes and feed restrictions on semen output in two meat-type strains of cockerels. Pages 220-231 in: Control of Fertility in Domestic Birds. (Les colloques de L'INRA, No. 54) ed. INRA, Tours, France.


MANUSCRIPT I. Effect of Plane of Rearing Nutrition of Broiler Breeder Males on Body Weight, Shank Length, Comb Height, Carcass Characteristics, and Fertility

ABSTRACT

Two experiments were conducted to evaluate the effects of two levels (High and Low) of cumulative nutrient intake during the rearing period to 21 wk of age on male broiler breeder performance. The High cumulative nutrition program supplied 33.5 Mcal ME and 1,730 g CP, while the Low cumulative nutrition program supplied 29.6 Mcal ME and 1,470 g CP to photostimulation at 21 wk of age. In Experiment I-1 two diets (HiDiet and LoDiet) were formulated and a single feeding program was used. In Experiment I-2, a single diet with two feeding programs (HiFeed and LoFeed) was used. In Experiment I-1 the two diets were blended from 22 to 24 wk to provide a gradual transition to a single common breeder diet that was fed during the breeder production period. At 21 wk of age in Experiment I-2, males were classified into Heavy or Light BW groups creating a 2 X 2 factorial design during the production period. The High plane of nutrition increased BW, shank length, and comb height during the rearing period but the differences disappeared after 28 wk of age. The Heavy males at 21 wk of age were also retrospectively the heaviest males at 8 wk of age. Both Low plane groups (LoDiet and LoFeed) exhibited better fertility during late production. The LoDiet group of males also produced broilers with higher BW at 42 d. A cumulative nutrient intake during the rearing period of 29.6 Mcal ME and 1,470 g CP resulted in a bird of adequate BW and physical attributes at 21 wk of age that was able to maintain good fertility throughout the production period and produce better performing broilers.

Key words: Broiler breeder males, rearing nutrition, fertility, broiler performance
INTRODUCTION

Genetic selection has been successfully used by the broiler industry to increase highly heritable variables such as BW, conformation, breast muscle yield, and feed efficiency. However, these genetic improvements may be accompanied by a correlated decline in reproductive variables (Goerzen et al., 1996; Barbato, 1999) such as delayed sexual maturity and reduced fertility (Siegel and Dunnington, 1985). The broiler breeder industry has used the relative conformation of males, or the extent of breast muscle "fleshing" in relation to skeletal growth, as a means to subjectively manage maturation and sexual activity. This practice has been supported by studies in the female (Zelenka et al., 1986) and the male (McGary et al., 2002; McGary et al., 2003) that suggested that carcass lean tissue, in particular breast muscle accretion, was a threshold trait required for the proper onset of sexual maturity. To control excessive BW and breast muscle development, low density diets have been commonly used in the poultry industry (Leeson and Summers, 1997;2000). In such a scenario, it has been shown that low CP diets fed to males during the rearing period had no effect on semen volume and spermatozoal concentration (Wilson et al., 1971; Vaughters et al., 1987), while low ME intakes were responsible for alterations in semen characteristics (Sexton et al., 1989a) and fertility (Sexton et al., 1989b; Cerolini et al., 1995). Although it has been attractive to use low density diets because of the potential of reduced feed cost in certain situations, it has also been demonstrated that there was a minimum cumulative nutrient intake that was required prior to photostimulation irrespective of BW (Walsh and Brake, 1997;1999; Peak, 2001). However, with the continued improvement in broiler BW and feed efficiency the threshold for cumulative intake of CP and ME may have changed. It has also been hypothesized that multiple thresholds including
minimum chronological age, BW, and body composition influence the onset of sexual maturity in females (Zelenka et al., 1984; Oruwari and Brody, 1988). Divergent studies have also shown that age, BW, and body composition may all be involved in the process of sexual maturation (Soller et al., 1984), or that these relationships were not readily apparent (Reddish and Lilburn, 2004b;a). These earlier reports emphasized the importance of the diet and feed program during broiler breeder rearing and the effects on carcass development. Additionally, since the rearing program affected subsequent reproductive performance, the progeny can also be affected (Attia et al., 1993; 1995). The objective of the present experiments was to study carcass and reproductive characteristics in commercial broiler breeder males fed High or Low planes of cumulative nutrition during the rearing period as related to the BW of those males at sexual maturity.

MATERIALS AND METHODS

Rearing Management. Two experiments were conducted to evaluate the effects of two levels (High and Low) of cumulative CP and ME intake during the rearing period on fertility, and broiler performance of commercially available broiler breeder males (Ross 344). In both experiments, day-old broiler breeder chicks were placed in a 20-pen growing house with 16 pens for females (Ross 308 SF) and 4 pens for males. Each 3.96 X 3.96 m pen was equipped with the equivalent of 750 cm of linear feeder space (six tube feeders) and two automatic drinkers. At placement, there were 68 females and 50 males in each female and male pen of Experiment I-1, respectively, and 96 females and 88 males in each female and male pen in Experiment I-2, respectively. All birds were brooded at an air temperature of 32°C with a heat source in each pen. Male BW and shank length were individually measured at 4, 8, 12,
16, and 20 wk of age during the rearing period, as well as comb height at 16 and 20 wk of age. Access to water was limited by a time clock and solenoid system sufficient to control litter moisture and allow the birds to have unlimited access to water while feed was present. After exposure to 23 h of light per day for one wk all birds were reared in a black-out house to 21 wk of age under a 8L:16D lighting program.

In Experiment I-1, at 21 wk of age, 15 males per pen were killed and portioned as would be done with broilers to determine carcass composition. Weights of feathers, ribs, testes, abdominal fat pad, Pectoralis major, Pectoralis minor, legs, and shanks plus feet were recorded. In Experiment I-2, 10 males per pen were killed at 21 wk of age to determine weights of the carcass, shanks plus feet, deboned breast meat, abdominal fat pad, and testes.

*Experimental Diets and Feeding Programs.* Two different cumulative programs providing 29,580 Mcal of ME and 1,470 g of CP or 33,500 Mcal of ME and 1,730 g of CP produced the High or Low planes of nutrition to 21 wk of age, respectively. In Experiment I-1, two diets were formulated (HiDiet and LoDiet) (Table I-1) to provide the cumulative nutrition treatments using a single feed allocation program (Figure I-1A). From 21 to 24 wk of age the HiDiet and LoDiet diets were proportionally blended to create a gradual transition to a single common breeder diet (2,925 kcal ME/kg and 15.4% CP) that was fed during the production period in both experiments. In Experiment I-2, during the rearing period, one single diet (Table I-1) was used with two different feed allocation programs (HiFeed and LoFeed) (Figure I-1A) to provide the same cumulative nutrition that was supplied during Experiment I-1.
<table>
<thead>
<tr>
<th>Ingredient and analysis</th>
<th>Starter Diet</th>
<th>Grower Diet Experiment I-1 (%)</th>
<th>Grower Diet Experiment I-2 (%)</th>
<th>Breeder Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LoDiet</td>
<td>HiDiet</td>
<td>LoDiet</td>
</tr>
<tr>
<td>Corn</td>
<td>60.35</td>
<td>56.90</td>
<td>69.43</td>
<td>64.85</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>21.45</td>
<td>10.35</td>
<td>11.96</td>
<td>15.31</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>13.00</td>
<td>25.00</td>
<td>3.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Corn gluten (60% CP)</td>
<td>-</td>
<td>-</td>
<td>7.28</td>
<td>-</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.56</td>
<td>1.51</td>
<td>1.80</td>
<td>1.58</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.33</td>
<td>1.31</td>
<td>1.18</td>
<td>1.27</td>
</tr>
<tr>
<td>Mineral premix(^1)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin premix(^2)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.64</td>
<td>0.64</td>
<td>0.56</td>
</tr>
<tr>
<td>Coccidiostat (Amprol)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>D,L-Methionine</td>
<td>0.13</td>
<td>0.10</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Selenium premix</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Threonine</td>
<td>0</td>
<td>0.05</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>-</td>
<td>0.14</td>
<td>0.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>1.0</td>
<td>1.90</td>
<td>3.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>-</td>
<td>0.48</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>0.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Totals</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated analysis\(^3\)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>17.66</td>
<td>14.00</td>
<td>16.70</td>
<td>15.18</td>
<td>15.36</td>
</tr>
<tr>
<td>ME, kcal/g</td>
<td>2.92</td>
<td>2.85</td>
<td>3.25</td>
<td>2.93</td>
<td>2.93</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.85</td>
<td>0.59</td>
<td>0.74</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Methionine + Cystine, %</td>
<td>0.71</td>
<td>0.51</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.95</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>2.75</td>
</tr>
<tr>
<td>Available Phosphorous, %</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.40</td>
</tr>
</tbody>
</table>

\(^1\)Mineral premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.

\(^2\)Vitamin premix contained the following per kilogram of diet: Vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; Vitamin E, 66 IU; Vitamin B-12, 34.6 ug; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; Vitamin k, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; and biotin, 252 ug.

\(^3\)Data presented as a percentage of dry matter.
Figure I-1. Male feeding programs during rearing (A) and production period (B) in Experiment I-1 and Experiment I-2. In Experiment I-1, Ross 344 males were fed a single feed program using two diets containing either High or Low nutrient density. In Experiment I-2 a single diet was used with either High or Low plane of nutrition. Both Experiments supplied the same cumulative nutrition to 21 wk of age.
Production Period. In both experiments, birds were moved at 21 wk of age to a curtain-sided, slat-litter house where the photoperiod was extended with artificial light to 14 h and 15 h at 22 and 24 wk of age, and to 15.5 and 16 h at 5% and 50% rate of lay, respectively. Supplemental light provided an intensity of 35 lux in the laying house during night hours. An average of 55 and 60 females for Experiments I-1 and I-2, respectively, along with 6 males were allocated to each of the 16 pens. Each breeder pen was equipped with 2/3 wood slats and 1/3 litter floors, two automatic drinkers, and four 12-hole galvanized nest boxes. In Experiment I-2, males were classified within the respective rearing program, according by BW at 21 wk of age, as either Heavy or Light. The BW of Heavy males within the LoFeed treatment ranged from 2,775 g to 3,272 g and within the HiFeed treatment ranged from 3,150 g to 3,552 g. Light males within the LoFeed treatment ranged from 2,575 g to 2,696 g and within the HiFeed treatment ranged from 2,765 g to 2,976 g. Male and female mortality were monitored daily and feed intake was adjusted to maintain the programmed daily feed allocation during the production period as described in Figure I-1B.

Male BW, comb height, and shank length were measured at 22, 24, 28, 32, 36, 40, 48, 56, and 64 wk of age. Shank length and comb height were determined only to 36 wk of age. Egg production, fertility, and hatchability of fertile eggs were determined. Eggs were collected twice daily and stored in a cooler at 18.6°C and 70% RH until incubated. Eggs laid on the floor and slats were collected separately and not incubated. Incubation analysis was conducted on the basis of weekly or biweekly sets of 60 eggs per replicate pen. All unhatched eggs were opened and examined macroscopically for evidence of embryological development. Fertility and stage of embryonic mortality were determined by a single experienced individual.
**Broiler Trials.** A broiler trial was conducted during each breeder experiment to determine if a progeny effect existed. The eggs were collected at 29 and 33 wk of age for Experiments I-1 and I-2, respectively. The chicks were sexed at hatching and male chicks (15/pen) were allocated to a 32-pen house, in Experiment I-1. In Experiment I-2, both female and male chicks were placed separately (10 chicks/pen) in a 64-pen house. Broiler diets were formulated to meet or exceed the NRC (1994) minimum requirements.

**Statistical Analyses.** A one-way ANOVA was conducted to analyze all data of Experiment I-1 and the rearing period data of Experiment I-2. For the production period of Experiment I-2, a two-way ANOVA design was used, with the rearing feed allocation program (HiFeed or LoFeed) and the BW classification (Heavy or Light) as main factors. Pen was the experimental unit and during the rearing period two treatments were randomly assigned to each of four pens, while in the production period 16 pens were divided among two or four treatments with 8 or 4 replicates per interaction cell for Experiments I-1 and I-2, respectively. The fertility and hatchability data were analyzed on a weekly or biweekly basis as well. Additionally, these latter data were analyzed on a cumulative and age-based quartile time period basis. For the broiler trial of Experiment I-1 a one-way ANOVA with sixteen replicates per treatment was used and a two-way ANOVA with eight replicates per interaction treatment was used for the broiler trial of Experiment I-2.

The general linear model (GLM) procedure of SAS Institute (2001) was used to analyze the broiler data and the continuous variables of the broiler breeder data. The repeated statement of SAS Institute (2001) was used for BW, shank length, and comb height data. Analyses of slopes for BW and shank length during the rearing period were conducted. Differences between the coefficient of variation (CV) for BW and the correlation between BW and shank
length were analyzed. The fertility data was analyzed as categorical data, where each individual egg was taken as a binomial event, either fertile or infertile, using the general model (GENMOD) procedure of SAS Institute (2001). To test the time effect and its interaction with the treatments a split plot design with time and its interactions in the subplot unit was conducted using PROC MIXED of SAS Institute (2001). Orthogonal contrasts were used to compare treatments probabilities (Giesbrecht and Gumpertz, 2004). Means were partitioned using LSMEANS and statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

RESULTS

Growth and Carcass Composition. The effect of two cumulative planes of nutrition during the rearing period on male BW is shown in Figure I-2. The High plane of nutrition, produced by either diet (HiDiet) in Experiment I-1 or feeding program (HiFeed) in Experiment I-2 significantly increased male BW during the rearing period (Figures I-2A and I-2B). No significant effect was observed after 28 or 32 wk of age in Experiments I-2 and I-1, respectively. Although male BW classification was conducted at 20 wk of age, retrospective analysis (Figure I-2C) showed that Heavy males at 28 wk of age were already significantly heavier than their pen mates at 8 wk of age. The Heavy BW group maintained significantly higher BW up to 38 wk but this effect disappeared later. The fact that the Light male group increased in BW after 38 wk of age appeared to be related to mortality of the lightest BW males between 38 to 44 wk of age (i.e. smaller males died). The effect of the rearing plane of cumulative nutrition (either due to diet or feeding program) and male BW classification at 21 wk of age on shank length and comb height is shown in Figures I-3 and I-4, respectively. The
Figure I-2. Male BW as affected by feeding program during the rearing period in Experiment I-1 (Panel A) and Experiment I-II (Panels B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (HiDiet and LoDiet, in Experiment I-1) or two feeding programs (HiFeed and LoFeed, in Experiment I-2). Males were classified as being in the 50% Heavy or 50% Light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Analyses of slopes during the rearing period (0 to 21 wk of age is shown in the right box. Transition period in Figure I-2A indicates when the HiDiet and LoDiet were blended in various proportions.

Analyses of slopes (0-21 wk)

HiDiet 588 ± 8.7
LoDiet 506 ± 11.2
$P$-value < 0.0146

Analyses of slopes (0-21 wk)

HiFeed 530 ± 43.07
LoFeed 461 ± 8.7
$P$-value > 0.05

Analyses of slopes (0-21 wk)

Heavy 588 ± 58.7
Light 526 ± 60.0
$P$-value < 0.057
Figure I-3. Male shank length as affected by feeding program during the rearing period in Experiment I-1 (A) and Experiment I-II (B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (Experiment I-1) or two feeding programs (Experiment I-2). Males were classified as being in the 50 % heavy and 50 % light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Analyses of slopes during the rearing period (0 to 21 wk of age) is shown in the right box. Transition period in Figure I-3A indicates when the HiDiet and LoDiet were blended in various proportions.
**Figure I-4.** Male comb height as affected by feeding program during the rearing period in Experiment I-1 (A) and Experiment I-II (B and C). Ross 344 males were provided High or Low planes of nutrition through either two diets (Experiment I-1) or two feeding programs (Experiment I-2). Males were classified as being in the 50% heavy and 50% light groups at 21 wk of age (Experiment I-2). Asterisk (*) represents significant differences ($P < 0.05$). Transition period in Figure I-4A indicates when the HiDiet and LoDiet were blended in various proportions.
Low plane of rearing nutrition resulted in males with shorter shanks (Figures I-3A and Figures I-3B) and shorter combs (Figures I-4A and I-4B) during the rearing period, however, similar comb heights and shank lengths were observed at 28 wk of age. In Experiment I-2, no significant interaction between the rearing feeding program and BW classification was found for any of the variables. However, the Heavy male group exhibited longer shanks at 8, 20, and 26 wk of age and greater comb height at 16 and 20 wk of age.

Overall, during the rearing period there was a high correlation (90.5%) between BW and shank length. From the repeated measures analysis it was observed that males grew in a linear manner in both experiments; however males from Experiment I-1 appeared to grow faster than males from Experiment I-2. The slope represented the rate of BW or shank length increment per unit of time (4 wk intervals) from 4 to 20 wk of age. In Experiment I-1, there was a significant difference between the slopes, 588 g versus 506 g, for the HiDiet and LoDiet treatments, respectively (Figure I-2A), while in Experiment I-2 significant differences were not found, 530 g versus 461 g (Figure I-2B), between the HiFeed and LoFeed treatments, respectively. The selected Heavy males at 21 wk of age tended to show higher slopes than the selected Light males, 588 g versus 526 g (Figure I-2C). Although the stocking density was higher in Experiment I-2, no significant differences were observed in the uniformity of the males, as measured by the CV (15.1% and 15.5%, for Experiments I-1 and I-2, respectively).

The effect of two cumulative planes of nutrition during the rearing period on carcass composition is shown in Table I-2. Males from the Low plane of nutrition were smaller and possessed proportionately lower carcass and portion weights. However, relative weights were analyzed to correct for this difference. In Experiment I-1, the LoDiet males possessed
significantly less relative abdominal fat, and apparently equivalent relative frame size, in spite of a lower BW, as evidenced by heavier shanks plus feet, and ribs. No differences were found for the relative weight of feathers, legs, *Pectoralis* major, *Pectoralis* minor, or testes (Table I-2). In contrast, in Experiment I-2, there were no significant differences observed for abdominal fat, shanks, testes, or breast meat on a relative basis (Table I-2).

**Fertility.** The effect of the male rearing diet on percentage fertility is shown in Table I-3 for Experiment I-1. Fertility experienced a significant decline after 36 wk of age (second quartile time period). However, the LoDiet treatment showed a reduced decline in fertility. The effect of the rearing feeding program and BW classification on fertility is shown in Table I-4 for Experiment I-2. Although the decline in fertility was less than in Experiment I-1, this decline occurred after the second quartile time period (45 wk of age), and in a similar manner the decline was less in the LoFeed plane of cumulative nutrient intake group. Overall either Low plane of nutrition (LoDiet and LoFeed) improved cumulative percentage fertility (Tables I-3 and I-4, respectively). On the other hand, the male BW classification did not produce a significant effect. A significant interaction between rearing feeding program and male BW classification was observed only during the fourth quartile time period when the HiFeed-Heavy combination group exhibited the lowest fertility (Table I-4).

**Progeny Performance.** The effect of cumulative nutrient intake of male broiler breeders during rearing and the male BW classification at 21 wk of age on broiler performance from early lay breeders is depicted in Table I-5. In the broiler trial from Experiment I-1, the male broilers from the LoDiet treatment, compared with male broilers from the HiDiet treatment, tended to have higher BW at 21 d (764 g *versus* 731 g; *P* < 0.1) and at 42 d of age (2,766 g *versus* 2,669 g; *P* < 0.06). Further, the broilers from the LoDiet treatment tended to exhibit
Table I-2. Male broiler breeder carcass composition as affected by plane of nutrition during the rearing period in Experiment I-1 and Experiment I-2.

<table>
<thead>
<tr>
<th>Carcass Characteristic</th>
<th>Rearing Plane</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g/100g BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feathers</td>
<td>HiDiet</td>
<td>2.20</td>
<td>1.96</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>1.96</td>
<td>1.96</td>
<td>0.14</td>
</tr>
<tr>
<td>Leg</td>
<td>HiDiet</td>
<td>36.80</td>
<td>36.40</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>36.40</td>
<td>36.40</td>
<td>0.30</td>
</tr>
<tr>
<td>Pectoralis major</td>
<td>HiDiet</td>
<td>17.97</td>
<td>14.62</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>14.62</td>
<td>14.62</td>
<td>0.47</td>
</tr>
<tr>
<td>Pectoralis minor</td>
<td>HiDiet</td>
<td>7.20</td>
<td>5.90</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>5.90</td>
<td>5.90</td>
<td>0.80</td>
</tr>
<tr>
<td>Testes</td>
<td>HiDiet</td>
<td>0.15</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>0.13</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Abdominal Fat Pad</td>
<td>HiDiet</td>
<td>0.59 A</td>
<td>0.20 B</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>0.20 B</td>
<td>0.20 B</td>
<td>0.08</td>
</tr>
<tr>
<td>Ribs</td>
<td>HiDiet</td>
<td>24.10 B</td>
<td>25.60 A</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>25.60 A</td>
<td>25.60 A</td>
<td>0.50</td>
</tr>
<tr>
<td>Shanks plus Feet</td>
<td>HiDiet</td>
<td>1.94 B</td>
<td>2.04 A</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>LoDiet</td>
<td>2.04 A</td>
<td>2.04 A</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Experiment I-2

<table>
<thead>
<tr>
<th>Carcass Characteristic</th>
<th>Rearing Plane</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HiFeed</td>
<td>LoFeed</td>
<td></td>
</tr>
<tr>
<td>Deboned Breast</td>
<td></td>
<td>22.50</td>
<td>22.90</td>
<td>0.39</td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td>0.15</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Abdominal Fat Pad</td>
<td></td>
<td>0.60</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Shanks plus Feet</td>
<td></td>
<td>2.01</td>
<td>2.02</td>
<td>0.06</td>
</tr>
</tbody>
</table>

A,B Means with different superscripts are significantly different (P < 0.01).
Table I-3. Broiler breeder fertility during the production period as affected by plane of nutrition during the rearing period in Experiment I-11.

<table>
<thead>
<tr>
<th>Plane Of Nutrition</th>
<th>Weeks Of Age Quartile (%)</th>
<th>Cumulative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiDiet</td>
<td>28-35 96.2 36-45 95.1 B 46-55 85.5 B 56-64 72.3 B</td>
<td>89.6 B</td>
</tr>
<tr>
<td>LoDiet</td>
<td>28-35 97.0 36-45 96.3 A 46-55 90.9 A 56-64 82.9 A</td>
<td>93.1 A</td>
</tr>
</tbody>
</table>

A, B Means with different superscripts are significantly different ($P < 0.01$).

1 Categorical analysis does not generate standard errors.

2 Time and plane of nutrition interaction was significant ($P <0.01$).
Table I-4. Broiler breeder fertility during the production period as affected by male feeding program during the rearing period and male body weight classification in Experiment I-21.

<table>
<thead>
<tr>
<th>Plane Of Nutrition</th>
<th>BW Classification</th>
<th>Weeks Of Age Quartile(^2) (%)</th>
<th>Cumulative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>28-35</td>
<td>36-45</td>
</tr>
<tr>
<td>HiFeed</td>
<td></td>
<td>96.3</td>
<td>96.8</td>
</tr>
<tr>
<td>LoFeed</td>
<td></td>
<td>96.9</td>
<td>97.0</td>
</tr>
<tr>
<td>Heavy</td>
<td></td>
<td>97.1</td>
<td>97.1</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td>96.1</td>
<td>96.7</td>
</tr>
</tbody>
</table>

**Interactions**

<table>
<thead>
<tr>
<th>HiFeed</th>
<th>Heavy</th>
<th>97.0</th>
<th>97.0</th>
<th>91.6</th>
<th>92.5</th>
<th>94.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiFeed</td>
<td>Light</td>
<td>95.7</td>
<td>96.6</td>
<td>92.4</td>
<td>94.6</td>
<td>94.7</td>
</tr>
<tr>
<td>LoFeed</td>
<td>Heavy</td>
<td>97.2</td>
<td>97.2</td>
<td>93.8</td>
<td>94.7</td>
<td>95.5</td>
</tr>
<tr>
<td>LoFeed</td>
<td>Light</td>
<td>96.6</td>
<td>96.7</td>
<td>92.6</td>
<td>93.0</td>
<td>94.4</td>
</tr>
</tbody>
</table>

| P value\(^3\) | 0.59  | 0.93   | 0.10   | 0.0001  | 0.0009  |

\(^A, B\) Means with different superscripts are significantly different (\(P < 0.01\)).
\(^1\) Categorical analysis does not generate standard errors.
\(^2\) Time and plane of nutrition interaction was significant (\(P < 0.01\)).
\(^3\) Probability values for interaction means between plane of nutrition and BW classification.
Table I-5. Effect of the plane of nutrition of male broiler breeders during the rearing period through two diets (HiDiet and LoDiet) or two feeding programs (HiFeed and HiLow) on broiler male performance as indicated by body weight (BW), adjusted feed conversion (AdjFCR), and feed intake. In Experiment I-2 broiler breeder males were classified by BW at 21 wk of age.

<table>
<thead>
<tr>
<th>Plane Of Nutrition</th>
<th>Experiment I-1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet</td>
<td>BW</td>
<td>AdjFCR</td>
<td>Feed Intake</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g)</td>
<td>(g:g)</td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 d 21 d 42 d</td>
<td>21 d</td>
<td>42 d</td>
<td>21 d</td>
<td>42 d</td>
</tr>
<tr>
<td>HiDiet</td>
<td>35 731 2,669</td>
<td>1.46 x</td>
<td>1.66</td>
<td>1,039</td>
<td>4,378</td>
</tr>
<tr>
<td>LoDiet</td>
<td>35 764 2,766</td>
<td>1.43 y</td>
<td>1.64</td>
<td>1,069</td>
<td>4,483</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2 12.8 32.4</td>
<td>0.008</td>
<td>0.014</td>
<td>15.7</td>
<td>49.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment I-2¹</th>
<th>Feed Program</th>
<th>0d 21d 49d</th>
<th>21d 49d</th>
<th>21d 49d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g)</td>
<td>(g:g)</td>
<td>(g)</td>
</tr>
<tr>
<td>HiFeed</td>
<td>36 955 3,465</td>
<td>1.38</td>
<td>1.87</td>
<td>1,271</td>
</tr>
<tr>
<td>LoFeed</td>
<td>35 954 3,470</td>
<td>1.39</td>
<td>1.88</td>
<td>1,296</td>
</tr>
<tr>
<td>SEM</td>
<td>0.3 6.6 32.0</td>
<td>0.009</td>
<td>0.011</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Male BW classification

|                  | Heavy 36 910 3,498 | 1.38 | 1.86 | 1,280 | 6,464 |
|                  | Light 36 906 3,347 | 1.39 | 1.88 | 1,270 | 6,393 |
|                  | SEM 0.29 6.6 32.0  | 0.009 | 0.011 | 12.8 | 74.0 |

¹ Means with different superscripts are significantly different (P < 0.06).
² Interaction between male rearing diet and BW classification in Experiment I-2 was not significant and interaction means are not shown.
better adjusted feed conversion (AdjFCR) at 21 d of age (1.43 g:g versus 1.46 g:g; \( P < 0.06 \)).

In contrast, the broiler trial from Experiment I-2 did not produce significant differences in any of the variables, and only male broiler data is shown for comparison purposes (Table I-5). No significant differences were observed for broiler mortality in either trial (data not shown).

**DISCUSSION**

Cumulative nutrient intake during the rearing period significantly affected growth variables such as BW, shank length, and comb height, but to a lesser extent carcass composition. In both experiments, regardless of the method used (diet or feeding program), males subject to the High plane of nutrition (either HiDiet or HiFeed) demonstrated, in general, significantly higher values for BW, shank length, and comb height. Upon retrospective analysis, males that were heavier at 21 wk of age were found to have been already heavier than their pen mates at 8 wk of age, reflecting the fact that BW at 8 wk has been an important tool in broiler selection (Siegel, 1963). After 21 wk of age significant differences in BW, shank length, and comb height tended to disappear as birds reached maturity. It has been believed that shank length could affect the intersexual cloacal distance during mating (McGary et al., 2003), but no significant effects of shank length on male fertility were found. In the present experiments no significant differences in either the slope of shank growth during the rearing period to 21 wk of age or in shank length after 28 wk of age were observed (Figure I-3). In Experiment I-2 a higher stocking density was used, a slower growth rate was observed, and the onset of sexual maturity was delayed three weeks with respect to Experiment I-1. In spite of these differences, the uniformity of the males was similar during the rearing period. Although a different approach was used, Zhang et al. (1999) also did not find significant
difference in BW uniformity during the rearing period due to either different CP level or different feeding regimens.

On the other hand, comb height was significantly less during late rearing on the Low plane of nutrition but no differences were found during the production period in Experiments I-1 and I-2 (Figures I-4A and I-4B). Additionally, the Heavy BW group of Experiment I-2 exhibited significantly greater comb height (Figure I-4C). However, these differences did not appear to affect reproductive performance, in agreement with a previous report that did not find differences in fertility between males classified by comb height (Romero-Sanchez et al., 2003). Although comb height may be influenced by the production of sex hormones (Eitan et al., 1998; Joseph et al., 2003), under the conditions of these experiments there appeared to be small differences in fertility. It has been reported that only with extremely low comb development would fertility problems become evident (Leonard and Zanette, 1998). On the other hand, female Galliforms of many species have been shown to prefer males with well developed ornaments such as combs and wattles because they were reliable indicators of livability (Rintamaki et al., 2000) and/or male dominance status (Graves et al., 1985; Holder and Mongomerie, 1993). However, grouping males by BW or by comb height (Romero-Sanchez et al., 2003) could have decreased variability in this trait, so that selection of males by females was not likely to occur within experimental units (pens). Although, it has been proposed that there was a trade-off between comb size and immunocompetence, mediated partially by testosterone (Verhulst et al., 1999), significant differences were not found for male mortality in either experiment.

Although males in the LoDiet treatment of Experiment I-1 exhibited significantly lower abdominal fat, the higher values of the HiDiet group would not be considered extremely fat
and were not considered to be a cause of lower fertility. On the other hand, the high
correlation between body fat and reproductive parameters reported in broiler breeder males
by Sexton et al. (1989a) supported the human theory that a minimum percentage body fat
was required for continued reproductive function (Frisch, 1984). In fact, broiler breeder
males not producing semen tended to have less carcass fat than males producing semen
(Wilson et al., 1987). Although there has been demonstrated a negative correlation between
abdominal fat pad size and fertility (Leeson and Summers, 2000), there have also been
reports showing inconsistent correlations among breeders from different BW classes and the
amount of absolute or relative abdominal fat (Zelenka et al., 1987; Renema et al., 1999).
In contrast with their reduced BW, males from the LoDiet group showed relatively equal
frame size as indicated by increased relative weights of the ribs and shank plus feet in
Experiment I-1. In a similar manner, it has been shown that birds with relatively larger frame
size but the same amount of breast meat “fleshing” had proportionally lower breast meat
relative to BW (Renema et al., 1999; Reddish and Lilburn, 2004a). These birds may have a
lower maintenance requirement for ME that would leave extra nutrients (primarily ME)
available to support fertility. Results from previous experiments have suggested that feed
restriction and its associated effects on BW and carcass development had a greater influence
on sexual development than genetic differences in carcass composition (Reddish and Lilburn,
2004a). Regarding testes size, no significant difference was found (Table I-2). In some
broiler breeder strains the relationship between testes weight and fertility was found to be
negative (McGray et al., 2002). Previous data have also shown that greater testicular weight
in broiler breeders (Janssen et al., 2000) or in other species (Fernandez-Abella et al., 1999)
may not necessarily correspond to increased spermatogenesis or higher androgen levels.
Although in poultry and other species the testis size has often been positively correlated with fertility, it appeared that there was a threshold for this relationship, after which no correlation with fertility was observed. The testes weights in the present experiments were similar to those reported by previous authors (Johnson, 1986; Etches, 1996). Therefore, it can be concluded that within different genotypes of commercial lines exhibiting extremes in breast meat, definitive relationships involving BW at sexual maturity, gross carcass measurements, and reproductive development were not readily apparent. These contradictory findings could be explained with a simplistic approach. Males with greater BW at 21 wk of age would have had a higher maintenance ME requirement and therefore fewer nutrients (primarily ME) would have been diverted to growth and reproduction. In fact, in Experiment I-1 when the male groups were divided into the 50% heaviest and the 50% lightest, it was observed that the 50% heaviest males from the HiDiet diet was the only group that did not continue to grow steadily after 28 wk of age (Figure I-5). To delineate this concept the ME intake was compared with the maintenance requirement (Figure I-6). The heaviest 50% of the males became more restricted from 24 to 36 wk of age. Although High BW has been implicated to be the main cause in declining fertility, McGray et al. (2003) did not find a correlation between fertility and male BW or spermatozoal penetration of ova in two different broiler breeder strains. Therefore, it seems unlikely that BW in and of itself directly impacted male mating ability within a normal range of BW as experienced in this study. In contrast, there was strong evidence to suggest that Heavy males could be easily over-restricted and stop mating as a result of a deficiency of ME. Thus, if we can accept the principle that the heaviest males were the first to cease of mating as a result of a ME deficiency, the genetic potential of these males would not be expected to appear in the progeny. Thus, this could
Figure I-5. Male body weight (BW) during the production period as affected by rearing diet in Experiment I-1. Ross 344 males were fed diets containing either High (HiDiet) or Low (LoDiet) nutrient density. Males received the same diet after 24 wk of age and were divided in the 50% heavy and 50% light within experimental groups to determine growth pattern. Transition period indicates when the HiDiet and LoDiet were blended in various proportions.
Figure I-6. Energy balance for males in HiDiet in Experiment I-1. Ross 344 males were fed a single diet providing 340 kcal/d of ME during the early production period. Males received the same diet after 24 wk of age and were divided in the 50% Heavy and 50% Light within experimental groups to determine growth pattern. The metabolizable energy requirement for maintenance (ME_m) at experimental conditions for the Heavy and Light group within the HiDiet treatment was estimated as function of the BW as ME_m = 1.45*BW^{0.65} * (1.78 – 0.012*T) where T is average temperature in °F (Combs, 1968).
explain how broiler performance tended to be improved by the LoDiet treatment in Experiment I-1. Fertility of the HiDiet group was numerically lower \((P < 0.08)\) during the time when the eggs were collected to conduct the broiler trial (Figure I-7). However, in Experiment I-2 there were no significant differences in fertility at the time the eggs were collected for the broiler trial and no differences were observed in broiler performance. It was important to remark that during the production period the males received consistent feed increments that could explain why the Heavy group did not exhibit decreased fertility. However, when the weekly data of Experiment I-2 were analyzed, the HiFeed-Heavy group combination exhibited significantly decreased fertility compared with the other treatment combinations during the early production period at 30 and 31 wk of age (data not shown for these specific times). Since the male feed allocation was increased with increasing age, further differences were only observed during the fourth quartile time period when a significant interaction \((P < 0.001)\) showed that the HiFeed-Heavy group had a reduced percentage fertility. In conclusion, the data showed that a cumulative nutrient intake during the rearing period of 29.6 Mcal of ME and 1,470 g of CP resulted in a broiler breeder male of adequate size with adequate cumulative nutrition at 21 wk of age to be able to maintain fertility and broiler progeny performance throughout the production period.
Figure I-7. Effect of the rearing broiler breeder male diet on fertility during the early production period and its effect on broiler male body weight (BW) at 42 d of age in Experiment I-1. Ross 344 males were fed diets containing either High (HiDiet) or Low (LoDiet) nutrient density. Plus symbol (+) indicates numeric difference that approach significance ($P < 0.1$) as determined by GLM procedure at each age.
REFERENCES


MANUSCRIPT II. Effect of Antioxidants (Selenium, Vitamin C, and Vitamin E) On Broiler Breeder Egg Production, Fertility, and Hatchability

ABSTRACT

Two experiments were conducted to evaluate an elevated antioxidant level (Two sources of selenium and/or Vitamins C and E) and two sources of selenium alone on broiler breeder (Ross 344 x 308) egg production, fertility, and hatchability. From 0 to 21 wk all birds received the same starter and grower diets after which pens were randomly assigned to either a High or Normal antioxidant diet in Experiment II-1. The High antioxidant diet was developed by supplementation of Vitamin C, additional supplementation of Vitamin E, and replacement of inorganic selenium (sodium selenite (NaSe)) with a high selenium yeast ((HiSe Yeast) Selplex®) source. In Experiment II-2, Ross 344 males with either the largest comb (LC) or smallest comb (SC) heights were divided into two groups that were then randomized among 16 pens and mated with Ross 308 females per pen. Breeders received a diet with either HiSe Yeast or NaSe as their source of supplemental selenium in a 2 X 2 factorial design. All males were weighed individually at 4, 12, 21, 24, 26, 28, 32, 36, 40, 48, 56, and 64 wk of age. Percentage fertility, fertile hatchability, and embryo mortality were evaluated on either a weekly or biweekly basis.

In Experiment II-1, the High antioxidant level significantly increased fertility on an overall basis. Further, after 38 wk of age, there was a precipitous decrease in fertility due to an inadequate feed allocation for males that apparently had less effect on the High antioxidant group. However, after the male feed allocation was increased at 54 wk of age the differences due to antioxidant level tended to disappear. In Experiment II-2, the LC group showed a
consistently higher BW. The LC males receiving NaSe exhibited a significantly lower fertility during early production that was related to a significantly heavier BW relative to the feed allocation. Similarly, in Experiment II-2, the SC males received more feed (more ME) relative to BW and responded with significantly increased BW (at 40 wk of age) and higher fertility during the early production period. An interaction between comb size and selenium source suggested that when higher BW males (LC) were slightly underfed after photostimulation, the HiSe Yeast had a “nutrient sparing” effect that allowed these males to maintain high fertility even when they did not gain BW in a consistent manner. The data further suggested that consistent feed increments that maintained a consistent male BW gain during the early phase of production played an important role in subsequent fertility.

*Key words*: antioxidants, selenium, broiler breeder, energy requirement, fertility, Vitamin C, Vitamin E
INTRODUCTION

The detrimental effects of oxidative damage on male poultry fertility have been described (Surai, 2002). In mammals, the seminal plasma has been shown to contain antioxidant machinery composed of a number of enzymes (superoxide dismutase, catalase, glutathione peroxidase (GSH-Px)), and free radical scavengers such as Vitamins C and E, hypotaurine, taurine, and albumin (Aumuller and Seitz, 1990; Aumuller et al., 1990; Lewis et al., 1997; Zini et al., 2002). For instance, the effects of selenium (Se) on fertility and reproductive performance have generally been attributed to its antioxidant function, as a component of the GSH-Px system (Allan et al., 1999). In a similar manner, Vitamins C and E have been shown to protect semen against oxidative damage (Yousef et al., 2003). Since the seminal plasma has been found to be scarce in the poultry male (Lake, 1969; Johnson, 1986; Lake and Ravie, 1987), several researchers have tried to demonstrate the beneficial effect of elevated levels of dietary antioxidants (Surai et al., 1998; Creel et al., 2001).

Edens (2002) reported that roosters fed selenium deficient diets had reduced ejaculate volumes and decreased spermatozoal concentrations relative to those receiving supplemental selenium (0.3 ppm) in their diets. It was also indicated that organic selenium from a high selenium yeast source could improve fertility when compared to inorganic selenium, because selenium from yeast was taken up more efficiently by tissues (Sunde, 1990; Schrauzer, 2000). However, the commercial field responses have not been consistent (Iwanier and Zachara, 1995; Mahan and Kim, 1996; Mahan and Parrett, 1996; Wolffram, 1999; Edens, 2002), suggesting that the effect of this antioxidant may be dependant upon other factors, such as the male feeding program and male sexual development. In fact, Walsh and Brake (1997) and Peak (2001) had previously demonstrated that female fertility was dependent upon
cumulative nutrition prior to photostimulation and, under commercial conditions, improper feeding relative to BW was a common problem (Brake, 2002). Since the effect of organic antioxidants could be influenced by the feed allocation program and sexual maturity, the objective of these experiments was to evaluate the effect of a combination of supplemental organic antioxidants (Experiment II-1) and the effect of selenium source alone and apparent sexual maturity, as evidenced by comb development (height), in the presence of a more than normally limited male feed allocation (Experiment II-2).

MATERIALS AND METHODS

Experiment II-1. An evaluation of the effects of an additional antioxidant package containing Vitamin C, Vitamin E, and high selenium yeast (HiSe Yeast (Selplex®, Alltech, Nicholasville, KY 40356)) during the broiler breeder production period on BW, egg production, fertility, and hatchability was conducted. Day-old chicks were placed in a 24-pen litter floored growing house with 12 pens for females and 12 pens for males. At placement, there were 220 females and 24 males in each female and male pen, respectively. After 23 h of light per day for one week all birds were reared to 21 wk of age with 8 h of light per day at an average intensity of 15 lux. An average of 200 females and 20 males were moved to two-thirds slat laying quarters at 21 wk of age and photostimulated with 14 h of light. The daylength was increased to 15 h 14 d later and then to 15.5 h and 16 h at 5% and 50% rate of lay, respectively. Natural light entered the house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux when natural light was not present. Feed was provided daily during the first week of age and then a 4/3 feed allocation program was used until 21 wk of age after which a daily feeding program
was employed. Male and female mortality were recorded daily and feed allocation adjusted accordingly. Male feed allocation was maintained during the production period at 110 g/male/d until 54 wk of age and then increased by 5 g/male/d (Figure II-1) after fertility had been observed to decline. The Normal antioxidant diet used during the production period was amended with additional quantities of Vitamins C and E and HiSe Yeast replaced sodium selenite ((NaSe) Na₂SeO₃) (Table II-1). Access to water was limited by a time clock and solenoid system sufficient to control litter moisture and allow the birds to have unlimited access to water until one hour after all feed was consumed and a similar amount on non-feed days during rearing. Water was limited to 8 h per day during the production period. Four 12-hole conventional nests were provided in each breeding pen. Twelve tube feeders for females were placed over the slat area while there were two tube feeders in the pine shavings litter area for the males. Separation of sexes was insured by special grills on the female feeder that prevented the non-dubbed males from accessing the female feeders. There were four bell-type drinkers in each of 12 breeding pens. Male BW was measured individually at 21, 24, 26, 32, 36, 40, 48, 56, and 64 wk of age. Eggs were collected twice daily from the nests and stored in an egg cooler at 18-20°C and 60% RH until incubated. Eggs laid on the floor and slats were collected separately but not incubated. Analysis of percentage fertility, hatchability, and embryo mortality was conducted weekly from 28 to 36 wk of age and biweekly from 38 to 64 wk of age by macroscopic examination of all unhatched eggs from sets of 180 eggs per pen on the respective weeks. At 28, 34, 40, 46, 52, and 58 wk of age individual eggs were weighed to the nearest 0.1 g, the contents removed and shells dried to constant weight. The eggshells were then weighed.
Figure II-1. Broiler breeder male feeding programs during the production period in Experiment II-1 and Experiment II-2. A typical commercial feeding program is shown for comparison. The feeding program employed in Experiment II-2 was meant to be slightly more restrictive than normal.
Table II-1. Composition of diets in Experiment II-1.

<table>
<thead>
<tr>
<th>Ingredient and analysis</th>
<th>Starter diet</th>
<th>Grower diet</th>
<th>Breeder diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antioxidant Level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Corn</td>
<td>65.10</td>
<td>68.00</td>
<td>64.13</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>22.20</td>
<td>17.00</td>
<td>19.10</td>
</tr>
<tr>
<td>Wheat midds</td>
<td>7.64</td>
<td>9.88</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>-</td>
<td>-</td>
<td>2.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.62</td>
<td>1.60</td>
<td>1.47</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.24</td>
<td>1.28</td>
<td>6.10</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>0.20</td>
<td>0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin premix²</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.58</td>
<td>0.41</td>
</tr>
<tr>
<td>Coccidiostat (Amprol)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.08</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Sodium selenite (NaSe)</td>
<td>0.10</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>Antioxidant package³</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>Mold inhibitor</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>-</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>1.00</td>
<td>1.00</td>
<td>1.10</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated analysis⁴

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>17.00</td>
<td>15.00</td>
<td>16.02</td>
<td>16.03</td>
</tr>
<tr>
<td>ME, kcal/g</td>
<td>2,925</td>
<td>2,925</td>
<td>2,912</td>
<td>2,918</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.88</td>
<td>0.75</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>Methionine + Cystine, %</td>
<td>0.70</td>
<td>0.80</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.90</td>
<td>0.90</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Available Phosphorus, %</td>
<td>0.45</td>
<td>0.45</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>Vitamin E, I.U/kg.</td>
<td>-</td>
<td>-</td>
<td>114.25</td>
<td>20.25</td>
</tr>
<tr>
<td>Vitamin C, mg/kg</td>
<td>-</td>
<td>-</td>
<td>120.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

¹Mineral Premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.
²Vitamin premix contained the following per kilogram of diet: Vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; Vitamin E, 66 IU; Vitamin B12, 34.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; Vitamin K, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; and biotin, 252 µg.
³Antioxidant package contained the following per kilogram: Vitamin E (106 IU); Vitamin C as Stay-C 35%; HiSe Yeast (Selplex ®) 0.1%; Ethoxyquin, 66, 127 mg . Stay-C dry mixture is a fine powder containing mono-, di- and triphosphate esters of L-ascorbic acid in a suitable carrier. It provides a minimum of 35% of ascorbic acid by weight equivalent to 150 grams of ascorbic acid per kg of dry mixture.
⁴Data expressed on a percentage of dry matter basis. Formulations were confirmed by proximate analysis.
Experiment II-2. An experiment was conducted to evaluate the effects of the interaction between two selenium sources (HiSe Yeast versus NaSe) and male apparent sexual maturity, as indicated by comb height, in the presence of a more than normally limited male feed allocation (Figure II-1) on broiler breeder fertility. A group of 200 males (Ross 344) and 1,100 females (Ross 308 slow-feathering) was reared under the same conditions as generally described by Brake and Baughman (1989). Briefly, day-old chicks were placed in a 20-pen growing house with 16 pens for females and 4 pens for males. Each 3.96 X 3.96 m pen was equipped with the equivalent of 750 cm of linear feeder space (six tube feeders) and two automatic drinkers. At placement there were 68 females and 50 males in each female and male pen, respectively. The feed management and lighting program was similar as described for Experiment II-1 above. At 21 wk of age an average of 55 females and 6 males were allocated to each of the 16 pens. From 200 males, 96 males were classified according to their comb height. The males with large sized combs (LC) and small sized combs (SC) were allocated to 8 pens each (6 males per pen) in such a manner as to ensure a similar male BW distribution within each comb height group among all of the pens in the respective treatments. Males and females were fed the same breeder diet (Table II-2) with either 500 mg/kg of HiSe Yeast or 1 g/kg of NaSe. Both diets were formulated to supply 0.3 ppm of elemental selenium. The male feed allocations were intended to be slightly more restrictive than might be normal in commercial practice (Figure II-1) in order to create a marginal ME deficiency during the early production period.

Male BW was measured at 4, 12, 21, 24, 26, 27, 29, and after 32 wk of age at the same ages indicated in Experiment II-1. Male shank length was measured from the tibiotarsal articulation to the foot pad after the latter was bent as if the foot were in contact with the
### Table II-2. Composition of diets in Experiment II-2.

| Ingredient and analysis | Starter diet | Grower diet | Breeder diet | Se source
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HiSe Yeast</td>
</tr>
<tr>
<td>Corn</td>
<td>65.10</td>
<td>68.00</td>
<td>69.60</td>
<td>69.60</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>22.20</td>
<td>17.00</td>
<td>20.10</td>
<td>20.10</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>7.64</td>
<td>9.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.62</td>
<td>1.60</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.24</td>
<td>1.28</td>
<td>6.10</td>
<td>6.10</td>
</tr>
<tr>
<td>Mineral premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin premix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.58</td>
<td>0.58</td>
<td>0.58</td>
</tr>
<tr>
<td>Coccidiostat (Amprol)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Sodium selenite (NaSe)</td>
<td>0.10</td>
<td>0.10</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>HiSe Yeast (Selplex ®)</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Sand</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>-</td>
<td>0.008</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.20</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>1.00</td>
<td>1.00</td>
<td>1.16</td>
<td>1.16</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Calculated analysis<sup>3</sup>**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>17.00</td>
<td>15.00</td>
<td>15.50</td>
<td>15.50</td>
<td></td>
</tr>
<tr>
<td>ME, kcal/g</td>
<td>2,925</td>
<td>2,925</td>
<td>2,925</td>
<td>2,925</td>
<td></td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.88</td>
<td>0.75</td>
<td>0.80</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Methionine + Cystine, %</td>
<td>0.70</td>
<td>0.80</td>
<td>0.75</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.90</td>
<td>0.90</td>
<td>2.70</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>Available Phosphorus, %</td>
<td>0.45</td>
<td>0.45</td>
<td>0.40</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Mineral Premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.

<sup>2</sup>Vitamin premix contained the following per kilogram of diet: Vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; Vitamin E, 66 IU; Vitamin B12, 34.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; vitamin K, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; and biotin, 252 µg; ethoxyquin 66 mg.

<sup>3</sup>Data expressed on a percentage of dry matter basis. Formulations were confirmed by proximate analysis.
ground at 21, 24, 28, 32, and 36 wk of age. The comb height was measured from the top of the head to the highest point of the comb at the same ages that shank length was measured. Eggs were collected and handled as described in Experiment II-1, except that only 60 eggs per pen were set during each respective week. Additionally, the internal egg quality was investigated by measuring albumen height (Benton et al., 2001) and yolk weight at 48 and 60 wk of age. Male and female mortality were recorded daily and feed allocations adjusted appropriately. At 64 wk of age, all remaining males from 3 pens per treatment combination were necropsied and the testes excised and weighed.

Statistical Analyses. The fertility and hatchability data were analyzed on either a weekly or biweekly basis. Additionally, these data were analyzed on a cumulative and age-based quartile time period basis. For Experiment II-1, a completely randomized design with two treatments and six replicates per treatment was used. For Experiment II-2, a completely randomized design with a factorial (2 X 2) arrangement of treatments was used. The main factors were comb height (LC or SC) and selenium source (HiSe Yeast or NaSe). The treatments were randomly distributed among 16 pens with 4 replicate pens per interaction cell. The general linear model (GLM) procedure with the repeated statement of SAS Institute (2001) was used to analyze the continuous variables. Percentage data was analyzed after arcsine transformation. The fertility data were analyzed as categorical data where each individual egg was taken as a binomial event, either fertile or infertile, using the general model (GENMOD) procedure of SAS Institute (2001). To test the time effect and its interaction with the treatments a split plot design with time and its interactions in the subplot was conducted using PROC MIXED of SAS Institute (2001). Orthogonal contrasts were used to compare treatments probabilities (Giesbrecht and Gumpertz, 2004). Means were
partitioned using LSMEANS and statements of statistical significance were based upon $P < 0.05$ unless otherwise indicated.

**RESULTS**

*Experiment II-1.* The effect of the antioxidant concentration level on male BW from 21 to 64 wk of age is shown in Figure II-2. The High antioxidant level produced a lower male BW at 48 wk of age but no significant effect was observed for female BW (data not shown). The effect of the antioxidant level on percentage fertility is shown in Figure II-3 and Table II-3. Figure II-3 details that percentage fertility declined after 38 wk of age, while Table II-3 shows the decline during the third quartile time period. However, the High antioxidant level ameliorated the decrease in fertility. Once the daily male feed allocation was increased by 5 g at 54 wk of age (about the end of the third quartile time period) fertility increased in both treatments and the difference between antioxidant levels tended to disappear. Overall, the High antioxidant level improved percentage fertility by 4.9% (Table II-3). No significant difference was observed for embryo mortality (data not shown). The effect of antioxidant level treatment on egg production and eggshell weight is shown in Figures II-4 and II-5, respectively. The High antioxidant level significantly increased egg production and numerically increased ($P < 0.10$) eggshell weight at 40 and 58 wk of age.

*Experiment II-2.* The effect of selenium source and comb height classification on male BW is shown in Figure II-6. The LC group exhibited significantly heavier BW from 21 to 32 wk of age but the differences thereafter decreased. No significant effect was observed for selenium source or the interaction with comb height. The correlation between BW and comb height
Figure II-2. Male BW as affected by antioxidant level (Control or High) during the production period in Experiment II-1. Asterisk (*) represents a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
Figure II-3. Fertility during the production period as affected by antioxidant level (Control or High) in Experiment II-1. The arrow indicates the age when the male feed allocation was increased by 5 g/male/d. Asterisk (*) represents a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
Table II-3. Broiler breeder fertility as affected by level of antioxidant in Experiment II-1

<table>
<thead>
<tr>
<th>Antioxidant Level</th>
<th>Weeks Of Age Quartile</th>
<th>Cumulative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28-35</td>
<td>36-45</td>
</tr>
<tr>
<td>High</td>
<td>94.6</td>
<td>95.5</td>
</tr>
<tr>
<td>Control</td>
<td>94.0</td>
<td>93.9</td>
</tr>
</tbody>
</table>

\(^{A, B}\) Means with different superscript are significantly different \((P < 0.01)\).
\(^{a, b}\) Means with different superscript are significantly different \((P < 0.05)\).

1 Categorical analysis does not generate standard errors.

2 Time by treatment (antioxidant level) interaction was significant \((P < 0.01)\).
Figure II-4. Egg production as affected by antioxidant level in Experiment II-1. Asterisk (*) represents a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
Figure II-5. Egg shell weight during the production period as affected by antioxidant level during the production period in Experiment II-1. Plus symbol (+) indicates a numeric difference that approached significance (P < 0.1) as determined by GLM procedure at each age.
Figure II-6. Male body weight (BW) during the rearing and production period as affected by male comb height classification and selenium source during the production period in Experiment II-2. Selenium was supplied as organic selenium (HiSe Yeast), or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 wk of age.
was 63%, based upon data from 21 to 36 wk of age. The effect of selenium source and comb height classification on shank length and comb height is shown in Figures II-7A and II-7B, respectively. Comb height followed a growth pattern that mirrored BW from 21 to 36 wk of age. No significant difference was observed for shank length, although the SC and HiSe Yeast combination group exhibited the lowest value from 21 to 36 wk of age. No significant difference was found for absolute or relative weights of the testes at 64 wk of age (data not shown). In Experiment II-2 there was high variability in the weekly fertility data so the effect of selenium source and comb height classification on percentage fertility was reported only on a quartile time period basis (Table II-4) for clarity. Percentage fertility exhibited consistently high values (>90%) during the complete laying period in any given treatment. Significant interactions between the comb height classification, selenium source, and flock age were observed. During the first quartile time period (28 to 35 wk of age), fertility was significantly lower with NaSe and a significant interaction revealed decreased fertility in the LC male group fed NaSe. No significant differences were observed during the second and third quartile time periods for percentage fertility. During the fourth quartile time period (56 to 64 wk of age) the NaSe group exhibited the highest fertility, while no significant interaction between comb height and selenium source was observed (Table II-4). Conversely, HiSe Yeast increased percentage total embryo mortality during the third quartile time period (46 to 55 wk of age) (Figure II-8A). Analyses were conducted for early, late, and pipped embryo mortality, but only the more representative total embryo mortality was presented for the sake of brevity (Figure II-8A). There were significant interactions in the third quartile time period, which showed that HiSe Yeast increased total embryo mortality in the SC group. This embryo mortality was due mainly to late mortality (data not shown) and
Figure II-7. Male shank length (Panel A) and comb height (Panel B) as affected by selenium source and male comb height classification in Experiment II-2. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 wk of age.
**Table II-4.** Broiler breeder fertility as affected by male comb height classification and selenium source during the laying period in Experiment II-2\(^1\). Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 wk of age.

<table>
<thead>
<tr>
<th>Comb Height Classification</th>
<th>Selenium Source</th>
<th>Weeks Of Age Quartile(^2) (%)</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>28-35</td>
<td>36-45</td>
</tr>
<tr>
<td>LC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiSe Yeast</td>
<td></td>
<td>98.1 (^a)</td>
<td>95.2</td>
</tr>
<tr>
<td>NaSe</td>
<td></td>
<td>96.1 (^b)</td>
<td>95.1</td>
</tr>
<tr>
<td>SC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiSe Yeast</td>
<td></td>
<td>97.6</td>
<td>94.9</td>
</tr>
<tr>
<td>NaSe</td>
<td></td>
<td>97.5</td>
<td>96.0</td>
</tr>
</tbody>
</table>

Interactions

<table>
<thead>
<tr>
<th></th>
<th>Selenium Source</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LC HiSe Yeast</td>
<td></td>
<td>98.6</td>
<td>95.6</td>
<td>95.5</td>
<td>92.8</td>
<td>95.5</td>
</tr>
<tr>
<td>LC NaSe</td>
<td></td>
<td>94.6</td>
<td>94.2</td>
<td>96.2</td>
<td>95.7</td>
<td>95.1</td>
</tr>
<tr>
<td>SC HiSe Yeast</td>
<td></td>
<td>97.6</td>
<td>94.9</td>
<td>94.6</td>
<td>92.0</td>
<td>94.7</td>
</tr>
<tr>
<td>SC NaSe</td>
<td></td>
<td>97.5</td>
<td>96.0</td>
<td>95.6</td>
<td>96.6</td>
<td>96.4</td>
</tr>
</tbody>
</table>

P-value\(^3\) 0.001 0.001 0.96 0.08 0.001

\(^A,B\) Means with different superscript are significantly different \((P < 0.01)\).
\(^a,b\) Means with different superscript are significantly different \((P < 0.05)\).
\(^1\) Categorical analysis does not generate standard errors.
\(^2\) Time by treatment (comb height and selenium source) interaction was significant \((P < 0.01)\).
\(^3\) Probability value for interaction term between comb height and selenium source.
Figure II-8. Total embryo mortality (Panel A) and percentage fertile hatchability (Panel B) as affected by selenium source and male comb height classification (Experiment II-2). Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 wk of age. Means with different superscripts are significantly different (P< 0.05). Time by treatment (comb height and selenium source) interaction was significant (P < 0.01).
reflected in the fact that HiSe Yeast, when compared to NaSe, significantly decreased fertile hatchability in the SC group (Figure II-8B). The HiSe Yeast-LC combination group exhibited an intermediate value for embryo mortality and fertile hatchability (Figures II-8A and II-8B).

The effect of selenium source on egg production during the production period and female BW at 48 wk of age is shown in Figure II-9. NaSe resulted in slightly better egg production, that was significant at 29, 38, 43, and 51 wk of age; but significant differences were not observed for eggshell weight or internal egg quality as indicated by albumen height and yolk weight (data not shown). HiSe Yeast significantly increased female BW at 48 wk of age when all females in the experiment were weighed.

**DISCUSSION**

The feeding of diets from 21 to 64 wk of age with two levels of antioxidants (differed in Vitamin E, Vitamin C, and source of selenium) in Experiment II-1 and with two different sources of selenium in Experiment II-2 was intended to evaluate the effect of the prolonged use of these antioxidants on male BW and reproductive performance. Because the same diets were supplied to both males and females, as would be the case commercially, it was not possible to completely separate sex effects with respect to fertility and/or hatchability. However, the present discussion has emphasized the male effect since the male feed program was the only change made at 54 wk of age when percentage fertility had declined (Experiment II-1) and males were classified at 21 wk of age according to their comb height as an indicator of sexual maturity (Experiment II-2) while females remained the same across all treatments. Specific female data such as BW, egg production, and egg shell quality were
Figure II-9. Egg production and female body weight (BW) at 48 wk of age, as affected by selenium source during the production period in Experiment II-2. Asterisk (*) represents a significant difference ($P < 0.05$) as determined by GLM procedure at each age. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe).
taken to demonstrate the effect of antioxidants on overall breeder performance and to delineate obvious female effects. Experiment II-1 initially showed that the High antioxidant level improved fertility. However, the effect appeared to be related to a deficient male feed allocation, as the significant difference disappeared after the male feed allocation was increased by 5 g/bird/d at 54 wk of age. At 48 wk of age the Normal antioxidant group had a significantly heavier male BW and apparently required more ME to sustain reproductive activity. Although, the detrimental effects of oxidative damage on poultry male fertility has been extensively described (Surai, 2002; Breque et al., 2003), under commercial conditions it has been clearly demonstrated that excessive feed restriction alone decreased the intake of macronutrients such as CP and ME and depressed fertility (Wilson et al., 1987a; Wilson et al., 1987b; Sexton et al., 1989b; Cerolini et al., 1995; Romero-Sanchez and Brake, 2005). Furthermore, it has been observed that males that slowly but consistently gained BW showed better reproductive performance (Cerolini et al., 1995).

Male broiler breeders must be physiologically and behaviorally mature to successfully elicit female sexual receptivity and copulate. Although only 16% of the variability in fertility was attributed to comb area, the comb area has been thought to be a reliable indicator of male fertility in some strains (McGary et al., 2002; McGary et al., 2003). In the present experiment the comb height was used as indicator of the comb area. Comb height has been shown to be highly correlated with comb area and this measurement, as described in Experiment II-2, has been shown to be practical under both commercial and experimental conditions (Joseph et al., 2003). Previous work by Zuk et al. (1990a; 1990b) and McGary et al. (2003) showed that although large comb (LC) males tended to have better reproductive performance, there was probably a minimum acceptable comb size, and once the comb
reached the “minimum” size there were no reproductive differences between small comb (SC) and large comb (LC) males. Comb size has been shown to be an indicator of sexual maturity as testosterone release was shown to be highly correlated with comb development (Deyhim et al., 1992; Rath et al., 1996). Testes size has also been shown to be related to comb size, although it has been shown that greater testicular weight in broiler breeders may not necessarily correspond to increased spermatogenesis (Janssen et al., 2000) or higher androgen levels (Fernandez-Abella et al., 1999). Furthermore, a negative relationship between testes weight and fertility has been found in some broiler breeder strains (McGary et al., 2002). In the present experiment no significant difference was observed for testes weight at 64 wk of age, while the SC group exhibited greater comb height than those reported to be subnormal by McGary et al. (2002; 2003). This could partially explain why grouping by comb height did not affect fertility in Experiment II-2.

A significant correlation between comb height and BW (0.63) was consistent with the fact that male BW was significantly higher in the LC group, although the difference tended to disappear with age. Testosterone, the major male reproductive hormone, has been shown to be affected by the interaction of ME intake and age (Sexton et al., 1989b). This study showed that testosterone decreased with age but the decline was less for diets with adequate ME levels (diets between 2,800 and 3,200 kcal/kg). In the same manner, Hulet and Brody (1986) reported increased semen production in turkey toms with higher ME intakes from 34 to 36 wk of age. On the other hand, excessive ME intake and obesity have been shown to be associated with low levels of testosterone (Barbato and Landau, 1974) and low levels of testosterone binding proteins (Amatruda et al., 1978).
The HiSe Yeast source used in these experiments has been reported to contain 50% selenomethionine, 15% selenocystine, 15% selenocysteine, 10% selenocystathione, and 10% methylselenocysteine (Mahan, 1999). Obviously, these several seleno-compounds and other residues in the commercial presentation could have had unexpected effects that differed from what might be expected from selenium alone. The significant interaction between selenium source and comb height showed that LC males, which had heavier BW, failed in maintain fertility under severely feed restricted conditions. HiSe Yeast improved fertility of LC males by up to 4% but did not affect the fertility of SC males during the first quartile time period. HiSe Yeast had a greater effect when feed intake was more restricted relative to male BW.

Figure II-10 illustrates the ME balance in males with different comb heights relative to their maintenance requirement with the current experimental temperatures (Experiment II-2). The data showed that the LC males were heavier and the ME intake was apparently marginal. So, in such a situation an energy-sparing effect was one explanation for the apparently beneficial effect of HiSe Yeast. On the other hand, the HiSe Yeast increased female BW at 48 wk of age and reduced overall egg production by potentially a similar mechanism. Higher female BW should require more ME and egg production could have been negatively affected as a result. To illustrate this effect, the ME required daily to maintain 100 g of BW and to produce a 60 g egg each day was calculated (Combs, 1968). It was determined that 7 kcal ME/d were required to maintain each additional 100 g of BW. This extra BW demanded ~50 kcal ME/bird/wk that represented the ME required to produce 0.25 egg/wk. Therefore, HiSe Yeast appeared to have shifted female energy metabolism somewhat from egg production to BW gain. Selenium has been shown to be part of many selenoproteins whose functions vary widely from antioxidant capacity to energy metabolism (Allan et al., 1999). One of the most
Figure II-10. Energy balance in males from 22 to 32 wk of age. Males were classified by comb height at 21 wk of age as either large comb (LC) or small comb (SC) males. This figure compares ME intake with ME required for maintenance (ME_m) at experimental temperature as function of the BW as $\text{ME}_m = 1.45 \times \text{BW}^{0.65} \times (1.78 - 0.012 \times T)$ where $T$ is average temperature in °F (Combs, 1968).
important selenoproteins has been shown to be type I iodothyronine deiodinase, which catalyzes the 5'-deiodination of thyroxine to tri-iodo-thyronine (Behne et al., 1990). Therefore, if organic selenium from HiSe Yeast was more effectively incorporated into tissue proteins and after protein degradation more selenium was available for iodothyronine deiodinase, in a manner similar to what has been shown to occur with other selenoproteins (Allan et al., 1999), the increased conversion rate of T4 to T3 could affect important metabolic and reproductive functions. In fact, dietary selenium has been suggested to improve BW gain in broilers though the conversion of T4 to T3 (Jianhua et al., 2000). When males with apparently early maturity and larger BW, as for the LC group, were slightly underfed after photostimulation, HiSe Yeast appeared to have a nutrient sparing effect that allowed the LC males to maintain high fertility even when they did not gain BW in a consistent manner. A similar effect may have been responsible for the subsequently increased female BW and poorer egg production.

In contrast with the results during the early production period, NaSe produced better percentage fertility than did HiSe Yeast during the fourth quartile time period (Table II-4). Additionally, HiSe Yeast increased embryo mortality (early, late, and pipped) during the third quartile time period (Figure II-7A). A significant interaction showed that the negative effect of HiSe Yeast on embryo mortality occurred in the SC males. However, fertility and fertile hatchability demonstrated that the HiSe Yeast group maintained performance above 92% (Table II-4) and 91% (Figure II-8B), respectively. With such good performance it was not likely that HiSe Yeast was truly detrimental to fertility and/or embryo mortality, although a significant effect was found. However, in light of the results, it was important to remember that organic selenium, such as selenomethionine, has been shown to be incorporated more
easily into some tissues. If there was an excess in the quantity of selenium being incorporated, sub-lethal toxicity symptoms could occur. In fact, it has been shown that biotransformation of organic selenium compounds by flavin monooxygenase enzymes can produce selenoxides (Palace et al., 2004). These selenoxides have been shown to produce seleninic acid (Chen and Ziegler, 1994; Rooseboom et al., 2001), a potent redox cycling compound that can elevate oxidative stress in vivo (Spallholz and Hoffman, 2002; Spallholz et al., 2004). In a similar manner, the presence of selenomethionine has been shown to be essential for superoxide radical production and thereafter methioninase activity may be responsible for the teratogenesis observed in offspring of adult fish that had ingested sub-lethal but elevated concentrations of selenomethionine (Palace et al., 2004). However, in the present experiments apparent macroscopic embryo malformations were not observed, nor would the inclusion level of selenomethionine be considered toxic. However, it remained possible that since organic selenium has been shown to be taken up more efficiently than NaSe, a lower concentration may need to be considered for organic sources than for inorganic sources to obtain the same selenium status in the animal.

Although many clinical studies have demonstrated the beneficial effects of antioxidants in select cases of male infertility, some studies failed to demonstrate the same benefit. In an extensive review in human studies, Agarwall et al. (2004) concluded that “the majority of the human studies suffered from lack of placebo-controlled, double-blind design, making it difficult to reach a definite conclusion. In addition, pregnancy, the most relevant outcome variable of fertility, was reported in only a few studies.”

In Experiment II-1 there was a trend towards improved eggshell weight, but in Experiment II-2 there was no effect. Therefore, the effect could be related to the higher vitamin
concentration of the antioxidant additive package in Experiment II-1. In particular, Vitamin C has been shown to play an important role in hydroxylation reactions (Leeson and Summers, 2001) that play a role in eggshell formation (Zapata and Gernat, 1995).

In conclusion, HiSe Yeast and antioxidant vitamins (Vitamins C and E) could have had an energy sparing effect as antioxidants ameliorated fertility problems due to marginal feed allocations, but when feed allocations were increased the antioxidant benefit tended to disappear. Thus, it was difficult to ascribe the beneficial effects to antioxidant properties alone. On the other hand, the data showed conflicting effects of selenium source on fertility, embryo mortality, and fertile hatchability during the production period.
REFERENCES


MANUSCRIPT III. Effect of Feeding Program and Dietary Crude Protein During The Rearing Period To 26 Weeks of Age On Body Weight and Fertility of Broiler Breeder Males

ABSTRACT

A 2 X 2 factorial experiment was conducted to compare the effects of two male broiler breeder feed allocation programs (Concave or Sigmoid) during the rearing period from 2 to 26 wk of age and the interaction with dietary crude protein (CP) level (12 or 17% CP) on male broiler breeder BW and subsequent reproductive performance. Day-old Ross 344 male broiler breeders were placed in 12 pens in a blackout rearing facility. From 0 to 2 wk all birds received a 17% CP starter feed after which pens were randomly assigned to the four treatment combinations. The cockerels were photostimulated at 21 wk of age when they were transferred to the production facility. All males were weighed individually at 4, 8, 12, 16, 20, 24, 26, 28, 32, 40, 48, 56, and 64 wk of age. To test the effect of the male feed restriction program on fertility at 49 wk of age the male feed allocation in all treatments was increased by 5 g/d.

No significant interaction of feeding program and CP level was observed for male BW or fertility at any age. As expected, the larger early increments in feed allocation to birds on the Sigmoid program corresponded to greater early BW gain while the more rapid feed increments during the late rearing period of the Concave program allowed these birds to have faster rates of BW gain later in the rearing period. By 26 wk differences in BW had disappeared. However, males on the Concave feeding program lost BW from 32 to 40 wk of age and had significantly lower BW from 40 to 48 wk of age corresponding to a more rapid decrease in fertility than birds from the Sigmoid feeding program. Low dietary CP (12 %)
during the rearing period to 26 wk of age decreased male BW from 8 to 32 wk of age, but no significant differences were observed later. The low dietary CP level during rearing improved fertility during the production period. Fertility declined more rapidly after 40 wk of age for male breeders that had received 17% CP diets during the rearing period. The subsequent increase in the male feed allocation of 5g /d across all treatments at 49 wk restored fertility and caused the differences within main factors of either feed allocation program or rearing CP level to diminish. The data suggested that BW during the early rearing period did not affect fertility but that heavy males towards the end of the rearing period from the Concave or 17% CP treatments had higher maintenance requirements and were unable to sustain fertility after 40 wk of age without an appropriate allocation of feed.

*Key words*: broiler breeders, feeding program, crude protein, fertility
INTRODUCTION

Continued genetic improvement for rapid broiler growth, feed efficiency, and meat yield has generally implied a need for constant adaptation of the management and feeding regimen of broiler breeder parent stock to avoid the potential negative effects on reproductive performance of overweight birds. Although quantitative and qualitative feed restriction methods have been shown to be appropriate to control broiler breeder BW (Mench, 2002), an excessive nutrient restriction of the male has remained a recurrent problem (Brake and Peak, 1999). Previous data has shown that female broiler breeders required a minimum cumulative nutrient intake prior to photostimulation to sustain subsequent egg production and fertility during the production period (Walsh and Brake, 1997; 1999). Further, de Reviers and coworkers (1990), Brake and Peak (1999), and Romero-Sanchez et al. (2004) showed that male broiler breeders may also require a minimum cumulative nutrient intake during rearing in order to sustain subsequent reproductive performance. Small differences in caloric intake during rearing of male breeders have been shown to affect both growth rate and body composition, while small differences in dietary CP allocation resulted in only slight differences in male broiler breeder BW (Vaughters et al., 1987). In female broiler breeders, the effect of different feeding programs and different CP levels was extensively researched by Walsh and Brake (1997; 1999). According to these previous data it appeared that in males the effect of feeding programs could interact with different dietary CP levels to affect fertility. The fertility of broiler breeder flocks has been reported to decline during the latter weeks of life (Creel et al., 1990; Walsh and Brake, 1997). This reduction in fertility has been largely attributed to progressive reductions in the mating efficiency and/or frequency of mating (Duncan et al., 1990), which may, in turn, be related to excessive male BW (Hocking,
1990; Hocking and Bernard, 2000) and lameness (Hocking and Duff, 1989). However, some evidence that a deficient ME intake towards the end of the production period could exacerbate the decline in late fertility was also reported by others (Parker and Arscott, 1964; Sexton et al., 1989b; Bramwell et al., 1996) and was primarily observed in broiler breeder males when BW exceeded 5 kg (Hocking and Bernard, 2000). The decline in fertility of heavy BW breeders was found under conditions of restricted feeding contradicted the findings that *ad libitum* feeding was beneficial to fertility of caged males (Sexton et al., 1989a; Cerolini et al., 1995). Based on these observations the convention of feed restriction of male broiler breeders to achieve a fixed BW target may actually be counterproductive as the increased nutrient requirements for both growth and reproductive function of modern genotypes may not be met. Therefore, the objective of this experiment was to determine if the pattern of feed allocation during the rearing period of male broiler breeders interacted with the dietary CP level to affect male broiler breeder BW and fertility during the subsequent production period.

**MATERIALS AND METHODS**

*General.* An experiment was conducted to evaluate the effects of the male feeding program and dietary CP during rearing (from 2 to 26 wk of age) on BW, fertility, and hatchability of Ross 308 SF female and Ross 344 male broiler breeders. There were 220 females and 24 males placed in each of 12 female or 12 male pens located in a blackout rearing house. After 23 h of light per day was used for one wk the lighting program was changed to 8L:16D at 20 lux and maintained to 21 wk. Broiler breeders were moved at 21 wk of age to a curtain-sided slat-litter house where the photoperiod was extended to 14 h and 15 h at 22 and 24 wk,
respectively, and to 15.5 h and 16 h at 5% and 50% rate of lay, respectively. Natural light entered the breeding house through open or translucent curtains during normal daylight hours. Supplemental light from a mixture of incandescent and high pressure sodium lamps provided an average intensity of 35 lux when natural light was not present in the breeder facility. Upon transfer of birds from the rearing to the production house, 200 females and 20 males were allocated to each of the 12 breeder pens while maintaining treatment identity. Each breeder pen was equipped with 2/3 wood slats and 1/3 litter floors. Feed was provided for daily consumption during the first two wk of age and then a 4/3 feed allocation program was used until 21 wk of age after which a daily feeding program was employed. Access to water was limited by a time clock and solenoid system sufficient to control litter moisture and allow the birds to have unlimited access to water until one hour after all feed was consumed during rearing and a similar amount on non-feed days. Water was limited to 8 h per day during the production period.

*Experimental Treatments and Measurements.* Two feeding programs (Concave and Sigmoid) (Figure III-1) and two dietary CP levels (12 and 17 %) during rearing (2 to 26 wk of age) were evaluated using a single level of ME (Table III-1). All males received the 17% CP diet for the first 2 wk. Male pens were then randomly assigned to one of the four treatments that were designed to provide equal cumulative intake of ME at 22 wk of age. A cumulative CP intake of 1,470 g was provided by the 12% CP diet, while the 17% CP diet provided 1,990 g. Romero-Sanchez et al. (2004) previously demonstrated 1,470 g CP during the rearing period to be adequate for maintaining fertility during the production period.
Figure III-1. Male feeding program during the rearing period to 26 wk of age. After 26 wk of age both feeding programs supplied the same amount of feed (110 g/d) until 49 wk when the allocation was increased by 5 g/male/d.
### Table III-1. Composition of rearing and breeder diets.

<table>
<thead>
<tr>
<th>Ingredient and Analysis</th>
<th>Male Rearing Diet</th>
<th>Breeder Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein Level (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>64.17</td>
<td>57.20</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>5.50</td>
<td>19.20</td>
</tr>
<tr>
<td>Wheat midds</td>
<td>24.00</td>
<td>2.30</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>-</td>
<td>15.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.51</td>
<td>1.45</td>
</tr>
<tr>
<td>Limestone fine</td>
<td>1.31</td>
<td>1.45</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin premix²</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.64</td>
<td>0.35</td>
</tr>
<tr>
<td>Coccidiostat (Amprol)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>D,L-Methionine</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>Selenium premix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Mold inhibitor</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Tallow</td>
<td>1.90</td>
<td>2.32</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>0.03</td>
<td>0.29</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated analysis³

<table>
<thead>
<tr>
<th></th>
<th>Male Rearing Diet</th>
<th>Breeder Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>12.00</td>
<td>17.00</td>
</tr>
<tr>
<td>ME, kcal/g</td>
<td>2.92</td>
<td>2.92</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.59</td>
<td>0.74</td>
</tr>
<tr>
<td>Methionine + Cystine, %</td>
<td>0.51</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Available phosphorous, %</td>
<td>0.45</td>
<td>0.45</td>
</tr>
</tbody>
</table>

¹ Mineral Premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.

² Vitamin premix contained the following per kilogram of diet: Vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; vitamin E, 66 IU; vitamin B12, 34.6 ug; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; vitamin K, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; and biotin, 252 ug.

³ Expressed on a percentage of dry matter basis. Formulations were confirmed by proximate analyses.
From 26 wk of age during the production period a constant feed allocation program of 110 g/male/d providing a ME intake of ~330 kcal/male/day was used (Bramwell et al., 1996). To test the effect of an increment in the male feed allocation during later production, a single feed increment of 5 g/male/d was applied at and after 49 wk of age after the fertility had been observed to decline.

BW of individual males was determined every 4 wk from 4 to 40 wk of age, after which measurements were taken every 8 wk to 64 wk of age. Male and female mortality were recorded daily. Eggs were collected twice daily and stored in a cooler at 18.6ºC and 70% RH until incubated. Eggs laid on the floor and slats were collected separately and not incubated. Fertility and hatchability analysis was carried out on the basis of biweekly sets of 180 eggs per replicate pen. All unhatched eggs were opened and examined macroscopically for evidence of embryological development by a single experienced individual.

Statistical Analyses. The experimental design was a completely randomized design with a 2 X 2 factorial structure, with three replicate pens per interaction cell. Comparisons of fertility and hatchability data were made between individual observations from the biweekly evaluations as well as on an overall cumulative basis and age-based quartile time period basis prior to analysis, respectively, as described previously (Walsh and Brake, 1997). The general linear model (GLM) procedure with the repeated statement of SAS (SAS Institute, 2001) was used to analyze the continuous variables. The fertility data was analyzed as categorical data, where each individual egg was taken as a binomial event, either fertile or infertile (Walsh and Brake, 1999), using the general model (GENMOD) procedure of SAS Institute (2001). To test the age effect and its interaction with the treatments a split plot design with age and its interactions in the subplot was conducted using PROC MIXED (SAS Institute,
Orthogonal contrasts were used to compare main factors and interaction probabilities (Giesbrecht and Gumpertz, 2004). Means were partitioned using LSMEANS and statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

**RESULTS**

Since no significant interaction between the feeding programs and dietary CP levels applied during the rearing period from 2 to 26 wk of age was found for male BW, only the main effects of either the Concave or Sigmoid feeding program or dietary CP levels of 12 or 17% on male BW are shown in Figures III-2A and III-2B, respectively. BW gain on either feeding program reflected the rate of feed allocation, with BW gain initially being higher on the Sigmoid program during the early rearing period and conversely higher during the latter rearing period for birds assigned to the Concave feeding program in proportion to the respective weekly feed allocation changes at those ages. By 26 wk differences in BW between feeding programs had disappeared and only re-appeared at 40 wk and 48 wk of age when male breeders on the Sigmoid program exhibited a heavier BW. Irrespective of the dietary feeding program, increasing the dietary CP level during rearing from 12% to 17% increased male BW after 4 wk of age. While differences in dietary CP level were only applied before 26 wk, the differences in BW remained apparent to 32 wk of age. Although cumulative mortality increased with time as expected, no significance differences were observed for percentage mortality. The effect of feeding program or dietary CP applied during the growing period from 2 to 26 wk of age on subsequent male fertility is shown in Figures III-3A and III-3B, respectively. The percentage fertility for each quartile time period and on cumulative basis is shown in Table III-2. The Sigmoid feeding program produced
Figure III-2. Male BW as affected by feeding program and crude protein (CP) level during the rearing period to 26 wk of age. Ross 344 males received two different feed programs, either Concave or Sigmoid, as shown in Figure III-1, and two different CP levels, either 12 or 17% as shown in Table III-1. Asterisk (*) represents significant difference ($P < 0.05$) as determined by GLM procedure at each age.
Figure III-3. Fertility during the production period as affected by feeding program and crude protein (CP) level during the rearing period to 26 wk of age. Ross 344 males received two different feed programs, either Concave or Sigmoid, as shown in Figure III-1, and two different CP levels, either 12 or 17% as shown in Table III-1. The arrow indicates the age when the feed male allocation was increased by 5 g/male/d. An asterisk (*) represents significant differences ($P < 0.05$) determined by GLM procedure at each age.
Table III-2. Broiler breeder fertility during the production period as affected by crude protein (CP) level and male feed allocation program during the rearing period (2 to 26 wk of age)\(^1\).

<table>
<thead>
<tr>
<th>Rearing Feed Program</th>
<th>Rearing Protein Level</th>
<th>Weeks of age quartile(^2) (%)</th>
<th>28-35</th>
<th>36-45</th>
<th>46-55</th>
<th>56-64</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concave</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>95.3</td>
<td>91.2</td>
<td>B</td>
<td>79.9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>95.6</td>
<td>93.7</td>
<td>A</td>
<td>87.1</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>96.3</td>
<td>93.4</td>
<td>A</td>
<td>86.3</td>
<td>A</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>94.6</td>
<td>91.5</td>
<td>B</td>
<td>80.7</td>
<td>B</td>
</tr>
<tr>
<td>Sigmoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>96.5</td>
<td>92.0</td>
<td></td>
<td>83.9</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>94.2</td>
<td>90.4</td>
<td></td>
<td>76.0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>96.1</td>
<td>94.8</td>
<td></td>
<td>88.7</td>
<td></td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concave</td>
<td>12</td>
<td></td>
<td>96.5</td>
<td>92.0</td>
<td></td>
<td>83.9</td>
<td></td>
</tr>
<tr>
<td>Concave</td>
<td>17</td>
<td></td>
<td>94.2</td>
<td>90.4</td>
<td></td>
<td>76.0</td>
<td></td>
</tr>
<tr>
<td>Sigmoid</td>
<td>12</td>
<td></td>
<td>96.1</td>
<td>94.8</td>
<td></td>
<td>88.7</td>
<td></td>
</tr>
<tr>
<td>Sigmoid</td>
<td>17</td>
<td></td>
<td>95.1</td>
<td>92.6</td>
<td></td>
<td>85.5</td>
<td></td>
</tr>
</tbody>
</table>

\(P\)-value\(^3\): 0.11 0.24 0.04 0.11 0.66

\(A,B\) Means with different superscript are significantly different \((P < 0.01)\).

\(a,b\) Means with different superscript are significantly different \((P < 0.05)\).

\(^1\) Categorical Analysis does not generate standard errors

\(^2\) Time by treatment (CP x Feeding program) interaction was significant \((P < 0.01)\).

\(^3\) Probability value for interaction term between rearing feed program and protein level.
better fertility during the second and third quartile time periods and on a cumulative basis. The categorical analyses showed that CP level had an effect on the fertility during the fourth quartile period time as well as on a cumulative basis. A significant interaction between feeding program and CP level during the third quartile time period showed that the Concave and 17% CP combination was more negatively affected (Table III-2). The decrease in fertility observed after 42 wk of age was greater for birds that had been reared on the Concave feeding program or the 17% CP rearing treatments. There was a greater decline in fertility after 42 wk of age (Figure III-3). The increase in the male feed allocation at 49 wk of age increased fertility in all treatments and resulted in the differences in fertility within either rearing feeding program or dietary CP level to disappear, showing a significant interaction with flock age (quartile time period) \( (P < 0.0001) \). Since differences in embryonic mortality were not significant, the hatchability data showed a similar pattern compared with fertility (data not shown for brevity).

**DISCUSSION**

Small changes in the feeding program during the early part of the rearing period resulted in rapid responses in male BW, while there appeared to be a lag in the BW response when similar changes in the feeding program were applied towards the end of the rearing period or during the early production period (16 to 26 wk of age). Males from the Concave feeding program that received more rapid feed increments during the late rearing and early production periods exhibited decreased fertility during the second and third quartile time periods compared with males from the Sigmoid feeding program. The better fertility that was observed with the Sigmoid feeding program was similar to previous work that showed that
replacement breeder pullets reared on feeding programs that provided slow and consistent feed increments late in the rearing period produced better fertility compared to those ones that received either rapid or decreasing feed increments (Walsh and Brake, 1997). These prior authors concluded that differences in late fertility were due to the shape of the female feed allocation program during the rearing period, since pullet BW at onset of lay was not different. An important observation in the present study was that the decline in late fertility observed with the Concave feeding program was restored by increasing the male feed allocation after 49 wk, clearly demonstrating that the quantity of feed for males played a more important role in determining late fertility than did the feed allocation program during the rearing period, at this particular point in time. Although there were no differences in BW between the Concave and Sigmoid feeding programs at 26 wk and no differences in fertility observed during the first quartile time period (early production period), the Concave feeding program appeared to increase the ME requirements of male breeders later in the production period as was evidenced by a decrease in BW at 40 and 48 wk. The decline in BW at 40 wk suggested that while both groups of birds received the same ME intake from 26 wk of age, the ME intake of birds that had received the Concave rearing program was below that required to support a modest BW gain and fertility. This was confirmed by the observation that the decreased fertility was restored by a 5 g increase in the daily feed allocation for these birds. Zhang et al. (1999) used two feeding regimes that created growth curves during the early rearing period similar to those created by the present Concave and Sigmoid feeding programs. Although fertility was not measured, it was found that changing from the starter to a grower diet at 0.45 or 0.68 kg BW did not significantly alter BW gain after 4 wk, semen volume, semen concentration, and percentage of males in semen production (Zhang et al.,
This indicated that the shape of the feeding program during the first part of the rearing period appeared to have a minor effect on the reproductive performance of male broiler breeders compared to the feeding program during later rearing. Although previous data had shown that different dietary CP levels (between 12 and 18%) during rearing did not affect male broiler breeder BW (Wilson et al., 1987a,b), in the present experiment the low CP level (12%) produced a lower BW during rearing. These data were in agreement with those reported by Zhang et al. (1999). Since male BW was not different due to feed allocation program at 26 wk or due to dietary CP level after 32 wk it was concluded that differences in male BW per se were not the cause of fertility differences later in the production period. The lower BW of males on the 12% CP diets to 26 wk resulted in these males consuming similar CP, when this was expressed per unit of BW, to the males receiving the 17% CP diets. Since the ME intake was the same, again the smaller males consuming the 12% CP diets consumed more ME per unit of BW. In this experiment the rearing feed allocation on the 12% CP diet was calculated to provide 1,470 g of cumulative CP at time of photostimulation. These data supported previous findings of Romero-Sanchez et al. (2004) that feeding low density breeder diets that contained reduced CP and ME levels, while meeting the minimum cumulative ME and CP recommendations of Brake and Peak (1999), resulted in good fertility. The increased BW on the 17% CP diets corresponded to a decline in early and late fertility and suggested that the larger BW males had received inadequate quantities of nutrients, probably ME, required to support that greater BW and continued reproductive performance. Zhang et al. (1999) also found a numerically higher semen concentration in caged males consuming 12% CP during the rearing period compared to those consuming 16% CP.
A major problem encountered in commercial broiler breeder flocks has been the dramatic decrease in fertility that commonly occurs in the latter part of the laying cycle, particularly after 50 wk of age (Kirk et al., 1980). The late decline in fertility has been thought to be largely a male fertility problem because fertility could be maintained by artificial insemination, although a greater number of spermatozoa was required (Brillard and McDaniel, 1986). Possible explanations for the decrease in late male fertility have been that males become very large and broad breasted towards the end of the laying year and may find it anatomically difficult to achieve cloacal contact with hens (Soller et al., 1965; Hocking and Duff, 1989). However, strong evidence has suggested that a deficiency in ME towards the end of the production period could also cause decreased fertility (Parker and Arscott, 1971; Sexton et al., 1989b; Bramwell et al., 1996). These observations were made mainly when the male BW was heavier than 5 kg (Hocking and Bernard, 2000). Duncan et al. (1990) showed that cockerels on their most severe feed restriction program (100 g/male/d) exhibited some reduction in fertility at 38 wk of age and a more substantial reduction at 58 wk of age. The fact that the cockerels on their next most severe restriction (115 g/male/d) also exhibited a reduction of fertility at 58 wk suggested that these two levels of restriction were too severe (Duncan et al., 1990) and that late fertility problems were somewhat a function of dietary ME allocation. An important observation in the present work was that males that received the 17% CP diets during rearing or received the more rapid feed increases during the late rearing period (Concave feed program) apparently required more feed (ME) later in production to sustain fertility. A significant interaction during the third quartile time period showed the combination Concave feeding program and 17% CP decreased significantly fertility (Table III-2). The hypothesis that late fertility was suppressed as a result of inadequate nutrient
intake relative to the requirements of the males was supported by our data that showed that an additional 5 g/male/d of feed that supplied ~15 kcal ME/male/d was sufficient to restore fertility of male broiler breeders that had been grown on the Concave feeding program. While increasing male feed allocation to meet the ME requirements of heavier birds was shown to be important, extremes in broiler breeder male BW should be avoided because very heavy male broiler breeders tend to have an increased incidence of musculo-skeletal diseases (Duff and Hocking, 1986) and it was possible that pain or mechanical weakness associated with these disorders could interfere with copulation (Hocking and Duff, 1989). The present experiment showed that increasing male broiler breeder feed intake during the late production period can be an important management tool for sustaining and/or restoring late fertility in male broiler breeders.
REFERENCES


MANUSCRIPT IV. Effect of Feed Allocation Program From 16 to 26 Weeks and Subsequent Feed Increments During The Production Period On Body Weight and Fertility of Broiler Breeder Males and Performance of The Broiler Progeny

ABSTRACT

Two experiments were conducted to evaluate different feed allocation programs from 16 to 26 wk of age and during the production period on broiler breeder male BW, fertility, and progeny performance. In Experiment IV-1, Ross 344 males were grown until 16 wk in a blackout rearing facility after which they were randomly assigned to three planes (Slow, Medium, or Fast) of feed allocation increase from 16 to 26 wk of age. These feed programs were designed to provide a gradual increase from 85 to 110 g/male/d. Feed allocation was also increased 5 g/male/d in a single increment at 55 wk of age after fertility had declined. In Experiment IV-2, a 2 X 2 factorial design evaluated the interaction between the Slow and Fast feeding programs described in Experiment IV-1 in combination with two male feeding programs during the production period (Constant or Increasing). In both experiments, birds were photostimulated at 21 wk of age when they were moved into the production facility. All birds were weighed individually at 4, 8, 12, 16, 20, 24, 26, 28, 32, 40, 48, 56 and 64 wk of age. Furthermore, in Experiment IV-2, at 32 and 50 wk of age broilers were hatched to evaluate the effect of male treatments on progeny performance. Percentage fertility and embryo mortality were evaluated biweekly.

In Experiment IV-1, the males on the Fast feed program exhibited higher mortality after 32 wk of age and lower fertility after 46 wk of age. However, fertility recovered after feed was increased in all treatments. In Experiment IV-2, the Constant program elicited lower fertility
from 36 to 55 wk of age and this resulted in a lower progeny BW and poorer adjusted feed conversion at 42 d of age. Males that received the Slow feed program from 16 to 26 wk of age gained BW slowly and apparently required less feed to sustain their BW throughout the production period. Increasing male feed allocation during the production period improved fertility and favorably impacted progeny performance.

*Key words:* broiler breeders, feeding program, fertility, broiler progeny
INTRODUCTION

A major problem encountered with commercial broiler breeder flocks has been the often dramatic decrease in fertility during the latter part of the laying period, particularly after 50 wk of age (Kirk et al., 1980; Walsh and Brake, 1997). Although there have been several physiological factors identified that could affect fertility, it has been generally considered that the reduction in fertility was caused by a decline in mating activity that in turn was largely attributable to the heavy BW and poor physical condition of older males (Hocking, 1990). However, other evidence has suggested that a ME deficit was a more likely cause of such male fertility problems (Buckner et al., 1986; Sexton et al., 1989a,b; Cerolini et al., 1995; Bramwell et al., 1996a). It has been further shown that the pattern of male feeding during the latter part of the rearing program affected late fertility to a greater extent than did the initial feed allocation pattern during the early rearing period (Zhang et al., 1999; Peak, 2001; Romero-Sanchez and Brake, 2005). The late rearing and the early production period (about 16 to 26 wk of age), when birds have been typically photostimulated, could be the most critical period in the development of sexual maturity in the broiler breeder male (Leeson and Summers, 1997; Brake, 2002). In fact, it has been suggested that during this period an internal metabolic signal triggers reproductive development in broiler breeder females (Renema et al., 1999; Joseph et al., 2000). It has also been demonstrated that the detrimental effects that an inappropriate feeding program had on late fertility could be reversed by increasing the male feed allocation during the late production period (Romero-Sanchez and Brake, 2005; 2006). In a similar manner, Cerolini et al. (1995) used a low density diet to show that an increase in male feed allocation from 110 to 130 g/male/d improved fertility. Therefore, we hypothesized that increasing the male feed allocation in a
consistent manner during the production period would prevent the typical late decline in fertility. The present investigation was conducted to further investigate the effects of the male broiler breeder feed allocation program from 16 to 26 wk of age on fertility and broiler performance and to determine if an interaction existed with the production feed allocation program.

MATERIALS AND METHODS

General. Two experiments were conducted to evaluate the effects of the male broiler breeder feeding program from 16 to 26 wk of age and feeding program during the subsequent production period on BW, fertility, hatchability, and performance of the broiler progeny. In both experiments, 220 female Ross 308 SF broiler breeder chicks or 24 male Ross 344 cockerels were placed, respectively, into each of 12 male or 12 female floor pens located within a blackout rearing house. After 23 h of light per day for one wk all birds were reared in a blackout house to 21 wk of age under a 8L:16D lighting program at 15 lux light intensity. From 0 to 2 wk all birds received a starter feed followed by a rearing diet to 21 wk and a production diet from 21 to 64 wk of age (Table IV-I). Broiler breeders were moved at 21 wk of age to a curtain-sided slat-litter house where the photoperiod was extended with artificial light to 14 h and 15 h at 22 and 24 wk, respectively, and to 15.5 h and 16 h at 5% and 50 % rate of lay, respectively. Natural light entered the breeding house through open or closed translucent curtains during normal daylight hours. Supplemental light provided an intensity of 35 lux when natural light was not present. An average of 200 females and 20 males were allocated to each of the 12 breeding pens at 21 wk of age. Each breeder pen was equipped with 2/3 wood slats and 1/3 litter floors. Male and female feed was added daily during the first week of age and then a 4/3 feed allocation program was used until 21 wk of
Table IV-1. Composition of broiler breeder and broiler diets.

<table>
<thead>
<tr>
<th>Ingredient and analysis</th>
<th>Broiler Breeder Diet</th>
<th>Broiler Diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starter</td>
<td>Rearing</td>
<td>Production (%)</td>
</tr>
<tr>
<td>Corn</td>
<td>65.11</td>
<td>68.00</td>
<td>64.40</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>22.21</td>
<td>17.00</td>
<td>19.20</td>
</tr>
<tr>
<td>Extruded soybean</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wheat midds</td>
<td>7.64</td>
<td>9.87</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>-</td>
<td>-</td>
<td>2.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.62</td>
<td>1.6</td>
<td>1.20</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.24</td>
<td>1.28</td>
<td>6.10</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>0.20</td>
<td>0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin premix²</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.58</td>
<td>0.41</td>
</tr>
<tr>
<td>Coccidiostat (Amprol)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.08</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Sodium selenite (NaSe)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein supplement³</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mold inhibitor</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>-</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.20</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>1.00</td>
<td>1.00</td>
<td>1.10</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated analysis ⁴

<table>
<thead>
<tr>
<th></th>
<th>Starter</th>
<th>Rearing</th>
<th>Production</th>
<th>Starter</th>
<th>Grower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>17.00</td>
<td>15.00</td>
<td>16.03</td>
<td>22.7</td>
<td>19.8</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>2,925</td>
<td>2,925</td>
<td>2,918</td>
<td>3,086</td>
<td>3,148</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.88</td>
<td>0.75</td>
<td>0.82</td>
<td>1.23</td>
<td>1.05</td>
</tr>
<tr>
<td>Methionine + Cystine, %</td>
<td>0.70</td>
<td>0.80</td>
<td>0.63</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.90</td>
<td>0.90</td>
<td>2.70</td>
<td>1.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Available Phosphorus, %</td>
<td>0.45</td>
<td>0.45</td>
<td>0.42</td>
<td>0.50</td>
<td>0.45</td>
</tr>
</tbody>
</table>

¹Mineral Premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.

²Vitamin premix contained the following per kilogram of diet: Vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; Vitamin E, 66 IU; Vitamin B12, 34.6 ug; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; Vitamin K, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; and biotin, 252 ug.

³Pro-Pack is a protein supplement containing: crude protein, 62.3%; available phosphorus, 3.24%; calcium, 6.37%; lysine, 3.87%; methionine, 1.78%.

⁴Data expressed on a percentage of dry matter basis. Formulations confirmed by proximate analyses.
age after which a daily feeding program was employed. Access to water was limited by a
time clock and solenoid system sufficient to control litter moisture and allow the birds to
have unlimited access to water until one hour after all feed was consumed during rearing and
a similar amount on non-feed days. Water was limited to 8 h per day during the production
period.

Experimental Feeding Program. In Experiments IV-1 and IV-2, from 16 to 26 wk of age,
either three or two different feed allocation programs were evaluated, respectively. In
Experiment VI-1, three planes (Fast, Medium, or Slow) of feeding were used to provide three
gradual increases from 85 to 110 g/day as shown in Figure IV-1A. In Experiment IV-2 only
the Fast and Slow programs were evaluated (Figure IV-1B). These feed allocation programs
were designed to not considerably affect the cumulative nutrition to 21 wk when birds were
photostimulated, as the differences between the Fast and the Slow programs were less than
260 kcal of ME and 15 g of CP. A more than adequate minimum intake of 31.46 Mcal of ME
and 1,669 g of CP was assured at 21 wk of age (Brake, 2002).

From 26 wk of age, during the production period, a single constant feed allocation (110
g/male/d) was used in Experiment IV-1. In Experiment IV-2, two different feed allocation
programs (Constant or Increasing) were applied after 26 wk of age to study the interaction
between the feed allocation program from 16 to 26 wk of age with the feed allocation
program during the production period. The Constant feeding program maintained 110
g/male/d throughout the production period while the Increasing feeding program provided
biweekly increments of 1 g from 26 to 42 wk and a similar increase every 4 wk from 42 to 62
wk until a daily feed intake of 123 g/male/d was reached (Figure IV-1B). Male BW was
measured individually at 16, 20, 24, 28, 32, 36, 40, 48, 56, and 64 wk of age. Male and
Figure IV-1. Male feeding programs from 16 to 26 wk of age and during the production period. Panel A shows Experiment IV-1 including three feeding programs (Fast, Medium and Slow) while panel B shows Experiment IV-2 using only the Fast and Slow programs from 16 to 26 wk of age and the Constant and Increasing programs during the production period.
female mortality were recorded daily. Eggs were collected twice daily and stored in a cooler at 18.6°C and 70% RH until incubated. Eggs laid on the floor and slats were collected separately and not incubated. Fertility and hatchability analysis was carried out on the basis of biweekly sets of 180 eggs per replicate pen. All unhatched eggs were opened and examined macroscopically by a single experienced individual for evidence of embryological development. Fertility and stage of embryonic mortality were determined on all unhatched eggs from each setting.

**Broiler Trials.** To test for effects of male breeder treatment on the performance of broiler progeny, two broiler trials were conducted for Experiment IV-1 and one broiler trial in Experiment IV-2. Eggs were collected at 30 and 50 wk of age in Experiments IV-1 and were stored as described above and incubated under standard conditions. In Experiment IV-2, eggs were collected at 50 wk of age. The chicks were sexed at hatch, while maintaining identity of the breeder treatments, and allocated to a 72-pen house with 12 (Experiment IV-1) or 9 (Experiment IV-2) replicate pens of 15 male or female chicks from each breeder treatment. A single starter or grower diet that met or exceeded the NRC (1994) minimum requirements was used during the starter and grower periods from 0-21 and 21-42 d, respectively (Table IV-1). No finisher diet was used for the sake of simplicity.

**Statistical Analyses.** From 16 to 26 wk of age a complete randomized design was employed in both experiments, using three or two treatments with four or six replicates, respectively for Experiments IV-1 and IV-2. The fertility and hatchability data were analyzed on a biweekly basis with particular attention to the time when the broiler trials were conducted. Additionally, all fertility data were summarized on an overall cumulative pen basis and into age-based quartile time periods prior to analysis. For Experiment VI-1 a completely
randomized design within age was conducted, where the 12 pens were divided into three treatments with four replicates per interaction cell. For Experiment IV-2 a completely randomized design with a 2 X 2 factorial arrangement was used to analyze for effects of the two feed allocation programs around the time of photostimulation (Fast or Slow) and two feed allocation programs during the production period (Constant or Increasing). The twelve pens were divided among the four interaction combinations with three replicates per interaction cell.

For the broiler trials, a broiler sex effect was added as an additional factor. For the broiler trial of Experiment IV-1 a completely randomized design with a 3 X 2 factorial arrangement with twelve replicates per treatment was used and for the broiler trial of Experiment IV-2 a completely randomized design with a 2 X 2 X 2 factorial arrangement with nine replicates per interaction treatment was used.

The general linear model (GLM) procedure of SAS Institute (2001) was used to analyze the broiler data and the continuous variables of the broiler breeder data. The repeated statement of SAS Institute (SAS Institute, 2001) was used for BW in the broiler breeder data. The fertility data was analyzed as categorical data, where each individual egg was taken as a binomial event, either fertile or infertile, using the general model (GENMOD) procedure of SAS Institute (2001). To test the time effect and its interaction with the treatments a split plot design with time and its interactions in the subplot was conducted using PROC MIXED (SAS Institute, 2001). Orthogonal contrasts were used to compare treatments probabilities (Giesbrecht and Gumpertz, 2004). Means were partitioned using LSMEANS and statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.
**RESULTS**

*Experiment IV-1.* The effect of the three feeding programs from 16 to 26 wk of age on male BW is shown in Figure IV-2. No significant differences were observed before 36 wk of age but the Low feeding program produced a significantly increased male BW at 40 wk of age. The effect of the feeding program from 16 to 26 wk of age on male fertility is shown in Figure IV-3 and Table IV-2. There was a significant decrease in percentage fertility after 38 wk of age but the Low feeding program exhibited a significantly reduced decrease relative to the High feeding program, which experienced the greatest decline in fertility, while the Medium feeding program produced an intermediate decline. Following an increase in the male feed allocation at 55 wk of age fertility increased in all three treatments and treatment differences diminished. A significant interaction with age was also observed. Overall, breeders that received the Slow feed increment program exhibited the highest fertility compared with the birds that received the Fast feed increment program, while the Medium feeding program produced intermediate values (Table IV-2). As no significant differences were found for embryo mortality at different stages, the hatchability data followed the same pattern as that of fertility (data not show for sake of brevity).

The effect of the feeding program from 16 to 26 wk of age on cumulative male mortality is depicted in Figure IV-4. No significant differences were found up to 32 wk of age. However, the Fast feeding program group experienced a significantly higher cumulative mortality to 48, 56, and 64 wk of age.

*Experiment IV-2.* The effect of male feeding program from 16 to 26 wk of age, feeding program during the subsequent laying period, and age on male BW is shown in Figure IV-5.
Figure IV-2. Male BW as affected by feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different fed programs, either Fast, Medium, or Slow. a,b Means with different superscript are significantly different ($P < 0.05$) as determined by GLM procedure at each age.
Figure IV-3 Fertility during the production period as affected by male feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different feed programs, either Fast, Medium, or Slow. The arrow indicates the age when the feed male allocation was increased by 5g/bird/d. a,b Means with different superscripts are significantly different ($P < 0.05$) as determined by GLM procedure at each age.
Table IV-2. Broiler breeder fertility as affected by male feeding program from 16 to 26 weeks of age in Experiment IV-1\(^1\).

<table>
<thead>
<tr>
<th>Male feeding program</th>
<th>28-35</th>
<th>36-45</th>
<th>46-55</th>
<th>56-64</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>93.8</td>
<td>93.4</td>
<td>71.2</td>
<td>77.5(^B)</td>
<td>84.0(^B)</td>
</tr>
<tr>
<td>Medium</td>
<td>93.7</td>
<td>95.4</td>
<td>75.3</td>
<td>82.0(^A)</td>
<td>86.5(^{AB})</td>
</tr>
<tr>
<td>Slow</td>
<td>95.4</td>
<td>95.5</td>
<td>79.9</td>
<td>84.4(^A)</td>
<td>88.8(^A)</td>
</tr>
</tbody>
</table>

\(^A,^B\) Means with different superscript are significantly different (P < 0.01).
\(^1\) Categorical Analysis does not generate standard errors
\(^2\) Time by treatment (Male feeding program) interaction (P < 0.01)
Figure IV-4. Male mortality as affected by feeding program from 16 to 26 wk of age in Experiment IV-1. Ross 344 males received a single growing diet using three different feed programs, either Fast, Medium, or Slow. \textsuperscript{a,b} Means with different superscripts are significantly different \((P < 0.05)\) as determined by GLM procedure at each age.
Figure IV-5. Male BW as affected by feeding program from 16 to 26 wk of age (Panel A) and during the subsequent production period (Panel B) in Experiment IV-2. Ross 344 males received a single growing diet using two different feed programs, either Fast or Slow as shown in Figure IV-1, followed by either a Constant or Increasing feed allocation during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
No significant differences were observed from 26 to 40 wk of age. However, the Increasing feeding program during the production period produced a significantly increased male BW at 48 wk of age and thereafter.

The effect of male feeding program from 16 to 26 wk of age, male feeding program during the subsequent production period, and age on male fertility is shown in Figure IV-6 and Table IV-3. Although the fertility values were greater than 90%, differences in fertility appeared during the second quartile time period (Table IV-3). Fertility decreased after 40 wk of age; however, the Slow feeding program from 16 to 26 wk of age and the Increasing program during the production period experienced a significantly lower decrease in fertility (Figure IV-6). Overall, the Fast and Constant feeding programs experienced the greatest decrease in fertility. No significant interactions were found (Table IV-3). Significant difference was found in male mortality at 32 wk of age, but no further differences were found (Figure IV-7).

*Effect On Progeny Performance.* Since significant differences were not observed in broiler performance when the breeder flock was young (30 wk of age), no data were included for the sake of brevity. However, when the breeder flock was 50 wk of age, significant differences were observed in broiler performance (Table IV-4). There was no significant effect of the feeding program from 16 to 26 wk of age or a significant interaction with feeding program in production observed. Broiler BW and adjusted feed conversion (AdjFCR) at 42 d of age were significantly improved by the Increasing feeding program employed during the broiler breeder production period. No significant effect on broiler feed consumption was observed.
Figure IV-6. Fertility as affected by male feeding program from 16 to 26 wk of age (Panel A) and during the production period (Panel B) in Experiment IV-2. Ross 344 males received a single growing diet using two different fed programs from 16 to 16 wk of age (Fast or Slow), and then a group received either the Constant or Increasing feed program during the production period.
Table IV-3. Broiler breeder fertility as affected by male feeding program from 16 to 26 weeks of age and during the production period in Experiment IV-2\(^1\).

<table>
<thead>
<tr>
<th>Breeder Feeding Program</th>
<th>Weeks of Age Quartile(^2) (%)</th>
<th>Cumulative (%A, B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28-35</td>
<td>36-45</td>
</tr>
<tr>
<td>Fast</td>
<td>96.7</td>
<td>95.5(^B)</td>
</tr>
<tr>
<td>Slow</td>
<td>97.1</td>
<td>96.5(^A)</td>
</tr>
<tr>
<td>Constant</td>
<td>96.9</td>
<td>95.4(^B)</td>
</tr>
<tr>
<td>Increasing</td>
<td>96.9</td>
<td>96.6(^A)</td>
</tr>
</tbody>
</table>

Interactions

<table>
<thead>
<tr>
<th>Breeder Feeding Program</th>
<th>Weeks of Age Quartile(^2) (%)</th>
<th>Cumulative (%A, B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>96.6</td>
<td>95.0</td>
</tr>
<tr>
<td>Fast Increasing</td>
<td>96.8</td>
<td>95.9</td>
</tr>
<tr>
<td>Slow</td>
<td>97.2</td>
<td>95.7</td>
</tr>
<tr>
<td>Slow Increasing</td>
<td>97.1</td>
<td>97.2</td>
</tr>
</tbody>
</table>

P-value \(^3\) NS

\(^{A,B}\) Means with different superscripts are significantly different \((P < 0.01)\).

\(^{a,b}\) Means with different superscripts are significantly different \((P < 0.05)\).

\(^1\) Categorical analysis does not generate standard errors.

\(^2\) Time by treatment (feeding program from 16 to 26 wk of age x production period) interaction was significant \((P < 0.01)\).

\(^3\) Probability value for interaction term between rearing period and production period were no significant \((NS, P > 0.1)\).
Figure IV-7. Male mortality as affected male feeding program from 16 to 26 wk of age and during production period in Experiment IV-2. Ross 344 males received a single growing diet using two different fed programs from 16 to 16 wk of age (Fast or Slow), and then a group received either the Constant or Increasing feed program during the production period. Means with different superscripts are significantly different ($P < 0.05$) as determined by GLM procedure at each age.
Table IV-4. Effect of the broiler breeder male feeding program from 16 to 26 wk of age and during the production period in Experiment IV-2 on broiler performance as measured by body weight (BW), adjusted feed conversion (AdjFCR), and feed intake.

<table>
<thead>
<tr>
<th>Breeder Feeding Programs</th>
<th>Broiler BW (g)</th>
<th>Broiler AdjFCR (g:g)</th>
<th>Broiler Feed Intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing Period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>45 a</td>
<td>996</td>
<td>2,588</td>
</tr>
<tr>
<td>Slow</td>
<td>44 b</td>
<td>945</td>
<td>2,562</td>
</tr>
<tr>
<td>SEM</td>
<td>0.16</td>
<td>5.0</td>
<td>18.88</td>
</tr>
</tbody>
</table>

| Production Program       |                |                      |                        |
|--------------------------|----------------|----------------------|                        |
| Constant                 | 45             | 997                  | 2,546 b                | 1.40 | 1.77 a| 1,396 | 4,473 |
| Increasing               | 45             | 953                  | 2,604 a                | 1.40 | 1.74 b| 1,334 | 4,555 |
| SEM                      | 0.16           | 5.0                  | 18.88                  | 0.00 | 0.00 | 6.59  | 41.19 |

\(^{ab}\) Means with different superscripts are significantly different \((P < 0.05)\).

Interaction between sex, feeding program from 16 to 26 wk of age, and production program was not significant \((P > 0.1)\) and the data are not shown.
DISCUSSION

Zhang et al. (1999) showed that changing feed from a starter to a grower diet at a mean BW of either 0.45 or 0.68 kg had no effect on BW gain after 4 wk of age. Although fertility was not measured, these authors found no significant differences between treatments for semen volume, semen concentration, or percentage of males in semen production. These data were similar to data from Peak (2001) and Romero-Sanchez et al. (2006) that showed that altering the male feeding program during the initial part of the rearing period did not affect broiler breeder male fertility during the subsequent production period. In contrast, changes in male feeding program during the latter part of the rearing period appeared to affect subsequent broiler breeder fertility (Peak, 2001; Romero-Sanchez and Brake, 2005). Therefore, three feeding programs were designed, representing different feeding management approaches around the time of photostimulation. These feeding programs were designed to provide a gradual feed increment from 85 to 110 g/male/d between 16 and 26 wk of age. The Fast and the Slow feeding programs used in Experiments IV-1 and IV-2 provided a similar feed allocation from 16 to 26 wk of age as was described for the Concave and Sigmoid programs, respectively, of Romero-Sanchez and Brake (2005), while the Medium program in Experiment IV-1 provided an intermediate feed increment program. The results of these experiments confirmed the previous data of Romero-Sanchez and Brake (2005) that male feed rearing program had very little effect on early fertility but that there was a progressive decrease in fertility after 38 wk of age that has previously been described (Kirk et al., 1980; Creel et al., 1990; Walsh and Brake, 1997). However, importantly, the relative decrease in late fertility was comparatively less for birds that had received the Slow feed program when compared to the Fast feed program, while fertility of birds on the Medium feed program was
intermediate. The results showed that increasing the male feed allocation program too rapidly during the period around photostimulation caused the BW of these birds to reach a plateau (Figure IV-2) at 36 wk of age that was indicative of inadequate nutrient intake to support further BW gains. The detrimental effect that this plateau in BW had on fertility of birds in the Fast and Medium groups was evident (Figure IV-3) and supported previous work by Peak (1996; 2001) and Romero-Sanchez and Brake (2005; 2006) that showed fertility to decline when nutrient intake was inadequate to support continued modest increases in BW.

Replacement of broiler breeder males (“spiking”) has previously been utilized as a strategy to overcome the adverse effects of increased male age on fertility (Wolanski et al., 2004). Although young males have been surmised to have a higher concentration of spermatozoa in ejaculates of greater volume, there was evidence that old males could have less dead spermatozoa and the same physiological capacities to fertilize the egg in vivo as young males (Bramwell et al., 1996b). This implied that if the physical impairments that males develop upon aging could be reduced, spiking may not be necessary. Spiking with old males has also been shown to improve fertility (Leeson and Summers, 1999), suggesting that the decline in late fertility may be associated with reduced libido rather than with physical problems caused by increasing breeder age. However, there was strong evidence that showed that sexual behavior may not be well correlated with fertilizing ability in the domestic cock (Penquite et al., 1930; Parker et al., 1940; Wood-Gush and Osborne, 1956; Siegel, 1972; Duncan et al., 1990). With this scenario, it appeared that there was not a clear pattern that correlated low fertility in old males with loss of libido. The fact was that, in this and previous experiments (Romero-Sanchez and Brake, 2005; 2006), an improvement in fertility was observed when the feed allocation was increased at 55 wk of age. This suggested that nutrient deprivation
(primarily ME) could be related to reduced libido and fertility. This conclusion would support previous findings that showed that fertility declined when male nutrient intake was limiting (Parker and Arscott, 1964; Sexton et al., 1989b; Cerolini et al., 1995).

The results of Zhang et al. (1999) showed that consistent BW gain after sexual maturity was required to optimize the number of spermatozoa per ejaculate and semen production, especially during the late breeding period after 46 wk of age. This finding was in agreement with data from Sexton et al. (1989a,b), who reported that full-fed males that possessed heavier BW also had higher spermatozoa per ejaculate than those that were restricted-fed from 17 to 56 wk of age. Zhang et al. (1999) showed that a greater BW gain after sexual maturity was associated with an increased number of spermatozoa per ejaculate. Similar results have been shown in turkeys (Bakst and Cecil, 1981; Revington et al., 1991). These former and our present data strongly suggested that feeding programs should be designed to maintain BW gain rather than be focused on the attainment of some fixed BW. While sufficient feed allocation appeared to be a prerequisite, overfeeding has also been shown to impair reproductive function. Cerolini et al. (1995) showed that ad libitum feeding of male broiler breeders increased BW but did not increase fat deposits. However, the highest mean percentage of males producing semen was recorded in groups at a moderate restriction of 357 kcal/male/d (130 g/d) compared to the ad libitum fed groups or groups that had been more severely restricted to either 302 or 330 kcal/male/d.

Some decline in fertility of broiler breeder flocks must be inevitable because of the normal aging process in females and males. As fertility has been shown to be maintained towards the end of the production period by artificial insemination it has been assumed that the decline in late fertility was predominantly a male problem (Brillard and McDaniel, 1986). Based on
that had difficulties achieving cloacal contact with hens (Soller et al., 1965; Hocking and Duff, 1989; Hocking et al., 1989). Other investigations have related fertility problems to leg problems of overweight males (Duff and Hocking, 1986) although leg problems were more frequently observed in adequately performing caged birds (Leeson and Summers, 1997). Based on these data, it would appear that the reduction in broiler breeder fertility that has been commonly observed in older commercial flocks could be attributed to a decreased mating efficiency and/or frequency (Duncan et al., 1990), which in turn, may be related to excessive male BW (Hocking and Bernard, 2000; Hocking and Robertson, 2000), lameness (Hocking and Duff, 1989; Hocking et al., 1989), or excessive restriction of nutrients (Cerolini et al., 1995). Further, the high correlation that had previously been observed between body fat and reproductive parameters has led several authors to conclude that a minimum percentage of body fat was required for optimum reproductive function (Sherman et al., 1975; Frisch et al., 1981). Importantly, the convention that modern meat-type males overeat and become too fat was not supported by the data of Cerolini et al. (1995) that showed no effects of ad libitum male feeding on the percentage fat pad. Restricted feeding of males in cages has also been shown to be detrimental to spermatozoal production, whereas ad libitum feeding was beneficial in such cases (Sexton et al., 1989a,b). The 110 g and 328 kcal ME/male/d that were supplied in Experiment IV-1 were similar to that supplied in a study by Bramwell et al. (1996a). Although these previous researchers reported a decrease in male fertility that was related to a reduction of male BW of about 1 kg, the males in Experiment IV-1 demonstrated a normal BW. It was important to note that in the work by Bramwell et al. (1996a) the average male at 33 wk of age was very heavy with an average
BW of 5.94 kg. The degree of restriction, relative to BW, in that experiment would have been more severe than in the present work, leading to a greater negative impact on reproductive performance. The present results supported those of Sexton et al. (1989a) that daily ME intake below the minimum level resulted in a decreased semen spermatozoal concentration. While effects on average male performance must be important when considering flock fertility, it was reasonable to assume that effects on fertility varied between individual males in relation to variations in individual BW versus individual nutrient intake. Presumably, the largest males in a flock would be more subject to nutrient deficiencies (primarily ME) than smaller males and would be the first to show reduced semen production and mating activity. Evidence to support this hypothesis was provided by the results of the broiler trials conducted in Experiment IV-2. When there were no differences in fertility between male breeder treatments there were also no differences in the performance of the broiler progeny. However, in the second broiler trial the treatments that showed a decline in broiler breeder fertility also showed reduced 42 d broiler performance (Figures IV-8 and IV-9). The most plausible interpretation of these data was that there was a reduction in the mating frequency of the heaviest broiler breeder males with the greatest potential to produce heavy BW progeny. Similar results have previously been shown by Attia et al. (1993; 1995) who provided broiler breeder males with different levels of daily ME intake and reported a significant increase in 6-wk BW of offspring sired by males provided the highest ME intake. These and our data suggested that while the overall decline in late broiler breeder fertility can be reduced by increased feed allocation to all birds in the flock, this practice may have the most benefit with the male broiler breeders that possessed the greatest BW. The benefits of
Figure IV-8. Effect of the male feeding program during the production period on broiler breeder fertility and its effect on progeny BW at 42 d in Experiment IV-2. Ross 344 broiler breeder males were fed a single diet following an Increasing or a Constant program during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
Figure IV-9. Effect of the male feeding program during the production period on broiler breeder fertility and its effect on the progeny adjusted feed conversion (AdjFCR) at 42 d in Experiment IV-2. Ross 344 broiler breeder males were fed a single diet following an Increasing or a Constant program during the production period. An asterisk (*) indicates a significant difference ($P < 0.05$) as determined by GLM procedure at each age.
providing sufficient nutrients to maintain mating behavior and libido of these birds was shown to not only increase the fertility of the flock as a whole but also to have positive effects of increasing the overall genetic potential of the broiler progeny for growth and feed efficiency.
REFERENCES


SUMMARY AND CONCLUSIONS

Previous data demonstrated that broiler breeder fertility can be significantly and consistently affected by the cumulative nutrient intake during rearing of females and males. However, male cumulative nutrient intake had not been as clearly evaluated as had that of the female prior to this study. Furthermore, interactions between male cumulative nutrient intake and feeding programs had not been sufficiently investigated as they had been for females. Also, much of the published literature had been conducted with males in cages where artificial insemination was employed to obtain fertility data. Although under the conditions of these experiments, which emulated commercial conditions, it was difficult to totally separate male effects from female effects during the production period, this study represented a significant step forward for male broiler breeder nutrition and feeding management.

The data of the present study showed that the Low plane of cumulative nutrition produced a male of similar carcass characteristics compared with males from the High plane. However, because the Low plane produced greater relative frame size at 21 wk of age as measured by heavier ribs and shanks plus feet relative, there must have been slightly less BW on a relative basis that would reduce the daily ME requirement and improved fertility was more apparent with feeding program employed (Experiment I-1). This effect can be viewed in a simplistic manner as a decreased maintenance requirement for the Low plane males that was appropriately covered by the specific feed allocation provided in this study. Assuming this scenario, it would be possible to obtain good percentage fertility from males with different carcass compositions and BW if those males received enough nutrients to meet maintenance and reproductive requirements appropriate for that BW, as long as the males possessed a carcass that did not interfere with mating, i.e. too much breast meat that caused balance
problems or excessively long legs. Although males on the Low plane of nutrition exhibited shorter comb height and shank length during the rearing period, there was no negative effect on the onset of sexual maturity when compared with males on the High plane of nutrition (Manuscript I) so the shorter shank length probably just reflected the slower growth of the lower BW Low plane males during rearing. Although feed restriction was more severe in the Low plane of nutrition, no significant effect was observed in male uniformity, even when the stocking density was greater in Experiment I-2.

Furthermore, the data of Manuscript I showed that a cumulative nutrient intake, regardless of the method employed to achieve this during the rearing period, of 29.6 Mcal of ME and 1,470 g of CP resulted in a broiler breeder male of adequate BW, frame, and physical attributes at 21 wk of age to be able to maintain optimum fertility and produce broiler progeny with the potential for rapid growth throughout the production period. However, the data did suggest that this level of cumulative nutrition was near the minimum.

In a similar manner, better fertility was observed with a 12% CP than with 17% CP rearing diet in Manuscript III. Males in the 12% CP diet also received 1,470 g of cumulative CP at 21 wk of age. Although the response was more evident through modification of diet (Experiment I-1) than with a different feeding program (Experiment I-2), the Low plane of nutrition consistently improved fertility under the conditions of management employed during the production period of these experiments. In general, males from the High plane of nutrition grew faster during the rearing period, but no significant differences in BW, shank length, comb height were observed after 28 wk of age, indicating that males that gained BW slowly but more consistently during the production period exhibited better fertility. Although High BW has been implicated as the main cause of declining fertility, McGary et al. (2003)
did not find a correlation between fertility and male BW or spermatozoal penetration of ova in two different broiler breeder strains. Therefore, it seemed unlikely that BW \textit{per se} directly impacted male mating ability within the normal BW range. In contrast, there was strong evidence to suggest that Heavy males could be easily over-restricted (ME deficient) and made to stop mating. This was observed in Experiment I-2, where the Heavy males reared on a High plane of nutrition (HiFeed) failed to sustain fertility during the fourth quartile time period as well as on a cumulative basis. In a different manner, but supporting the cumulative nutrient during the rearing period theory, the Light males reared on the Low plane of cumulative nutrition (LoFeed) might have not received enough nutrients during the rearing period to fully sustain fertility through the fourth quartile time period (Experiment I-2), even with an adequate feed allocation during the production period. Additionally, males that were fed the 17% CP diet with a Concave feed program during the rearing period showed a subsequent loss of BW and a greater decline in fertility during the third quartile period time probably due to a higher energy requirement that was not cover by the current feeding program (Manuscript III). This decline in fertility could probably have been prevented by simply increasing the daily feed allocation in some appropriate manner, as demonstrated by a subsequent experiment (Experiment IV-2).

No significant effects on fertility were observed due to differences in male comb height. Although fertility problems in males with extremely low comb development had been reported (Leonard and Zanette, 1998), once the comb reached the “minimum” size there were no fertility differences between males that possessed either small or large combs. Female \textit{Galliforms} of many species have been shown to prefer males with well developed ornaments such as combs and wattles because they were apparently reliable indicators of livability
(Rintamaki, et al., 2000) and/or male dominance status (Graves, et al., 1985, Holder and Mongomerie, 1993). However, grouping males by BW or by comb height would have decreased variability in this trait so that selection by females was not likely to occur within the experimental units (pens) used in these experiments.

In previous attempts to improve broiler breeder male fertility, several antioxidants have been used and three were evaluated in these experiments (Manuscript II). Organic selenium (high selenium yeast (HiSe Yeast)) and antioxidant vitamins (Vitamins C and E) appeared to have had an energy sparing effect when the daily ME allocations were marginal relative to BW. Antioxidants protected against large decreases in fertility when feed allocations were marginal, but when feed allocations were increased the antioxidant benefit tended to disappear, supporting the supposition that a beneficial energy sparing effect was present when feed allocations were marginal. On the other hand, the data showed contradictory effects of selenium source on fertility, embryonic mortality, and fertile hatchability during the production period. HiSe Yeast was beneficial at some points in the reproductive cycle and detrimental at other times (Experiment II-2) maybe relative to whether there was, or was not, a significant energy deficiency at a given time. An energy sparing effect when nutrient allocations were adequate could increase BW and induce the well known negative effects of excess BW. HiSe Yeast has been shown to be incorporated more efficiently in many proteins. This characteristic could partially explain its ambiguous role, improving metabolic and reproductive functions (Jianhua et al., 2000), but at some point negatively affecting fertility (Palace, et al., 2004). Supporting this hypothesis, increased egg production and fertility was observed when HiSe Yeast was added together with higher concentrations of Vitamins C and E, but decreased egg production and increased female BW when added alone. Therefore, a
different requirement for selenium yeast should be considered depending upon feeding programs and dietary formulations. Further, the reported energy sparing effect of Vitamin C, in the face of low planes of nutrition and stress, could have overcome some of the negative effects of HiSe Yeast when that dietary combination was used.

In Manuscript IV, the data suggested that slow and consistent feed increases from 16 to 26 wk of age, around the time of photostimulation and during the early phase of the production period, played an important role in subsequent male BW and fertility. The males that received Fast feed increments during this time period tended to gain BW more rapidly and apparently subsequently required more feed (i.e. ME) to sustain their BW and maintain fertility. During the production period, an increase in the male feed allocation of 5 g/male/d for all treatments at 49 wk of age (Manuscript III) or 54 wk of age (Manuscript IV) restored fertility and caused the differences due to either feed allocation program from 16 to 26 wk of age or dietary CP during the rearing period to disappear. The data suggested that BW during the early rearing period did not have a very obvious effect on fertility but that heavier males towards the end of the rearing period due to either the Concave and/or 17% CP rearing treatments had higher maintenance requirements for ME and were unable to sustain fertility after 40 wk of age without an appropriate increase in the allocation of feed (i.e. ME). To prove this point, when the male feed allocation was slowly increased during the production period in Manuscript IV (Experiment IV-2) there was an improvement in fertility and a favorable impact on broiler progeny performance. Of interest was the fact that either rearing or production feeding programs that produced males with higher fertility tended to result in improved broiler progeny performance. This indicated that the males most probably adversely affected by less than adequate nutrient intake (i.e. ME) were the higher BW males.
that had the ability to produce better performing progeny (i.e. higher BW and better FCR). On the other hand, when there were no differences in fertility there were also no differences in broiler performance. These data supported data from Attia et al. (1995), who observed a decline in broiler performance from broiler breeder males that had received low daily ME allocation when compared to broiler breeder males receiving higher ME allocations.

In conclusion, providing sufficient nutrients to broiler breeder males through a programmed increase in feed allocation with increasing age apparently maintained mating activity and libido of genetically superior males as evidenced by increased fertility of the flock as a whole and the positive effects of increased growth and feed efficiency of the broiler progeny.
REFERENCES


