ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.242.247

Research Article
Protein and Caloric Intake on the Reproductive Performance Parameters of Hyline W-36 Parent Stock Males

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Abstract

Background and Objective: Reproduction in poultry has been studied for many years and is a crucial factor to consider when selecting and raising parental lines. Considerable work has focused on broiler breeder males, which has led to the development of feed restriction practices, as well as sex separate rearing, however very little research has been done on layer-type males. In broiler breeders a negative correlation has been found to exist between the Body Weights (BW) and sexual activity of the male. This study was conducted to explore the role of diet during grow out on reproductive traits in leghorn type males. **Materials and Methods:** Hyline parent stock males were raised on 3 different diets Low (12% CP) Control (18% CP) and High (24% CP). As birds reached sexual maturity, Body Weight (BW), testis size, semen volume, sperm concentration and histology were measured. **Results:** This trial demonstrated significant ($p<0.0001$) differences in body weights between the diets. The mean BW of birds were 1214.87, 1435.41 and 1475.96 g fed on the Low diet, control and high diet, respectively. There were significant differences observed in testicle weights. The Low group had a significantly lower mean weight as compared with Control and High groups. Differences in semen volume were observed between the dietary treatment groups, as well as, differences in sperm concentrations at 18-20 weeks of age. Significant differences in development were seen between High and Low groups upon histological examination and scoring on a 0-6 scale, 0 = Immature (no spermatozoa) to 6 = Fully mature (spermatozoa present in all tubules) of the testes high (3.9) low (1.2). **Conclusion:** These results suggest that feeding high nutrient dense diets during rearing does not result in a decrease in production as observed in broiler breeder males and that low nutrient diets are not as beneficial in layer males as in broiler breeder males.

Key words: Leghorn, reproduction, semen, testis weight, testis score

Received: March 03, 2017 Accepted: May 17, 2017 Published: June 15, 2017

Citation: Zachary S. Lowman, McCaide T. Wooten, Christopher M. Ashwell, Kenneth E. Anderson and H. John Barnes, 2017. Protein and caloric intake on the reproductive performance parameters of Hy-line W-36 parent stock males. Int. J. Poult. Sci., 16: 242-247.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reproduction in poultry has been studied for numerous years and is a crucial factor to consider in poultry reproduction in commercial parental lines. Considerable work has focused on broiler breeder males, which has led to the development of feed restriction practices, as well as sex separate rearing. Previous studies using broiler breeders have examined the relationship between nutrition and reproductive performance of the birds, however, very little research has been done on layer-type males. Decreases in semen production have been observed in broiler breeder males that have been placed on lower caloric diets. It has been shown that the decrease in semen production becomes more evident when caloric intake drops below 330 kcal day^{-1} [1]. There are several studies that report decreases in not only semen volume, but decreases in sperm concentration per ejaculate also[2]. In broiler breeders a negative correlation has been found to exist between the Body Weight (BW) and sexual activity of the male[3]. There have also been reports that there is no apparent relationship between testes weight and semen production in broiler breeder males[4]. Wilson *et al*.[5] reported that broiler breeder males fed diets containing Crude Protein (CP) levels from 9-15% showed no significant difference in semen volume or concentration when collected at 48 and 49 weeks of age. Hocking and Benard[6] reported that feeding broiler breeder males on higher levels of CP (16%) did not change semen volume or concentration as compared to the low CP (12%) group and that the average testes size was smaller in the birds fed the higher CP diet than the low CP diet. Simply losing weight does not solve the issue, in fact it is very well documented that decreases in male BW during production have detrimental effects on reproduction and result in fertility issues[1,7,8]. Debates continue as to whether decreases in reproductive performance in the males may be attributed to a reduction in protein or due to a reduction in caloric intake. Parker and Arscott[9] demonstrated that white leghorn males when placed on low energy diets demonstrated a decrease in semen volume, as well as a decrease in fertilizing capacity of the sperm. Sexton *et al*.[10] proved similar findings in broiler breeder males on low caloric diets, these birds' demonstrated reductions in BW, semen weight and sperm concentrations. Broiler breeders on low calorie diets have been shown to have decreased sperm penetration ability[1]. Decreases in protein intake have been shown by researchers to have no negative impact on fertility or semen volume in white leghorn males[11] or in broiler breeders[12]. However, Romero-Sanchez *et al*.[13] demonstrated that changes in protein levels in the latter part of rearing period do have some impact on fertility in broiler breeders. Fertility has been shown to decline when the nutrient intake is inadequate to support the current BW[13].

Reductions in nutrient intake have also been shown to effect hormone production; fasted young male chickens have decreased levels of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)[14]. Birds that were fed diets were deficient in essential fatty acids also exhibited decreased levels of LH[15]. Rearing birds on protein and caloric restricted diets have been shown to have decreased testicle growth, which was attributed to the decreased levels of LH[16].

Since there has been very little research aimed at the effects of crude protein levels and metabolizable energy in layer type birds. A pilot study was conducted to compare the layer male response to similar research that has been conducted in broiler breeders.

MATERIALS AND METHODS

One hundred and twenty day old Hyline W-36 Parent Stock males were obtained from Hyline International, Dallas Center, IA., USA. All birds were reared and handled in accordance with proceeding described in the Guide for the Care and Use of Agricultural Animals in Research and Teaching[17]. The chicks were dubbed and vaccinated at the hatchery with HVT/IBD, Rispens, SB1. Upon delivery the chicks were neck tagged and placed in a petersime brooder. At 5 weeks the cockerels were moved to grow out batteries (Alternative Design, Siloam Springs, AR., USA). Forty birds were assigned to one of the 3 diet groups Low, Control and High. The Low group was fed a starter diet that contained 12% CP and 1000 kcal kg^{-1}, Control diet contained 18% CP and 1360 kcal kg^{-1} and the High diet contained 24% CP and 1480 kcal kg^{-1}. The birds were fed the respective starter for 10 weeks, they then were placed on a respective layer diet consisting of the Low containing 12% CP and 1000 kcal kg^{-1}, Control diet contained 18% CP and 1360 kcal kg^{-1} and the High diet contained 24% CP and 1480 kcal kg^{-1}. The birds were allowed *ad libitum* feed and water for the entire trial. When the birds were 15 weeks old the males were euthanized by cervical dislocation. The birds were weighed and the right testicle was removed and weighed and then the left was removed and weighed. A random sample of 5 testicles from each group were selected, cut in half horizontal and were placed in 10% buffered formalin for 24 h and then placed in 70% ethanol at 4 F. The testicles were then trimmed and embedded in paraffin, cut to a thickness of 5 μ and affixed to a slide. The sample was dewaxed and stained with hematoxylin-eosin in order to see the seminiferous tubules. The testicles were staged on a 1-6 scale based on maturation and development of seminiferous tubules (Table 1).

Table 1: Testicle scoring rubric

Scores	Stage of development	Descriptions
0	Immature	Seminiferous epithelium is composed of spermatogonia and Sertoli cells. There is no differentiation
1	V. early development	Most tubules have only spermatogonia and Sertoli cells, there are spermatocytes and possibly later stages in a few tubules
2	Early development	Most tubules show early epithelial development
3	Intermediate development	Tubules show early development with most having more advanced development to the early or infrequently, the late spermatid stages
4	Late development	Most tubules have late spermatids, but few, if any spermatozoa
5	V. late development	Spermatozoa are present in some tubules and in epididymal ducts
6	Mature	Spermatozoa are in almost all tubules and are abundant in the epididymis

Four males from each treatment were saved for semen collection using the abdominal massage method described by Burrows and Quin[18]. Birds were only stimulated 2 times during each collection to avoid damage and bleeding of phallus. Semen was collected in 1.5 mL tubes immediately from the ejaculatory groves as soon as the phallus was erect. Great care was taken to avoid feces, urates or blood contamination. The males were collected once a week from 16 weeks of age through 21 weeks of age. At each collection time point the semen volume was measured and sperm content was determined by collecting semen in a capillary tube, sealing off one end with cryoseal and then centrifuging the sample in a clinical centrifuge for 5 min. The hematocrit tube was then placed in a hematocrit reader and the percentage of packed sperm were recorded. The weights were analyzed using JMP Pro 10 one-way ANOVA (SAS Institute Inc. Cary, NC., USA). There were significant differences ($p \leq 0.05$) in body weights amongst the three different diets.

RESULTS AND DISCUSSION

The birds fed on the Low diet had significantly lower ($p<0.0001$) mean BW (1214.87 g) than the other treatment groups. The control group had a mean BW (1435.41 g) which was 40 g lighter than the High group. The High group had 1475.96 g mean BW. However, these two groups did not differ significantly from each other (Table 2). These results agree with the findings of Zhang *et al.*[19] and Parker and McSpadden[20], who reported that the crude protein levels significantly change the body weights of the birds.

There were significant differences in testicle weights between the treatments. The left and right testicles followed the same trends between the treatments. Mean testicle weight of low group of left testicle was 1.1 g and of right testicle was 1.39 g which differed significantly from the other two treatment groups. In the control group weight of the left testicle was 4.9 g and of right testicle was 5.4 g. Weight of left testicle was 6.2 g and of right testicle was 7.09 g in the high group.

Table 2: Body weights for hyline W-36 males according to diets

Diets	Body weight (g)	n	SE
Low	1214.87^{B}	21	20.25
Control	1435.41^{A}	29	23.08
High	1475.96^{A}	34	18.70

^{AB}Denotes significant differences at $p \leq 0.05$ within a column

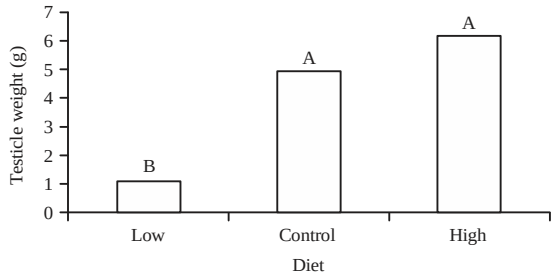

Fig. 1: Weight of left testicle by diets

^{A,B,C}Denotes significant differences within columns at $p \leq 0.05$. Low (n = 21), Control (n = 29) and High (n = 34)

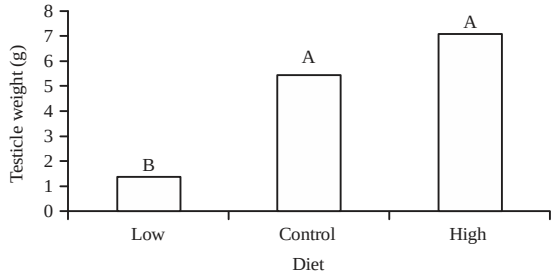

Fig. 2: Weight of right testicle by diets

^{A,B,C}Denotes significant differences within columns at $p \leq 0.05$ Low (n = 21), Control (n = 29) and High (n = 34)

The weights for the control group even though several grams lower did not differ significantly from the high group (Fig. 1, 2). There was no significant difference between the weights of the left and right testicles in any of the treatments. The total testicle weights were similar to the left and right break down weights. The low group had a significantly lower ($p<0.0001$) mean testicle weight from the other two treatments. There was not a significant difference in total testicle weight between the control and high diet group, yet

the Control group was slightly lighter than the High treatment group. (Fig. 3). Similar results were reported by Vizcarra *et al.*[21], where broiler breeder males were raised on full feed or restricted feed, the males on the full feed diet had significantly higher testicle weights upon necropsy than did the males on the restricted diet[21]. Wilson *et al.*[5] found a positive correlation between body weight and testicle weight. However, in contradiction to Hocking and Bernard[6], who reported that caged broiler breeders males fed 16% CP diets have smaller testicle size than the males fed the 12% CP diet.

Differences in semen volume were observed between the dietary treatment groups. There were significant differences in semen volumes seen at 4 of the 5 collection times. The males on the High diet always produced the largest volume of semen ranging from 310.9 µL on the initial collection and increasing to 603.1 µL of semen on the last collection (Table 3). The low group ranged from 93.8 µL on the initial collection to 385.9 µL on the fifth collection, this was significantly lower than the high groups at all time points except on wk 18 in which the volume was still lower, just not significantly different (Table 3). The volume of the control group ranged from 207.8 µL on the first collection and increased to 582.8 µL on the last collection. The only time point at which there were significant differences between the control group and either of the treatment groups was at 16 weeks, where the control group was significantly less than the high group and higher than the low group (Table 3). There were also differences in sperm concentrations with in the semen. The high group had significantly higher spermatocyte readings than the low group at weeks 18, 19 and 20, with a range starting at 5.70% topping out at 11.90% on the 5th week. The control group was intermediate and did not differ significantly from either treatment groups. The low group had a range from .95% sperm on week 16 and increased to 3.11% sperm on week 20 (Table 4). These findings agree with the results of Romero-Sanchez *et al.*[22], who observed broiler breeder birds on restricted diets produced lower levels of less concentrated semen. These trials however looked at changing the diets after rearing, where as the present study examined the effects of CP and energy levels from brooding to production. Sexton *et al.*[2] also showed that decreases in caloric intake resulted in decreased semen volume and sperm concentration. However, Wilson *et al.*[5] found that growing broiler breeder males on CP levels ranging from 9-15% resulted in no significant difference in semen volume or concentration from the males. Hocking and Bernard[6] found that caged broiler breeders have the exact opposite response to increased levels of CP, as well as demonstrating that broiler breeder males in production that were fed 16% CP diets had lower semen concentrations than

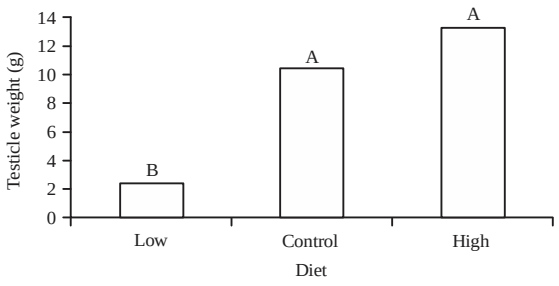

Fig. 3: Total testicle weight by diets
[A,B,C]Denotes significant differences within columns at $p \leq 0.05$. Low (n = 21), Control (n = 29), High (n = 34)

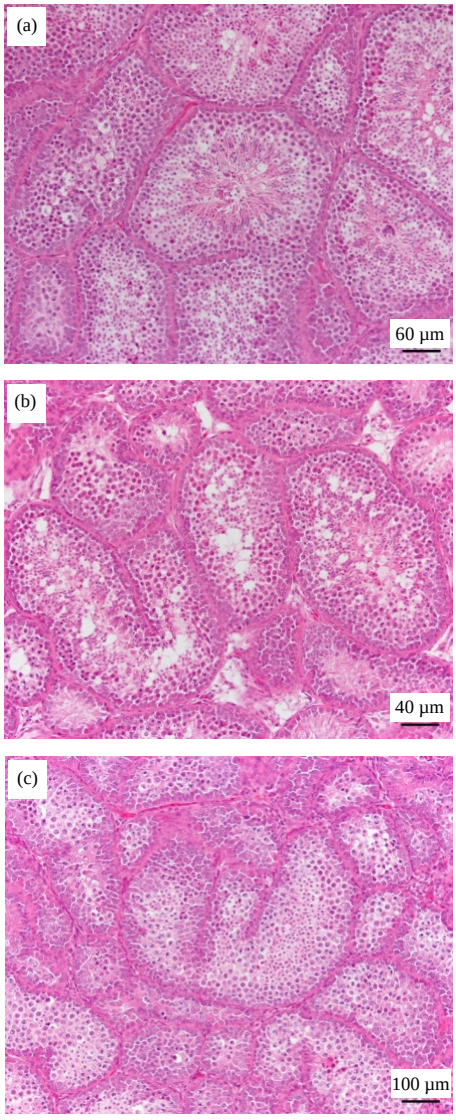

Fig. 4(a-c): All histology images stained with H and E, viewed under 140X (a) Late testicular development, (b) Mid-testicular development and (c) Early testicular development

Table 3: Mean semen volume (μL) per treatment

Weeks	Low diet	Control diet	High diet
16	93.8^{C}	207.8^{B}	310.9^{A}
17	262.5^{B}	393.8^{AB}	509.4^{A}
18	325.0^{A}	481.3^{A}	498.4^{A}
19	356.3^{B}	621.9^{AB}	670.3^{A}
20	385.9^{B}	582.8^{AB}	603.1^{A}

^{A,B,C}Denotes significant differences within columns at $p \leq 0.05$

Table 4: Mean spermatocyte reading per treatment

Weeks	Low (CP/ME)	Control (CP/ME)	High (CP/ME)
16	0.95^{A}	1.34^{A}	5.70^{A}
17	2.13^{A}	2.78^{A}	7.25^{A}
18	1.78^{B}	4.61^{AB}	10.95^{A}
19	1.60^{B}	6.07^{AB}	11.49^{A}
20	3.11^{B}	8.71^{AB}	11.90^{A}

^{A,B,C}Denotes significant differences within columns at $p \leq 0.05$

Table 5: Mean testicle scores by treatment

Diets	Mean testicle score	SE	N
Low	1.2^{B}	0.3	5
Control	3.2^{AB}	0.3	5
High	3.9^{A}	0.3	5

^{A,B}Denotes significant differences within columns at $p \leq 0.05$

males fed 12% CP. These results differ from the current study, most likely due to the differences between broiler breeder males and layer type males.

Significant differences were observed from the histological examination and scoring using testicle scoring rubric (Table 1). The testicles from the high group on average were the most mature. The development of the spermatogenic epithelium ranged from intermediate to very late stages of development. Several of the tubules contained spermatozoa (Fig. 4a). Testicles from the low group demonstrated very early epithelial differentiation. Approximately 1 out of every 100 seminiferous tubules showed some degree of differentiation, with the majority being in the spermatocyte stage (Fig. 4b). Histological examination of testicles from the Control group revealed intermediate development of spermatogenic epithelium (Fig. 4c). There were a few diffuse and clumps of spermatozoa in rete testis and efferent ducts. The high group had significantly ($p<0.0144$) higher mean score (3.9), than that of the average score (1.2) of the low group. No significant difference was observed between the control group score and the scores of the high or low CP groups (Table 5). From this trial it appears that changes in dietary CP and caloric intake can significantly influence testicular development and maturation. It appears that feeding layer males with high CP and ME diets do not result in the same negative effects observed when broiler breeder males are fed similar diets. Finally, it appears that feeding layer type males low CP and ME diets do not have the same positive effects on reproductive performance as in broiler breeder males.

SIGNIFICANCE STATEMENTS

Reproduction in the commercial poultry industry is of utmost importance and has been studied for many years. There is a wide range of research focusing on the effects diet has on reproduction in broiler breeder males; however, there is little published research focusing on these effects in layer type males. From these data it is evident that layer type males must be fed much differently than broiler breeder type males. The low cost, low CP and ME diets sometimes used in the layer industry could cause detrimental effects on reproductive performance in males. In contradiction to broiler breeder males, high energy and protein diets appear to be beneficial to reproductive performance of layer type males.

ACKNOWLEDGMENT

The authors would like to thank the North Carolina Agriculture Foundation for providing support for this project.

REFERENCES

1. Bramwell, R.K., C.D. McDaniel, W.H. Burke, J.L. Wilson and B. Howarth, 1996. Influence of male broiler breeder dietary energy intake on reproduction and progeny growth. Poult. Sci., 75: 767-775.
2. Sexton, K.J., J.A. Renden, D.N. Marple and R.J. Kemppainen, 1989. Effects of ad libitum and restricted feeding on semen quantity and quality, body composition and blood chemistry of caged broiler breeder males. Poult. Sci., 68: 569-576.
3. Burke, W.H. and J.M. Mauldin, 1985. Reproductive characteristics of broiler breeder males from flocks with low fertility. Poult. Sci., 64: 37-37.
4. Brown, H.B. and M.G. McCartney, 1983. Effects of dietary restriction on reproductive performance of broiler breeder males. Poult. Sci., 62: 1885-1888.
5. Wilson, J.L., M. Krista, G.R. McDaniel and C.D. Sutton, 1988. Correlation of broiler breeder male semen production and testes morphology. Poult. Sci., 67: 660-668.
6. Hocking, P.M. and R. Bernard, 1997. Effects of dietary crude protein content and food intake on the production of semen in two lines of broiler breeder males. Br. Poult. Sci., 38: 199-202.
7. Harris, Jr. G.C., J.A. Benson and R.S. Sellers, 1984. The influence of daylength, body weight and age on the reproductive ability of broiler breeder cockerels. Poult. Sci., 63: 1705-1710.
8. Duncan, I.J.H., P.M. Hocking and E. Seawright, 1990. Sexual behaviour and fertility in broiler breeder domestic fowl. Applied Anim. Behav. Sci., 26: 201-213.

9. Parker, J.E. and G.H. Arscott, 1964. Energy intake and fertility of male chickens. J. Nutr., 82: 183-187.
10. Sexton, K.J., J.A. Renden, D.N. Marple and R.J. Kemppainen, 1989. Effects of dietary energy on semen production, fertility, plasma testosterone and carcass composition of broiler-breeder males in cages. Poult. Sci., 68: 1688-1694.
11. Arscott, G.H. and J.E. Parker, 1963. Dietary protein and fertility of male chickens. J. Nutr., 80: 311-314.
12. Wilson, J.L., G.R. McDaniel and C.D. Sutton, 1987. Dietary protein levels for broiler breeder males. Poult. Sci., 66: 237-242.
13. Romero-Sanchez, H., P.W. Plumstead and J. Brake, 2007. Feeding broiler breeder males. 3. Effect of feed allocation program from sixteen to twenty-six weeks and subsequent feed increments during the production period on body weight and fertility. Poult. Sci., 86: 775-781.
14. Scanes, C.G., S. Harvey and A. Chadwick, 1976. Plasma luteinizing and follicle stimulating hormone concentration in fasted immature male chickens. IRCS Med. Sci., 4: 371-371.
15. Engster, H.M., L.B. Carew Jr. and F.J. Cunningham, 1978. Effects of an essential fatty acid deficiency, pair-feeding and level of dietary corn oil on the hypothalamic-pituitary-gonadal axis and other physiological parameters in the male chicken. J. Nutr., 108: 889-900.
16. Buonomo, F.C., P. Griminger and C.G. Scanes, 1982. Effects of gradation in protein-calorie restriction on the hypothalo-pituitary-gonadal axis in young domestic fowl. Poult. Sci., 61: 800-803.
17. FASS., 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd Edn., Federation of Animal Science Societies, Champaign, IL., USA.
18. Burrows, W.H. and J.P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and Turkey. Poult. Sci., 16: 19-24.
19. Zhang, X., W.D. Berry, G.R. McDaniel, D.A. Roland, P. Liu, C. Calvert and R. Wilhite, 1999. Body weight and semen production of broiler breeder males as influenced by crude protein levels and feeding regimens during rearing. Poult. Sci., 78: 190-196.
20. Parker, J.E. and B.J. McSpadden, 1943. Influence of feed restriction on fertility in male domestic fowls. Poult. Sci., 22: 170-177.
21. Vizcarra, J.A., J.D. Kirby and D.L. Kreider, 2010. Testis development and gonadotropin secretion in broiler breeder males. Poult. Sci., 89: 328-334.
22. Romero-Sanchez, H., P.W. Plumstead and J. Brake, 2007. Feeding broiler breeder males. 1. Effect of feeding program and dietary crude protein during rearing on body weight and fertility of broiler breeder males. Poult. Sci., 86: 168-174.