The effect of dietary crude protein on the fertility of male broiler breeders

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Abstract

Reports on the influence of dietary crude protein on male broiler breeder fertility are not consistent, and therefore an experiment was conducted to determine the effect of three isoenergetic diets containing 10.5%, 12.6% and 15% crude protein, respectively, on Ross broiler breeder male fertility from 26 to 41 weeks of age. Feed allocation was the same for each treatment, and was done according to breeder recommendations. Fertility was assessed by determining the number of sperm trapped in the outer perivitelline layer of eggs laid after artificial insemination with a fixed volume of semen collected from 12 males per treatment. Semen concentration, motility and morphology were also determined. Crude protein intake had a significant effect on the rate of decline in fertility post insemination. This resulted in a longer predicted length of the fertile period over all ages when eggs were fertilised with sperm obtained from males that received 12.6% CP diets (14.5 d) than males that received 10.5% and 15% CP (7 and 8.6 d respectively). There was, however, no treatment effect on the measures of live sperm with normal motility or morphology.

Keywords: Cockerel, insemination, perivitelline membrane, nutrition, reproduction
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Introduction

The management of the body weight of broiler breeders is essential to maintain fertility, and is often achieved through crude protein (CP) level and feed restriction (Zhang *et al.*, 1999). Obesity in female broiler breeders is associated with poor fertility due to ovarian dysfunction (Chen *et al.*, 2006), and is evident in reduced egg production, lower liveability and reduced egg shell quality (Wilson & Harms, 1986). Obesity in broiler breeder males, achieved when males were force-fed to achieve a heavy carcass weight, significantly reduced relative testis weights, serum testosterone concentration and the total number of sperm recovered from the vas deferens (Nir *et al.*, 1975). However, Cerolini *et al.* (1995) reported an increase in egg fertility after males had a lesser degree of feed restriction or were fed *ad libitum*, compared to those with more severe feed restriction, but with no increase in fat deposits, body weight or testes weights observed at 55 weeks of age. Sperm motility and the percentage of live sperm were also significantly increased with *ad libitum* feeding, which corresponded to an intake of 15.6 g CP/d, although the volume of semen was reduced. Birds with a greater degree of feed restriction experienced a reduction in fertility and libido (Duncan *et al.*, 1990) and an increase in feed allocation was reported to have a beneficial effect on fertility of male broiler breeders (Romero-Sanchez *et al.*, 2007). Anatomical implications may play a role in the decline in fertility of males. These could include musculo-skeletal lesions (Hocking & Duff, 1989), which could diminish the ability to copulate satisfactorily, a lower frequency of copulations and more incomplete matings from males with a greater weight of breast muscle (Hocking & Bernard, 1997b), and reduced fertility as a consequence of large bulk (Duncan *et al.*, 1990).

No difference in semen concentration or testis weight at 65 weeks of age was reported for males fed either 12% or 14% CP to achieve either 90% or 100% of the recommended body weight target, respectively (Fontana *et al.*, 1990). Semen volume was reported to be higher in males fed 12% vs. 16% CP from 28 to 36 weeks of age, but sperm concentration was not affected (Zhang *et al.*, 1999). No effects on semen volume or metabolic activity of the spermatozoa were observed in males fed different CP diets from 80 to 400 g CP/kg (Hocking, 1989). No differences in semen volume, sperm concentration, total sperm yield and the proportion

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of males producing semen were observed from males fed either 8% or 12% CP (Revington *et al.*, 1991). No effects of feeding 9%, 12% or 15% protein were observed on semen volume, concentration, number of spermatozoa ejaculated or spermatogenic activity (assessed by means of histological evaluation of the seminiferous epithelial area, tubule diameter and epithelial height) (Wilson *et al.*, 1988). There were no differences in fertility between males fed 12% or 16% CP (Hocking *et al.*, 1997b). There was also no effect of the level of protein (11% or 16% CP) observed on sexual behaviour (Duncan *et al.*, 1990). However, the proportion of males producing semen declined with increasing CP content from 8% to 40% (Hocking, 1989). Males on 11% CP diets had higher fertility (hatching eggs) than those on 16% CP diets (Hocking, 1990), and males fed 16% CP diets as opposed to 12% CP diets had a reduced semen concentration (Hocking & Bernard, 1997a).

The influence of the male broiler breeder in egg fertility is of greater importance than the contribution of the female (Wolc *et al.*, 2009). This is due to the mating ratio's where a number of females are inseminated by each male, usually in a ratio of 1 male : 10 females. Egg fertility will also depend on the fertilising ability of the spermatozoa produced by each male, with only fecund sperm occupying the sperm storage tubules (Bakst *et al.*, 1994).

Thus, the results of feeding different levels of CP on male fertility are varied, as are the measures of semen quality to quantify the effects on fertility. The objective of this experiment was to determine the effect of different levels of dietary CP on various aspects of semen quality (i.e. morphology concentration, motility), as well as sperm interaction with the egg, assessed by the number of sperm trapped in the outer perivitelline membrane. Since there is no non-invasive way to determine the number of spermatozoa residing in the sperm storage tubules (Brillard & Bakst, 1990), the number of spermatozoa in the perivitelline membrane is considered to be potentially a more accurate indicator of the fertilising potential of the sperm, and represents the number of sperm surrounding the ovum at the time of fertilisation (Wishart, 1987) and reflects the number of sperm in the uterovaginal sperm storage tubules (Brillard, 1993; Wishart, 1997).

Materials and Methods

Fifty two Ross 788 broiler breeder males were randomly allocated to individual cages (60 cm wide x 44 cm deep x 60 cm high) at 22 weeks of age. They were fed a commercial broiler breeder pelleted feed (140 g CP/kg and 11.5 MJ/kg AME) until 26 weeks of age. During this time, each male was trained for semen collection using the abdominal massage method (Burrows & Quinn, 1937), and 36 males that responded positively were selected and allocated to one of the following three isoenergetic dietary treatments differing in CP at 26 weeks of age; low (10.5% CP), medium (12.6% CP) and high (15.0% CP). The analysed nutrient contents of the diets are presented in Table 1. Feed allocation was the same for each treatment and was adjusted weekly, based on overall mean body weight, to adhere to the recommended growth curve (Aviagen, 2005). Fresh, clean water was provided *ad libitum*.

Males were trained to produce semen samples on a weekly basis, and these samples were used at 27, 29, 31, 37, 39 and 41 weeks for artificial insemination and evaluation of semen parameters. The semen used for artificial insemination was diluted 50 : 50 with Tyrode's solution, and a maximum of four commercial egg-type hybrid hens per male were inseminated with a fixed volume of 0.4 mL, a greater than normal dose. This was done to ensure that the number of spermatozoa would not be a limiting factor, and that the results would be a function of sperm quality. Eggs were collected on d 2, 3, 4, 6, 7, 8 and 14 post-insemination, and stored in a cold room (average 14 ºC, and 55% relative humidity).

Within a week of collection eggs were brought to room temperature, cracked open and the yolk separated from the albumen. Excess albumen was removed by rolling the yolk on paper towel. A square of approximately 1 x 1 cm of the perivitelline membrane over the germinal disc was cut and placed in phosphate buffer solution (PBS) to remove adherent yolk, and then stretched out on a glass microscope slide. It was then stained with a 1 μg/mL solution of diamidinophenylindole in PBS, and covered with a cover slip (Wishart, 1987). The slides were placed in a light-tight container, and examined under fluorescent microscopy within 3 h. Sperm nuclei embedded in the outer perivitelline membrane in 20 random fields of view were counted and totalled, and the number of sperm/mm^2 of membrane calculated.

Semen parameters evaluated included sperm morphology, motility and concentration, respectively. Individual semen samples from roosters were assessed for morphology at 27, 29, 31, 37 and 39 weeks of age. Semen samples were fixed in 3% glutaraldehyde, allowing subsequent visualisation with the use of a light microscope at 100 X magnification, with an oil immersion lens (Bakst, 2010b). Three hundred spermatozoa

were counted per slide, and the percentage of normal and abnormal (bent, swellings in the head/midpiece region and coiled heads) spermatozoa were recorded. The number of live normal motile sperm/mL was calculated as a function of the concentration and normal motility records assessed at 27, 29, 31, 37, and 41 weeks. Motility was assessed immediately after collection by diluting each sample with Tyrode's solution and placing it on a pre-warmed microscope slide and examined under a light microscope at 40 X magnification (Wishart & Bakst, 2010). A mean score of the percentage progressive motile sperm was assigned to each sample after assessment in three random areas of the slide. Sperm concentration was measured with the use of a haemocytometer (Bakst, 2010a).

Table 1 The analysed nutrient contents of the three experimental diets

Nutrient	Unit	Low	Medium	High
AMEn_adult	MJ/kg	10.41	10.39	9.97
Crude protein	%	10.46	12.61	15.00
Dry matter	%	88.60	86.70	87.40
Lysine	%	0.30	0.43	0.51
Methionine + cystine	%	0.09	0.19	0.24
Threonine	%	0.18	0.30	0.45
Arginine	%	0.42	0.38	0.81
Isoleucine	%	0.28	0.39	0.51
Leucine	%	0.64	0.82	1.13
Histidine	%	0.30	0.40	0.40
Phenylalanine	%	0.29	0.46	0.53
Tyrosine	%	0.22	0.28	0.34
Phenylalanine + tyrosine	%	0.51	0.74	0.87
Valine	%	0.36	0.42	0.69
Calcium	%	0.82	0.83	1.11
Available phosphorus	%	0.55	0.61	0.61
Selenium	ng/g	210.80	223.47	242.64

As the data for sperm trapped for mm^2 of perivitelline membrane were not normally distributed, a square root transformation was performed. Standard curve (exponential) regression was performed to determine the response of the square root-transformed number of sperm/mm^2 of perivitelline membrane to days post-insemination at each age, grouped by CP treatment. This was also performed on data with ages combined. The length of the fertile period was calculated from the combined regression for each treatment group using the regression equations for each treatment, and by substituting 1 sperm/mm^2 of perivitelline membrane as the number required for a fertile egg. This was based on a value between reported estimates of 0.43 sperm/mm^2 (Brillard & Antoine, 1990), 0.4 sperm/mm^2 (Wishart, 1987) and >3 sperm/mm^2 (Wishart, 1997) required for an egg to be fertile.

Semen concentration, morphology and motility measurements were subjected to a general ANOVA. Genstat 14th edition (2012) was used for all statistical analyses.

Ethical approval was obtained prior to the experiment from the Animal Ethics committee of UKZN (reference: AE/GOUS/05).

Results and Discussion

There was a significant decline in the number of sperm trapped in the outer perivitelline membrane with day post-insemination at every age (P <0.001), and with ages combined (P <0.001) (Figure 1). This was expected, as fecund spermatozoa fill the sperm storage tubules after copulation/insemination (Bakst *et al.*, 1994), and are released sequentially to provide fertile eggs for a limited period (Brillard, 1993). There was a

significant influence of CP treatment on the regression curve at 29 weeks (P = 0.5), 31 weeks (P <0.05), and 41 weeks (P <0.003), with the highest number of sperm in the outer perivitelline membrane, and a longer fertile period observed from birds on the 12.5% CP diet. The fertile period was calculated to be 7, 14.5 and 8.6 d from birds on 10.5%, 12.6% and 15.0% CP diets respectively (Figure 1).

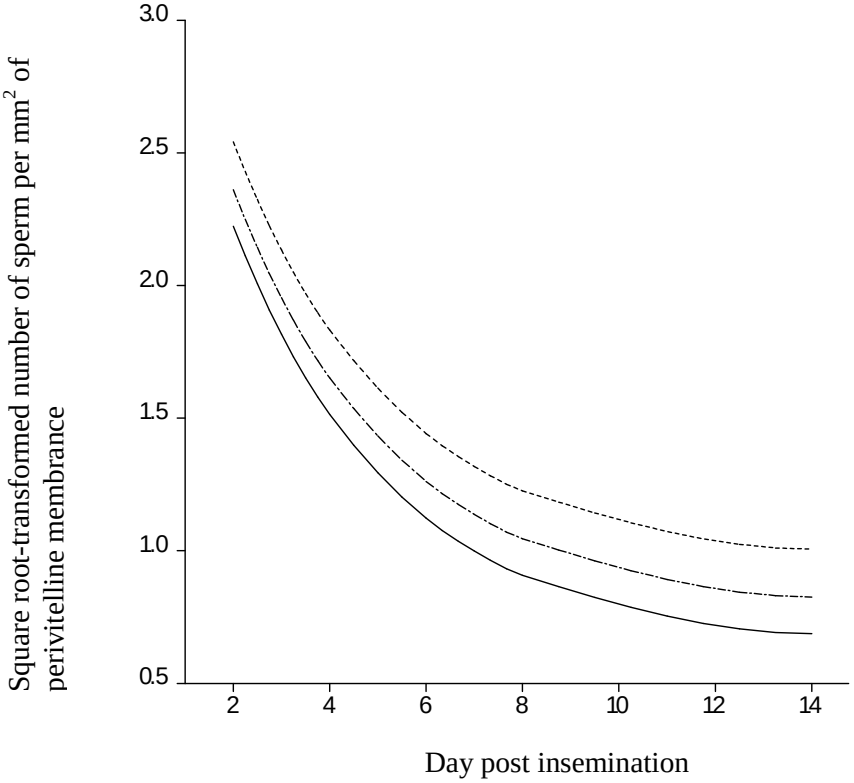

Figure 1 The square root-transformed number of sperm per mm^2 of perivitelline membrane of eggs obtained from laying hens on d 2, 3, 4, 6, 7, 8 and 14 post-insemination across all ages on dietary treatments of low (—), medium (--) or high (−·−) CP level (R^2 = 0.29).

There was no difference in the percentage of normal sperm morphology or the number of live sperm with normal motility from males on different treatments at any age (Table 2).

Although no differences were observed in the numbers of live sperm with normal motility or morphology from males fed different CP concentrations, males fed 12.6% CP produced sperm that resulted in a longer fertile period than those fed 10.5% or 15% CP, as predicted from the number of sperm trapped in the outer perivitelline membrane. The method of determining the number of sperm in the perivitelline membrane is preferable to the estimation of egg fertility, as it is a more sensitive estimate on a continuous scale compared to the binary measurement of fertile vs. infertile sperm that hatchability would estimate. Fontana *et al*. (1990) found no difference in the sperm concentration of *ad libitum*-fed birds or restricted birds, but fertility as measured by hatchability, was significantly lower in the *ad libitum*-fed group, which suggests that low fertility is a function of the actual fertilising ability of the sperm cells and not the inability to produce spermatozoa, which is estimated by the sperm concentration method.

In a natural mating situation, the difference observed in the length of the fertile period may not translate to decreased flock fertility if mating frequency is more frequent than the length of the fertile period, but it does highlight the greater fertilising potential of sperm from males fed 12.6% CP, which can potentially have an influence on fertility towards the end of the production cycle when mating frequency decreases (Duncan *et al.*, 1990).

Table 2 The number of live sperm with normal motility and the percentage normal morphology from males fed dietary low, medium or high crude protein treatments 26 to 41 weeks of age

Age (weeks)	Low	Medium	High	
	Live normal motile sperm/mL x 10^6 (n)			
27	45.6 ± 15.4 (5)	70.5 ± 13.9 (6)	95.8 ± 20.4 (6)	NS
29	26.0 ± 13.9 (5)	27.6 ± 12.8 (8)	74.3 ± 31.7 (4)	NS
31	43.6 ± 8.4 (5)	113.5 ± 41.1 (6)	43.8 ± 17.7 (5)	NS
37	168.2 ± 41.0 (4)	170.3 ± 50.6 (5)	118.0 ± 55.8 (4)	NS
41	202.7 ± 27.1 (5)	160.3 ± 38.6 (5)	174.1 ± 38.4 (5)	NS
	Normal morphology (%)			
27	95.8 ± 0.7 (5)	94.8 ± 0.7 (6)	94.6 ± 0.5 (6)	NS
29	97.3 ± 0.5 (3)	96.6 ± 0.7 (3)	97.3 (1)	NS
31	-	95.6 ± 0.9 (3)	96.7 ± 0.4 (2)	NS
37	91.3 ± 2.7 (3)	93.0 ± 1.6 (4)	89.6 ± 2.8 (2)	NS
39	91.5 (1)	93.0 (1)	91.6 (1)	

Conclusions

The length of the fertile period of oviposited eggs after artificial insemination with spermatozoa from males fed different levels of CP was extended for males fed a diet containing 12.6% crude protein, as predicted by the number of sperm in the perivitelline membrane of oviposited eggs measured at specific intervals after artificial insemination. Although no differences in other measures of semen quality (i.e. live sperm with normal motility and sperm morphology) were observed, the number of sperm in the perivitelline membrane can potentially be considered a more accurate indicator of the actual fertilising ability of the sperm. Therefore fertility may be negatively affected when crude protein levels below 12.6% are used to feed male broiler breeders or if rations higher in crude protein (typically female broiler breeder rations) are fed. A greater range of CP treatments could allow for a more accurate estimation of the most beneficial level of CP in the diet to maximise fertility in broiler breeder males.

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