

Research Note: The effect of crude protein and calcium intake on fertility of male broiler breeders

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ABSTRACT This experiment investigated the effect of excess crude protein and/or calcium on male broiler breeder fertility. Forty-eight broiler breeder males, from a group of 60 that consistently produced a semen sample were allocated to one of 4 dietary treatments, formulated to provide either the male or female recommendation for crude protein and calcium, or their combinations. Birds were provided a constant daily feed allocation. Semen samples were collected and assessed for concentration and sperm mobility index. Commercial laying hens were also inseminated and the points of sperm hydrolysis counted in the membranes of the eggs produced after insemination. Dietary crude protein had no significant effect on sperm concentration at any age, but significantly poorer sperm concentration was seen in the males fed high calcium at 42 and 57 wk of age, and a significant interaction was observed at 60 wk of age,

with the poorest sperm concentration from birds fed high crude protein and high calcium. There was no significant effect at any age of crude protein or calcium on sperm mobility index. The birds fed levels of crude protein and calcium recommended for males had significantly more points of sperm hydrolysis at 40 wk of age compared to all other dietary treatments, which indicates better fertilizing potential. While there were a few incidences of birds fed a ration with levels of crude protein and calcium recommended for males showing superior sperm quality, this was not a consistent trend across all ages or measures of fertility. Therefore, males fed a female ration at the correct allocation to ensure adherence to the bodyweight curve should not exhibit reduced (or improved) fertility, however, there were instances where birds fed the male recommended concentration of CP and Ca had improved measures of sperm quality.

Key words: semen quality, insemination, poultry

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INTRODUCTION

Separate sex feeding in broiler breeder production is an important practice to provide the correct feed allocation for males and females to allow adherence to the growth curve provided by the primary breeder. While this is widely practiced, there are producers that use the female ration to feed males. Due to egg production, the female requirements for crude protein (CP) and calcium (Ca) are higher than that of the male, so feeding a lower quantity of a female ration to males may still oversupply these nutrients to the male.

Provision of CP to broiler breeders above the requirement was shown to result in a shorter fertile period (Tyler and Bekker, 2012) and a reduction in sperm

concentration and testicular function (Hocking and Bernard, 1997). Ca has an important regulatory role in gamete function, and Nguyen et al. (2016) showed the importance of various Ca transport channels in birds (store-operated Ca²⁺ channels and high voltage-activated channels) in triggering sperm motility and the acrosome reaction, essential for fertilization. However, there is little research on the effects of an oversupply of Ca to broiler breeder males on fertility, and it is possible that excess Ca might interfere with the availability of other minerals.

Oversupply of nutrients is also costly. CP that is not utilized requires an energy cost to eliminate the excess from the body in the form of uric acid, which may also put pressure on the kidneys. Likewise, excess dietary Ca can cause renal problems through the need for elimination. Excess nitrogen losses in animal waste also pose an environmental risk.

It would also be useful to understand whether there is an interaction (positive or negative) of CP and Ca when these nutrients are provided in excess to broiler breeder males. Therefore, the objective of this experiment was to evaluate the effect of 4 diets, made up of 2 levels of CP and 2 levels

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of Ca (based on male and female requirements), on semen quality in the production phase of male broiler breeders.

MATERIALS AND METHODS

Sixty Ross 308 broiler breeder males were obtained at 24 wk of age (WOA). Each bird was placed in an individual cage (60 cm × 35 cm × 40 cm) on the floor with a layer of shavings. Each cage had a feed trough and nipple drinker. The birds were fed a standard breeder male ration with a restricted feed allocation provided every morning, which was adjusted weekly based on weekly individual body weight, in an attempt to meet the breeder recommended growth curve, until 32 WOA. During this time, each bird was trained for semen collection 2 to 3 times a week. Water was provided ad libitum. Forty-eight of the 60 males that most consistently produced a semen sample were selected for the experiment. At 32 WOA, semen samples were collected for measurement of sperm mobility (see [Namntu, 2016](#)) using a Turkey Mobility Analyzer 591B (Animal Reproduction Systems, Chino, CA), calibrated for use in chickens. The birds were then ranked according to sperm mobility index and allocated to one of 4 dietary treatments in a 2 × 2 factorial design with 2 levels of CP and 2 levels of Ca. Birds were assigned to one of 6 rooms with 8 cages per room, with 2 males per room on each dietary treatment until 61 WOA, where individual birds were considered the experimental unit.

Dietary treatments, formulated to be isocaloric, had analyzed values (see [Namntu, 2016](#)) of HP:LC (14.5% CP, 1.02% Ca), HP:HC (14.7% CP, 3.04% Ca), LP:LC (11.9% CP, 0.74% Ca) and LP:HC (12.4% CP, 3.05% Ca). Diets were mixed in one batch and a representative sample used for analysis. The limestone used had a particle size of 2 mm. No medications were included. Birds on all treatments received a feed intake of 140 g per day from 32 to 61 WOA when the experiment ended. Photoperiod during rearing was 9L:15D and during the experimental period was 13L:11D for all birds. The rooms had forced ventilation and temperatures were maintained within the breeder recommendation.

Semen samples were collected once a week, and at 36, 39, 42, 45, 49, 53, 57, and 60 WOA these were used for assessment of sperm concentration and sperm mobility index. Semen samples were also collected at 37, 40, 55, and 59 WOA from 6 random individual males per treatment. The samples were diluted with Poultry Motility buffer (Animal Reproduction Systems, Chino, CA) to contain 150×10^7 sperm/mL. Seventy-two commercial Lohmann egg-type hens were used to test the fertilizing ability of the sperm. This was to reduce the female bias on fertility as commercial egg-type hens are known to have high fertility. This measure also provides a quantitative measure of fertility rather than the binary measure of an egg being fertile or not. The hens were 41 WOA at first insemination and housed in cages (50 cm wide × 50 cm deep × 50 cm high) containing 3 birds per cage, with all birds in a cage inseminated with a 50 μ L volume of semen from the same male, resulting in 18

hens per treatment. Eggs were collected on days 2, 3, 4, and 8 post-artificial insemination (PAI) and stored in a cold room at an average of 14°C for no more than 2 weeks before being assessed for the number of points of sperm hydrolysis in the inner perivitelline layer (IPVL) (see [Namntu, 2016](#)). The slides were examined on the same day they were prepared using a light microscope (4 × magnification) and captured with a digital camera. The points of sperm hydrolysis (PSH) were counted in a 2 × 2 mm square of yolk membrane around the germinal disc and the number of points of sperm hydrolysis per mm² of IPVL was calculated. Ethical approval for this study was granted by the UKZN Animal Ethics Committee (Ref 063/14/Animal).

The sperm concentration and sperm mobility index were subjected to an unbalanced general ANOVA for each age (since not all birds produced a semen sample on the day of measurement) with sperm concentration and sperm mobility index as factors. Treatment means were compared using Duncan's multiple range test. Data were tested for normality with the Shapiro-Wilks test and that of the IPVL PSH were found not to be normally distributed. The data were log-transformed by adding a constant (to avoid a log of 0) and performing a log transformation. The log number of IPVL PSH from eggs collected on d 2, 3, 4, and 8 PAI were subjected to a general ANOVA at each age using an unbalanced treatment structure (because some eggs broke during analysis and not all hens laid an egg on the day of collection, resulting in an unequal number of eggs for each treatment). The mean log IPVL PSH was compared using Duncan's multiple range test. Although the ANOVA was performed in log-transformed data, the results presented are actual data. All statistical analyses were performed using GenStat 17 ([VSN International Ltd, 2014](#)).

RESULTS AND DISCUSSION

Dietary CP had no significant effect on sperm concentration at any age. There was an effect of Ca on sperm concentration at 42 ($P < 0.05$) and 57 ($P = 0.07$) WOA, with high Ca resulting in reduced sperm concentration. There was also a significant interaction at 60 WOA ($P < 0.05$) where it was observed that the HP: HC group had significantly lower sperm concentration. Thus, although there was no consistent trend over time, there were incidences where HC negatively impacted sperm concentration and particularly in those fed HP: HC ([Table 1](#)).

[Graaf et al. \(2018\)](#) observed no significant effect of dietary CP in the range of 9.7 to 13% on sperm concentration, and [Tyler and Bekker \(2012\)](#) observed no difference in the live motile sperm concentration in male breeders fed 10.5%, 12.6 or 15% CP. However, [Hocking and Bernard \(1997\)](#) found reduced sperm concentration from broiler breeder males fed 16% vs. 12% CP.

Whilst no literature on the effect of Ca on sperm quality were found, [Karaca et al. \(2002\)](#) did find that males classified as those with the best sperm quality index had higher seminal plasma Ca levels, necessary for motility.

Table 1. Sperm concentration and sperm mobility index of male broiler breeders fed different CP and Ca from 32 to 61 wk of age.

Diet	Weeks of age							
	36	39	42	45	49	53	57	60
Sperm concentration (sperm/mL $\times 10^6$)								
HP:LC	7.96	7.96	8.30 ^a	8.73	9.26	9.46	10.35 ^a	11.33 ^a
HP:HC	7.44	6.99	6.72 ^b	8.53	7.46	8.25	8.34 ^b	8.93 ^b
LP:LC	8.30	7.71	8.22 ^a	8.35	9.03	10.11	9.28 ^{ab}	10.26 ^{ab}
LP:HC	7.40	8.13	7.50 ^{ab}	8.65	8.67	9.66	8.96 ^{ab}	10.72 ^{ab}
Sperm mobility index								
HP:LC	16.6	15.5	11.8	14.6	15.7	21.1	18.8	13.6
HP:HC	11.8	11.8	18.4	11.6	17.3	20.5	25.8	13.9
LP:LC	13.0	10.2	12.0	11.3	15.6	18.8	21.2	13.2
LP:HC	11.2	10.3	11.7	11.6	14.4	18.4	21.1	13.7

^{ab}Means with different superscripts within a column are significantly different ($P < 0.05$).

Abbreviations: CA, calcium; CP, crude protein.

Roosters fed extra Ca produced sperm with improved cryosurvivability due to increased Ca in seminal plasma and lowered blood cholesterol (Kanyingi and Maeda, 2010). Thus, while Ca is important in the seminal plasma, there is an indication that high levels in the diet could impact sperm concentration negatively.

There was no significant effect at any age of CP or Ca on sperm mobility index (Table 1). Although sperm mobility was shown to be a good indicator of fertilizing potential (Froman and Feltmann, 1998), the phenotypic expression of sperm mobility genotype of a bird is thought not to be influenced by environmental factors. Graaf et al. (2018) also found no response in sperm mobility to CP intake at any age during the production period of broiler breeder males. Froman and Feltmann (1998) showed that breeder males classified as having average or high sperm mobility phenotype remained so over time. Males in this study were ranked by mobility phenotype before allocating to treatments, therefore it is likely that dietary treatment showed no significant effect.

There was a general decline in the PSH of the IPVL with age (Table 2) but no consistent pattern with

Table 2. Mean IPVL PSH with days post-AI at each age (The ANOVA and mean comparisons for IPVL PSH were performed on log-transformed data, but actual PSH are shown in the table.).

Age (wk)	DPAI	HP: LC	HP: HC	LP: LC	LP: HC
37	2	11.2	12.6	11.7	16.1
	3	7.3 ^b	10.9 ^a	7.8 ^b	9.4 ^a
	4	7.0	8.7	6.8	8.0
	8	3.2 ^a	2.8 ^{ab}	2.4 ^{ab}	1.8 ^b
40	2	8.2	8.7	8.7	12.6
	3	8.8 ^b	8.8 ^b	14.3 ^a	8.7 ^b
	4	7.0 ^b	5.6 ^b	9.8 ^a	7.2 ^b
	8	2.6 ^b	2.5 ^b	3.8 ^a	2.5 ^b
55	2	6.7 ^c	16.0 ^a	9.4 ^b	6.9 ^c
	3	6.3	8.3	8.3	5.4
	4	3.0	3.1	4.9	3.4
	8	2.9	2.8	2.9	1.7
59	2	3.7	6.8	4.8	5.4
	3	7.0	3.5	8.8	6.9
	4	3.3 ^{ab}	2.1 ^b	4.8 ^a	2.8
	8	2.1	1.8	2.0	1.3

^{ab}Means with different superscripts within a row are significantly different ($P < 0.05$).

Abbreviations: IPVL, inner perivitelline layer; PSH, points of sperm hydrolysis.

diet across ages. Wishart (1997) showed that eggs had maximum fertility when more than 6 spermatozoa per mm² penetrated the IPVL around the germinal disc, so in most cases, the mean PSH showed that eggs were not fertile 8 d PAI, and as the males aged, eggs were shown to be infertile even 4 d PAI. The LP:LC had significantly more PSH at 40 WOA 3, 4, and 8 d PAI compared to all other dietary treatments. At 55 WOA birds on this treatment also produced significantly more PSH than the HP:LC and LP:HC treatments at d 2 PAI as well as 4 d PAI at 59 WOA. While this pattern was not observed across all days PAI and across all ages, it does appear that the birds on the LP:LC diet tended to produce sperm with superior fertilizing potential.

Hence, there were no consistent patterns observed in a dietary effect on sperm quality, indicating that males fed a female ration at the correct allocation to ensure adherence to the bodyweight curve should not exhibit reduced (or improved) fertility, however there were instances where birds fed the male recommended concentration of CP and Ca had improved measures of sperm quality.

Potential limitations to this study include that broiler breeder males were caged individually so there are no behavioral considerations considered, such as hierarchy set up or mating behavior, that are known to influence fertility.

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DISCLOSURES

Dr NC Tyler has no conflict of interest to report.

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