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Effect of crude protein levels and organic selenium supplementation in the diets fed during the breeding season on reproductive parameters of red-winged tinamous (*Rhynchotus rufescens*)

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Egg weight, fertility, nutrition, semen quality, shell thickness.

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ABSTRACT

There is little information on the nutrition of red-winged tinamous (*Rhynchotus rufescens*) reared in captivity, and their nutritional requirements still need to be determined. This study aimed at determining dietary crude protein requirements and testing four organic selenium supplementation levels in the diet of red-winged tinamous during the breeding season. Birds were housed in a conventional broiler house divided in 16 boxes with one male and three females each. Iso-energy (2800kcal ME/kg) pelleted feeds, based on corn and soybean meal, were supplied in tube feeders. In the first experiment, treatments consisted of four different diets containing different crude protein (CP) contents (15, 18, 21, or 24%) and in the second experiment, the four diets contained equal protein level (22.5%) and four different organic selenium levels (0, 0.2, 0.4, or 0.8ppm). Data were analyzed by the least square method. The best egg weight and eggshell thickness were obtained with 22.5% dietary CP. Organic selenium did not influence the studied reproductive traits of red-winged tinamous (*Rhynchotus rufescens*) males or females.

INTRODUCTION

The consumption of meat of wild animals has increased in several countries, including Brazil. Research studies carried out with red-winged tinamous (*Rhynchotus rufescens*) have shown that these birds can be adapted to captivity due to their broad geographic dispersion, and omnivorous feeding habits. It was demonstrated that they can be habituated to the consumption of meal and pelleted feeds (Hoshiba *et al*., 2003), present good growth rates (Tholon & Queiroz, 2007), and excellent carcass and breast meat yield (Moro *et al*., 2006) when reared in captivity.

If inadequately fed, birds will shortly suffer nutritional deficiencies demonstrated by impaired productive and reproductive performance. A balanced poultry diet must include macro and trace nutrients in amounts sufficient to allow birds to grow, breed, and maintain their metabolic functions.

Low-protein diets cause reduced body weight gain and feed intake and worse feed conversion ratio (Malheiros *et al*., 2003), influencing egg production and size. Poultry have limited capacity of storing protein, and egg size is highly dependent on protein intake (Pesti, 1992). Therefore, the success of the domestication of a determined animal species depends on the determination of its nutritional requirements, including protein requirements.

There are very few studies on tinamou nutrition. In an attempt to determine the nutritional requirements of that species, Moro *et al*. (2002) used the recommendations for pheasants, but did not obtain satisfactory

results. On the other hand, the Japanese quail (*Coturnix coturnix japonica*), which also belongs to the Phasianidae family, could be a better initial model for the determination of protein requirements in red-winged tinamous (*Rhynchotus rufescens*). Crude protein levels recommended in literature for Japanese quails range between 16 and 25%, and the differences are possibly due to genetic, experimental, or climatic variations that may affect quail performance (Silva *et al*., 2007).

Minerals are essential for life maintenance, and are involved in several metabolic processes in the body. Minerals can be divided into macro and trace minerals, which classification is based on the amount required for individual metabolic functions.

Most trace minerals metabolically function as enzyme co-factors or components. Among trace minerals, selenium presents flexible oxi-reduction capacity as it is part of the active center of the enzyme glutathione-peroxidase, which protects cell membranes from the oxidative attack by free radicals (Ortolani, 2002). Selenium is also part of the enzyme iodothyronine-deiodinase, responsible for the conversion of thyroxin into its active form (Silva *et al*., 2007).

According to Hess *et al*. (2000), inorganic selenium sources (sodium selenite and calcium selenide) are traditionally used in poultry nutrition, starting in 1974, when the FDA approved their inclusion in animal feeds (Echevarria *et al*., 1988; Leeson & Summers, 1997). However, research has shown that when selenium is incorporated into organic molecules as amino acids and peptides, such as found in plants, grains, and yeasts, its antioxidant action is more effective than that of inorganic selenium forms, promoting higher intestinal absorption, better performance and feathering, and mineral tissue retention. It must also be mentioned that organic trace minerals are more available than their inorganic counterparts. Organic trace minerals can also be biosynthesized, as in the case of selenomethionine and selenocystine, which are produced by adding inorganic selenium and yeast to a culture medium. The yeast incorporates selenium instead of sulfur in the amino acids methionine or cystine (Hynes & Kelly, 1995).

In addition to protecting cells against oxidative processes and to prevent metabolic and infectious diseases, selenium is also important for maintaining sperm integrity. According to Edens (2004), cockerels supplemented with organic selenium presented better semen quality, with a lower number of sperm abnormality, followed by those supplemented with sodium selenite or non-supplemented. The substitution of inorganic by organic selenium improved eggshell quality as shown by its higher weight and thickness (Klecker *et al*., 2001), as well as egg production (Rutz *et al*., 2003).

This study aimed at evaluating the effects of crude protein levels and the use of organic selenium in the diets of red-winged tinamous (*Rhynchotus rufescens*), fed during the breeding season.

MATERIAL AND METHODS

Two experiments were carried out in the Wild Animal Sector of the Animal Science Department of FCAV-UNESP, Jaboticabal, SP, Brazil. The experiment for evaluating dietary crude protein levels during the breeding season was conducted between November 2006 and February 2007 (EXP1). Dietary organic selenium evaluation was carried out between November 2007 and February 2008 (EXP2).

In EXP1, birds were in their third breeding season, and were 28 to 36 months old. In EXP2, birds were 40 to 48 months old, and were in their fourth breeding season.

The following parameters were evaluated in both experiments: egg weight and dimensions, egg production, eggshell thickness, fertility, sperm motility, sperm vigor, semen volume, semen aspect, sperm concentration, sperm abnormalities, hatchability, and hatching rate.

The studied tinamous were housed in a conventional masonry poultry house with concrete floor and asbestos tiles. Birds were housed in 16 pens, with one male and three females each. Pens were separated by a 40-cm wall, and covered with 2.0m x 1.0m x 2.1m wire mesh to isolate the experimental birds from other wild birds in order to minimize disease transmission. The floor was covered with coast-cross (*Cynodon dactylon*) hay litter. Pens were equipped with tube feeders and bell drinkers. Feed and water were offered *ad libitum*. Feeds were pelleted and based on corn and soybean meal. A lighting program of 18 hours of light daily, starting in August, was applied in both experiments.

In EXP1, four iso-energy diets (2,800kcal ME/kg feed) were formulated with four different crude protein levels (15, 18, 21, and 24%) based on the studies of Moro *et al*. (2002). The requirements of all other nutrients followed the recommendations of the NRC (1994), and nutritional ingredient composition was according to Rostagno *et al*. (2000). In EXP2, iso-energy (2800kcal

ME/kg) and iso-protein (22.5% CP) were used, and contained four different concentrations of organic selenium (Sel-Plex®): 0, 0.2, 0.4, and 0.8ppm (Tables 1 and 2).

Table 1 - Ingredient and calculated nutritional composition of the diets of breeding red-winged tinamous (*Rhynchotus rufescens*) containing different crude protein levels (R1, R2, R3, and R4) - EXP1, or different selenium levels ** (R5) - EXP2.

Laying diets	EXP1				EXP2
Ingredients (%)	R1	R2	R3	R4	R5
Corn	62.84	52.90	44.55	40.87	42.72
Soybean meal	17.81	26.24	34.86	43.91	39.38
Wheat midds	10.00	10.00	8.58	3.27	5.91
Calcitic limestone	5.50	5.45	5.39	5.31	5.35
Dicalcium phosphate	1.66	1.62	1.60	1.61	1.61
Salt	0.40	0.40	0.40	0.40	0.40
Mineral supplement*	0.20	0.20	0.20	0.20	0.20
Vitamin supplement*	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.09	0.01	-	-	-
Vegetable oil	1.07	2.74	4.00	4.00	4.00
Inert material	0.12	0.12	0.12	0.12	0.12
Total	99.99	99.99	99.99	99.99	100.00
Calculated composition					
Crude protein (%)	15	18	21	24	22.50
Metab. energy (kcal/kg)	2800	2800	2800	2800	2800
Fat (%)	2.59	2.59	2.59	2.59	2.59
Crude fiber (%)	3.00	3.00	3.00	3.00	3.00
Calcium (%)	2.50	2.50	2.50	2.50	2.50
Available P (%)	0.40	0.40	0.40	0.40	0.40
Methionine (%)	0.33	0.32	0.32	0.36	0.32
Methionine + Cystine (%)	0.60	0.60	0.67	0.75	0.71
Lysine (%)	0.71	0.93	1.15	1.37	1.26

*Composition described in Table 2, ** Sel-Plex® - Alltech do Brasil - Agroindustrial Ltda. Curitiba, PR.

Table 2 - Composition of the vitamin and mineral supplements used in the diets of red-winged tinamous (Rhynchotus rufescens) containing different crude protein levels (R1, R2, R3, and R4) - EXP1, or different selenium levels ** (R5) - EXP2.

Vitamins	EXP1	EXP2
Vitamin A (UI)	3500000	3500000
Vitamin D3 (UI)	700000	700000
Vitamin AND (mg)	2500	2500
Vitamin K3 (mg)	670	670
Vitamin B12 (mcg)	6000	6000
Thiamin (mg)	_	_
Vitamin B2 (mg)	1500	1500
Pyridoxine (mg)	_	_
Biotin (mg)	_	_
Calcium pantothenate (mg)	2500	2500
Niacin (mg)	6000	6000
Antioxidant (mg)	20	20
Vehicle qsp.(g)	1000	1000
Trace minerals	**EXP1**	**EXP2**
Iron (mg)	15000	15000
Copper (mg)	12000	12000
Manganese (mg)	35000	35000
Zinc (mg)	30000	30000
Iodine (mg)	600	600
Selenium (mg)	70	70
Vehicle qsp.(g)	1000	1000

*Sel-Plex® - Alltech do Brasil - Agroindustrial Ltda. - Curitiba - PR.

Eggs were collected four times daily and identified with a tag per box, laying order, and collection date. Eggs were weighed in a digital scale with decimal precision, and their length and width were measured using a pachymeter. Eggs were submitted to artificial incubation, and eggs that did not hatch were submitted to embryo diagnosis. Three eggs per replicate were collected to measure eggshell thickness, using a digital micrometer (Mitutoyo, 0.001-mm resolution) in fragments removed from the tip, equator, and bottom of the egg, based on which average eggshell thickness was calculated.

Semen was collected six times in both experiments. In EXP2, two additional collections were made before feed adaptation. Before semen collection, cockerels' feathers around the cloaca were removed, and the area was cleaned to prevent semen loss and contamination. Semen was collect by digital pressure of the base of the phallus and of the *vas deferens* (Cavalcante *et al*., 2004).

Immediately after collection, semen was analyzed for semen volume, aspect, sperm vigor, and motility. Semen volume was described by direct observation in a capillary tube. Semen aspect was scored as two for milky, one for semi-milky, and zero for transparent (Cavalcante *et al*., 2004). Sperm motility (% moving sperm) and sperm vigor (progressive movement; 0 to 5 score scale) were evaluated by placing a 5-µL aliquot of the semen diluted in saline solution at 1:10 between a glass slide and coverslip, and observation under optical microscope at 400X magnification (Tiaimin, model TN212B).

After these analyses, a 2-µL aliquot of each semen sample was diluted in 998-µL saline formalin at 37°C, and stored in a plastic microtube identified with collection date, male number, and pen in a refrigerator at 5°C for subsequent sperm concentration and morphology analyses.

Sperm concentration was determined by counting in a Neubauer hemocytometer under optical microscope at 400X magnification. The obtained number was used in the sperm concentration formula, according to the criteria recommended by the Brazilian College of Animal Reproduction.

Sperm morphology was described by analyzing 200 cells in wet-mount smear under differential interference phase contrast microscopy at 1000x magnification, according to Barth & Oko (1989). Cells were classified as normal or abnormal as to head, intermediate piece, and tail.

Fertility, hatchability, and hatching rates were calculated as:

Fertility rate = (n. fertile eggs/n. incubated eggs) x 100

Hatchability = (n. hatched eggs/n. fertile eggs) x 100

Hatching rate = (n. hatched eggs/n. incubated eggs) x 100

Using the Guided Data Analysis and Analyst of the Statistical Analysis System Software (SAS 9.1, Institute, Cary, North Carolina, USA), data were tested for residue normality and variance homogeneity. The following traits did not comply with those assumptions: fertility rate, sperm motility, tail defects, intermediate-piece defects, acrosome defects, total defects, and normal sperm. Therefore, data were transformed according to the formula:

YT=ARCSIN (SQRT ((Y+1.5) /100));

where:
YT=transformed trait,
Y = observed trait,
ARCSIN= arcsine,
SQRT= square root.

The traits were submitted to analysis of variance according to a completely randomized experimental design with four treatments (crude protein or selenium levels) of four replicates each, employing the least square method. When traits presented significant differences among treatments, degrees of freedom were detailed in ordinary polynomials, and linear, quadratic, and cubic regression (p=0.10). Means were compared by the test of Duncan (p=0.10).

In EXP2, a split-plot experimental design was applied to evaluate the 4 different treatments (0, 0.2, 0.4, 0.8 ppm organic selenium - Sel-Plex®) and the 8 different semen collection times. Means per treatment and per semen collection times, as well as semen aspect and semen volume and sperm motility, sperm vigor, concentration, and abnormalities (head, intermediate piece, tail, and total defects) were analyzed. Collected data were submitted to analysis of variance by the least square method, according to the following model:

$$Y_{ijkl} = \mu + NS_i + R_j(NS_i) + TP_k + (NS * TP)_{ijk} + e_{ijkl},$$

em que:
Y_{ijkl} = analyzed trait,
μ = general mean,
NS_i = effect of the ith selenium level (i = 0, 0.2, 0.4, 0.8),
$R_j(NS_i)$ = effect of the jth replicate (j=1.2.3.4) within the ith selenium level,
TP_k = effect of the kth collection time (k=1,...8)
$(NS * TP)_{ijk}$ = effect of the interaction between the kth collection time with the ith selenium level,
e_{ijkl} = effect of the random error, assumed as normally and independently distributed.

RESULTS

Dietary protein levels

In EXP1, egg weight and eggshell thickness were influenced (p<0.1) by dietary crude protein levels (Table 3). The experimental design explained 52% of the variation observed in these traits, which coefficients of variation were 5.7 and 3.9%, respectively. The heaviest eggs were obtained with 21% crude protein level; however, they were not significantly different (p>0.1) from those from birds fed 15 and 24% crude protein. Eggshell thickness was different between the eggs produced by birds fed 15 and 18% crude protein (p<0.1), but these were not different from those fed 21 and 24% crude protein (Table 3).

The decomposition of the degrees of freedom showed significant effect of the cubic regression (p<0.1) for egg weight and eggshell thickness. Figures 1 and 2 present the regression curves and their respective equations.

Table 3 - Mean and standard error of egg weight (g) and eggshell thickness (mm) of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season, as a function of dietary crude protein levels.

Treatment	Egg weight (g)	Eggshell thickness (mm)
15% CP	59.43 ± 1.04 ab	0.277 ± 0.005 a
18% CP	55.59 ± 3.24 a	0.254 ± 0.004 b
21% CP	63.33 ± 1.41 b	0.275 ± 0.004 ab
24% CP	58.12 ± 1.32 ab	0.272 ± 0.007 ab

Means in the same column followed by the same letter are not different by the test of Duncan (p<0.10).

Dietary selenium levels

The different dietary selenium levels did not statistically influence (p>0.1) none of the evaluated traits. However, as this is a pioneer study, mean values obtained for red-winged tinamous (Table 4) per treatment (Tables 5 and 6) are presented.

Table 4 - General mean, number of observations (N) and standard deviation of reproductive traits of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season.

Trait	N	General mean	Standard deviation
Egg weight (g)	16	56.77	3.36
Egg length (cm)	15	5.45	1.40
Egg width (cm)	16	4.12	0.80
Eggshell thickness (mm)	13	0.24	0.02
Egg production/female	16	14.52	10.67
Feed intake/bird/day (g)	16	45.18	7.67
Fertility (%)	16	50.73	22.24
Hatchability (%)	16	48.07	24.93
Hatching rate (%)	16	26.76	19.11
Sperm motility (%)	16	73.75	12.78
Sperm vigor (0-5)	16	3.41	0.86
Semen volume (µl)	16	23.25	12.29
Semen aspect (0-2)	15	3.16 (1.72) *	1.33 (0.47) *
Sperm concentration (10^9xcell/ml)	16	1.26	0.45
Normal sperm (%)	16	59.72	17.19
Head defects (%)	14	2.58	2.3
Tail defects (%)	16	11.87	3.71
Intermediate piece defects (%)	16	0.73 (6.91) *	0.34 (5.77) *
Total sperm defects (%)	16	40.28	17.19

*Values between parentheses are transformed data.

There was no significant effect of dietary selenium levels ($p>0.1$) on male or semen traits. However, collection time significantly affected ($p<0.1$) sperm motility, sperm vigor, sperm concentration, semen volume, normal sperm cells, head defects, and tail defects (Tables 7 and 8). There

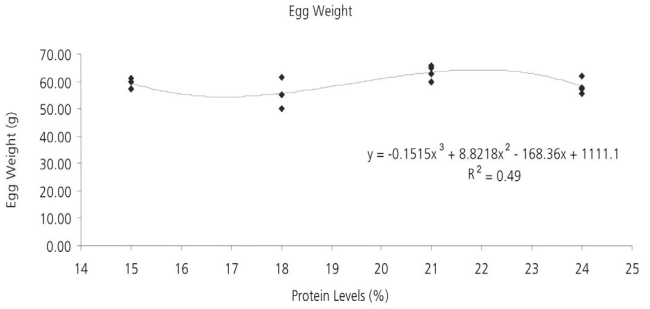

Figure 1 - Egg weight trend (g) as a function of crude protein (%) content in the diet of breeding red-winged tinamous (*Rhynchotus rufescens*).

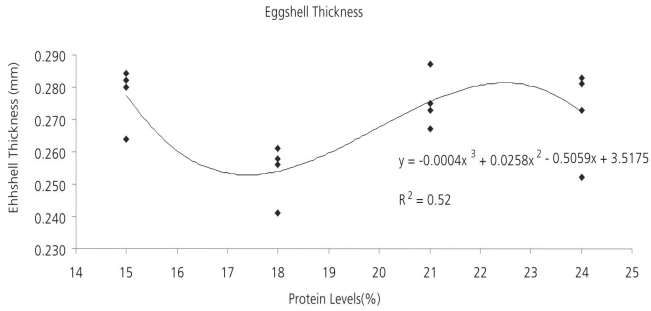

Figure 2 - Eggshell thickness trend (mm) as a function of crude protein (%) content in the diet of breeding red-winged tinamous (*Rhynchotus rufescens*).

Table 5 - Mean and standard error of egg weight (g), egg length (cm), egg width (cm), egg production/female, fertility (%), hatchability (%), hatching rate (%), eggshell thickness (mm), and feed intake/bird/day (g) of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season as a function of dietary selenium levels.

Traits	0	0.2	0.4	0.8
Egg weight (g)	54.54 ± 0.87	58.89 ± 1.35	56.93 ± 1.69	56.73 ± 2.41
Egg length (cm)	5.45 ± 0.59	5.57 ± 0.97	5.52 ± 0.61	5.39 ± 0.06
Egg width (cm)	4.06 ± 0.42	5.16 ± 0.56	4.15 ± 0.29	4.10 ± 0.13
Egg production/female	13.83 ± 6.33	14.67 ± 4.84	11.58 ± 3.54	18.00 ± 6.15
Fertility (%)	58.86 ± 11.61	47.83 ± 13.79	48.96 ± 11.83	47.25 ± 5.46
Hatchability (%)	51.98 ± 10.87	42.28 ± 14.74	54.79 ± 8.17	43.22 ± 14.81
Hatching rate (%)	32.65 ± 9.92	25.80 ± 11.23	29.12 ± 9.81	19.49 ± 6.68
Eggshell thickness (mm)	0.22 ± 0.003	0.24 ± 0.010	0.25 ± 0.013	0.23 ± 0.009
Feed intake/bird/day (g)	44.00 ± 4.56	50.43 ± 5.00	44.32 ± 2.94	41.94 ± 2.06

Table 6 - Mean and standard error of sperm motility (%), sperm sperm vigor (0-5), semen volume (µl), semen aspect (0-2), sperm concentration (109 cells/ml), normal sperm cells (%), total defects (%), head defects (%), tail defects (%), and intermediate piece defects (%) of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season, as a function of dietary selenium levels.

Traits	0	0.2	0.4	0.8
Sperm motility (%)	80.00 ± 5.40	67.50 ± 5.95	76.25 ± 5.54	71.15 ± 8.26
Sperm vigor	3.37 ± 0.24	3.25 ± 0.32	3.75 ± 0.48	3.25 ± 0.59
Semen volume (µl)	23.62 ± 5.10	31.86 ± 10.35	22.9 ± 3.65	14.62 ± 2.14
Semen aspect (0-2)	1.45 ± 0.36	1.65 ± 0.24	1.85 ± 0.15	2.00 ± 0.00
Sperm concentr. (109cell/ml)	1.40 ± 0.34	1.28 ± 0.16	1.14 ± 0.21	1.21 ± 0.13
Normal cells (%)	57.72 ± 11.18	66.04 ± 1.60	63.99 ± 6.44	51.11 ± 11.24
Total defects (%)	42.27 ± 11.18	33.95 ± 1.60	36.00 ± 6.44	48.88 ± 11.24
Head defects (%)	2.87 ± 0.91	1.76 ± 0.60	1.58 ± 0.79	2.01 ± 0.60
Tail defects (%)	11.89 ± 2.93	9.79 ± 0.91	14.37 ± 1.92	11.42 ± 0.60
Intermediate piece defects (%)	9.24 ± 4.36	5.40 ± 1.60	7.48 ± 3.28	5.49 ± 0.97

selenium levels and semen collection time only on sperm head defects (p<0.1).

DISCUSSION

Dietary crude protein levels significantly affected egg weight and eggshell thickness (p<0.1), with mean and standard deviation as 59.35±3.38g and 0.27±0.01mm, respectively. The coefficients of determination, expressing the fitness of the model to the parameters, were 50% and 52%, respectively, indicating that the statistical models explained most of the variation. The coefficients of variation were low (5.7% and 3.9%), demonstrating that there was little variation among individuals for these parameters.

In the study of Moro *et al*. (2002), red-winged tinamous were fed meal diets containing 15% CP and 2800kcal ME/kg, and their egg weights ranged between 47.5 and 54.6g, but were not statistically different. Bruneli *et al*. (2005) obtained an average weight of red-winged tinamous (*Rhynchotus rufescens*) eggs of 52.6g during the breeding season (October to January), which is the phase of highest egg production, and therefore, of lighter eggs. Nakage *et al*. (2002), also working with red-winged tinamous, observed that egg with weights of 51 to 55g presented 0.22mm eggshell thickness, as well as those weighing, in average, between 56 and 60g, value that are similar to those obtained in the present study. In the studies of Nakage *et al*. (2002) and Bruneli *et al*. (2005), birds were fed diets containing the same crude protein and energy content as that of Moro *et al*. (2002), but in the pelleted form, instead of meal. Feed physical form influenced egg weight in those studies, which were carried out after that of Moro *et al*. (2002), because tinamous present a very selective feeding behavior. When fed meal diets, tinamous tend to select larger particles, leaving the smaller one, which usually contain trace minerals. Pelleted feeds prevent particle selection, promoting the ingestion of all feed nutrients, which results in better reproductive performance of these birds. In addition, when there is particle selection, the true levels of crude protein (CP) and energy intake are not known, interfering with the determination of the birds' nutritional requirements.

It was shown that higher dietary protein (Parsons *et al*.,1993; Leeson, 1989), lipid (Grobas *et al*., 1999; Wu *et al*., 2005), methionine (Keshavarz, 1995), lysine (Zimmerman, 1997), and linoleic acid (Smith & Pourezza, 1989) increases egg weight. On the other hand, when the diet is deficient in protein, egg size tends to be reduced, while egg production level is maintained because of the bird's drive to perpetuate its species. Indeed, in the present experiment, birds

Table 7 - Mean and standard error of sperm concentration (109cells/mL), semen volume (µL), sperm motility (%), and sperm vigor (1-5) of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season as a function of dietary selenium and semen collection time.

Collection time	Concentration (10^9sptz/mL)	Semen volume (µL)	Sperm motility (%)	Sperm vigor (1-5)
-30	0.4+0.22 c	15.27+4.77c	63.64+5.86bcd	2.36+0.35bc
-15	0.95+0.12 b	10.92+4.39c	56.15+5.39cd	2.54+0.33bc
15	1.01+0.09 b	8.56+5.28c	51.11+6.48d	2.00+0.39c
30	1.03+0.15 b	14.00+4.57c	65.38+5.39cd	3.00+0.31abc
45	1.11+0.15 b	35.46+4.39a	73.08+5.39abc	3.31+0.33ab
60	1.07+0.11 b	29.36+4.77ab	83.64+5.86a	3.73+0.35a
75	1.42+0.12 b	21.87+3.96abc	74.37+4.86ab	3.62+0.29a
90	2.23+0.22 a	17.00+4.39bc	63.85+5.39bcd	2.69+0.33abc

Means followed by the same letter in the same column are not significantly different by the test of Duncan (p<0.10).

Table 8 - Mean and standard error of normal sperm cells (%), tail defects (%) and head defects (%) of the semen of red-winged tinamous (*Rhynchotus rufescens*) during the breeding season as a function of dietary selenium and semen collection time.

Collection time	Normal cells (%)	Tail defects (%)	Head defects (%)
-30	73.38+6.71ab	30.15+5.40abc	11.38+12.28b
-15	52.33+8.06bc	20.50+6.16bcd	43.20+14.00ab
15	75.62+8.55a	16.50+6.89cd	25.75+15.65ab
30	46.86+6.64c	40.93+5.21a	56.50+11.83a
45	46.78+6.46c	35.78+5.21ab	60.28+11.83a
60	70.62+6.98ab	11.67+5.62d	27.83+12.78ab
75	65.11+6.46abc	9.64+5.21d	41.93+11.83ab
90	66.85+7.65abc	19.30+6.16bcd	37.40+14.00ab

Means followed by the same letter in the same column are not significantly different by the test of Duncan (p<0.10).

fed 21% CP produced heavier eggs than those fed 18% CP; however, egg weight decreased when 24% CP was fed (Table 3). These results are partially consistent with the findings of Perly (1979), Murakami & Furlan (2002), and Pinto *et al*. (2002), who mentioned that egg weight is highly dependent on daily protein intake in layer chickens, and can be used as a reference to ensure amino acid requirements. Calderon & Jensen (1990) verified that methionine significantly increased egg production when commercial layers were fed 13, 16, or 19% CP. Sulfur amino acid requirements were estimated as 0.48-0.55% in feeds containing 13% crude protein, 0.59-0.61% in diets with 16% CP, and 0.67-0.70% in diets with 19% CP. It should be mentioned that lysine and methionine+cystine requirements were met in the present study.

Red-winged tinamous fed 15% CP produced eggs with thicker eggshells as compared to those fed the 18% CP diets (Table 3). This result is different from literature findings, such as those of Harms (1983) and Koelkebeck *et al*. (1991), who did not observe any differences in the specific gravity of eggs laid by birds fed different protein levels. Aiming at evaluating the effect of increasing dietary protein levels on the morphometrical characteristics of the oviduct of Japanese quails, Artoni *et al*. (2001) showed that 24% crude protein increased the thickness of the glandular layer of the magnum, isthmus, and shell gland, which may result in heavier and thicker eggs.

The decomposition of the degrees of freedom of treatments showed a cubic effect (P<0.1) on egg weight and eggshell thickness, with the highest values for these parameters obtained at 22.5% CP (Figures 1 and 2). Heavier eggs are related to heavier hatchling weight, and thicker eggshells to higher egg resistance to environmental harsh conditions (Nakagi *et al*., 2002). Egg production and egg size are highly dependent on daily protein intake, as birds can only store this nutrient for a limited period of time (Pesti, 1992). Similar results were reported by Singh & Narayan (2002), who recommended 22% crude protein (CP) for quails during lay, and by Pinto *et al*. (2002), who suggested 22.42% CP dietary level. Murakami *et al*. (1993) found higher egg weight in quails fed 20% crude protein (CP).

In EXP2, the different organic selenium supplementation levels did not significantly influence any of the evaluated parameters (P>0.1), perhaps because bird requirements were already supplied by the selenium contained in the trace mineral premix. Also, this result may be explained by the fact that birds were not submitted to any challenge that may have required higher dietary selenium levels. Similar results were reported by Pan *et al*. (2004), Xavier *et al*. (2004), De Lange & Elferink (2004), and Klecker *et al*. (2001) who also found that egg production was not affected by dietary selenium addition.

Klecker *et al*. (2001) observed that the replacement of inorganic selenium by organic selenium in the diet increased eggshell thickness as compared to the controls. Xavier *et al*. (2004) and Pan *et al*. (2004) despite reporting better eggshell quality, did not find significant differences in their results, which is consistent with the findings of the present study, where eggshell thickness was not statistically different among treatments, but numerically increased in 5 to 14% with increasing organic selenium levels relative to the control treatment not fed organic selenium.

Dietary organic selenium levels did not influence any trait of the semen of red-winged tinamous (P>0.10). Different results were obtained by Tavian *et al*. (2008), who reported higher sperm cell (52.31% *vs.* 38.96%), lower total defect (47.69% *vs.* 61.04%), and lower intermediate-piece defect (10.54% *vs.* 17.47%) percentages (P<0.001) in the semen of red-winged tinamous fed liquid selenium and vitamin E.

Cavalcante *et al*. (2004), feeding red-winged tinamous (*Rhynchotus rufescens*) pelleted feeds with 15% CP, obtained lower mean semen volume (14.13uL), sperm motility (66%), sperm vigor (2.30) and sperm concentration (0.93 x 109cells/mL) as compared to the present study. Those authors also reported higher rates of sperm morphological changes than here. However, the birds used in their study were in their first breeding season (12 to 18 months old), which may have increased the percentage of sperm defects as, according to Correa & Arceo (1995), animals with immature testicles may present higher levels of sperm abnormalities. Moreover, this difference could be also explained by the longer conditioning time of the birds in the present experiment as compared to that described by Cavalcante *et al*. (2004), allowing better adaptation of birds to handling, that is, reducing their stress. Stress may compromise reproductive performance due to the physiological and behavioral changes it causes.

The morphological changes found by Cavalcante *et al*. (2004) in red-winged tinamous were defects of the acrosome (0.79%), head (10.45%), intermediate piece (16.06%), tails (14.99%), and others (1.28%). In the present study, the most common defects were folded intermediate piece, folded and coiled tail, and strongly coiled tail. A lower percentage of acrosomal and intermediate-piece defects were also observed as

compared to Cavalcante *et al*. (2004), but higher rates of head defects, and intact sperm heads are essential for fertilization.

Silva *et al*. (2003) studied the supplementation of cockerel diets containing inorganic selenium with organic selenium or not, and reported that, when organic selenium was supplied, sperm abnormality rates tended to decrease, particularly intermediate-piece defects, and normal sperm cell rates tended to increase in the ejacultate. Similar to our results, those authors also observed higher head defect values.

Collection time significantly influenced (P<0.1) sperm motility, sperm vigor, concentration, semen volume, normal sperm cells, head defects, and tail defects, which improved along the breeding season (Tables 7 and 8) and presented the best performance during the summer (December and January). The improvement of semen traits along the breeding season was expected, as red-winged tinamous present breeding seasonality, which extends from September to April, and fertile egg production peak between December and February, according to Bruneli *et al*. (2005).

Conclusions

A crude protein content of 22.5% must be provided in the diet of breeding red-winged tinamous for the production of the heaviest eggs and thickest eggshell.

The supplementation of organic selenium in the diet of male and female red-winged tinamous is not required during the breeding season.

The semen quality of red-winged tinamous improved along the breeding season, and presented the best results during the summer.

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