



The effect of different feeding schedules on egg quality, blood, and bone parameters in broiler breeders

A. Londero^{1,3}, A.P. Rosa¹, C.E.B. Vivas¹, C. Orso¹, M.O. Fernandes¹, S.J. Paixão¹, C.B.S. Giacomini¹, C.M. Andrade², H.E. Palma²

¹Poultry Science Laboratory, Department of Animal Science, Federal University of Santa Maria, RS, Brazil.

²Clinic Veterinary Laboratory, Department of Small Animal Clinic, Federal University of Santa Maria, RS, Brazil.

Abstract

The aim of this study was to evaluate different feeding schedules and their relationship to calcium (Ca) and phosphorus (P) plasma levels and egg quality of broiler breeders. The feeding schedules were: single feeding at 8:00 AM; twice daily feeding (50% at 8:00 AM and 50% at 3:00 PM) and single feeding at 3:00 PM. 330 female and 45 male Cobb 500 broiler breeders were used from 40 to 60 weeks of age. The experimental design was completely randomized, consisting of 3 treatments with 5 replicates each, with 22 females and 3 males. The nutritional requirements were according to guideline recommendations. The following parameters were assessed weekly: total egg production, egg specific gravity, egg and eggshell weight, and eggshell thickness. Ca and P plasma levels, tibia weight and its length and Seedor index were evaluated at the end of the study. To determine the effect of feeding schedules, the total calcium and phosphorus plasma at oviposition time and 21 h after oviposition was evaluated. At 60 weeks of age, tibias were taken from the right leg, dried, measured and weighted. There were no differences among treatments regarding egg production, tibia length and Seedor index. Hens fed at 8:00 AM showed higher tibia weight than hens fed twice daily. Broiler breeders fed at 3:00 PM had higher egg ($P = 0.0105$) and shell ($P = 0.0066$) weight than others, and hens fed at 3:00 PM had more specific gravity ($P = 0.0219$) and eggshell thickness ($P = 0.0419$) than hens fed at 8:00 AM. Ca ($P = 0.758$) and P ($P = 0.811$) plasma levels were higher in hens fed at 8:00 AM than hens fed at 3:00 PM after 21 h of oviposition. Broiler breeders fed at 3:00 PM had better egg quality without any change in production, as well as these hens showing higher available Ca and P plasma levels.

Keywords: bone, calcium, egg production, eggshell, phosphorus.

Introduction

Broiler breeders are continuously selected for weight gain. To regulate this gain, limit health risks, and also maintain high fertility, husbandry practices for the parent stock of broiler chickens encompass a high degree of feed restriction (Renema and Robinson,

2004). Quantitative feed restriction is a common management technique used to increase the reproductive efficiency of broiler breeder hens (Moradi *et al.*, 2013).

A restriction of feed intake to approximately 50 to 60% of *ad libitum* intake is a practical approach to reduce metabolic disorders and improve egg production in broiler breeder hens (Chen *et al.*, 2006). Feed restriction is typically performed as once a day feeding during the laying period. This feed is rapidly consumed (about 4 h); therefore, the hens fast for a long time before their next feeding (Spradley *et al.*, 2008). The practice of feeding only once a day cannot supply the nutritional needs of hens, particularly for eggshell formation (Bootwalla *et al.*, 1983), which normally starts in the afternoon or evening. This is because broiler breeders are unable to retain calcium from the crop to the lower digestive tract at a uniform rate (Farmer *et al.*, 1983a). It may be possible to improve eggshell quality if nutrients are supplied at times that correspond more closely to periods of eggshell deposition by changing the time of feed intake (Backhouse and Gous, 2005).

Calcium is one of the major elements required for maintenance and egg production in hens. The hen will obtain the required calcium from the diet via the intestine and also from medullary bone reserves (Halls, 2005). The probable course for Ca ingested by hens in the morning would be the small intestine-blood-gland of eggshell and then the eggshell. Therefore, hens with a scheduled feeding in the afternoon until the early calcifications of eggshell can deposit the Ca directly into the egg (Calderon, 1994).

On the other hand, medullary bone is found in sexually mature and egg producing hens. It is the primary bone calcium reserve for eggshell formation. Medullary bone calcium can be deposited and released in response to changes in calcium supply and demand during eggshell formation. Intestinal calcium absorption reaches over 70% when the shell gland is actively forming the shell. An average of 4 g of calcium is required in the diet to maintain good shell quality since only 50-60% of dietary calcium is actually used in the shell formation (Halls, 2005). Phosphorus is another main component in eggshell formation. Eggshells contain very little phosphorus, but this mineral interacts with calcium during bone formation. The calcium bone

³Corresponding author: vetelondero@hotmail.com
Phone: +55(55)322-08269; Fax: +55(55)3220-8269

Received: June 15, 2015

Accepted: January 6, 2016



reserves are replenished during the time when the shell gland is in the inactive state (Halls, 2005). According to McCormack *et al.* (2001), feed restriction in broiler breeders results in changes in the relative growth of different body components, but there is little information on the impact of this on bone structure and composition.

The metabolic and structural function of these minerals in bone and eggshell formation are essential to poultry production. Thus, feeding at a later time or twice a day can retain them within the gut of the hen and provide a longer acting source of calcium, which can reduce the need for calcium resorption from the medullary bones. Therefore, the aim of this study was to evaluate the effect of different feeding schedules in broiler breeders on egg quality, Ca and P plasma levels and bone parameters.

Materials and Methods

This experiment was carried out in the Poultry Science Laboratory (LAVIC) of the Federal University

of Santa Maria (UFSM). This study was based on compliance with the Welfare Standards and was approved by the Ethics Committee of UFSM. 330 broiler breeder hens and 45 roosters were used in the trial. They were placed in an open-sided house with a wood shaving floor. The broiler breeders were reared following the Cobb 500 broiler breeder according to nutritional recommendations (Rostagno *et al.*, 2011). From 28 to 40 weeks of age, the three groups were fed at different schedules and the performance was evaluated. The selected hens were placed in 15 pens, each pen had 4.615 m² (3.24 x 1.42 m) and was equipped with an automatic drinker, one tube feeder for the breeder hens, and a trough-type feeder for the roosters. Hens were fed with corn-soybean-based mash diets (Table 1). The feed supply was strictly controlled, in accordance to the recommendations of the breeder company. Water was *ad libitum* and 16:30h light/day photoperiod was applied, with artificial lighting complementing natural lighting.

Table 1. Diet composition and nutritional levels used¹.

Ingredients	(%)
Corn	69.06
Soybean meal 46%	21.15
Soybean oil	-
Dicalcium Phosphate	1.60
Limestone 38% Ca	6.99
Salt	0.40
Premix ¹	0.50
DL-Methionine 99%	0.08
Calculated Nutritional Composition	
Crude Protein (%)	15.50
Metabolizable Energy (kcal/kg)	2800
Calcium (%)	3.20
Available Phosphorus (%)	0.39

¹Mineral and Vitamin Premix: Levels per kg of diet: Vit. A 10,450 IU, Vit. E 38 mg, Vit. D₃ 1662.5 IU, Vit. K₃ 4.75 mg; Nicotinic Acid 42.5 mg, Vit. B₁ 2.37 mg, Vit. B₁₂ 19 µg, Vit B₂ 9.5 mg, 4.75 mg Vit B₆, Folic Acid 1.18 mg, Biotin 0.19mg, Choline 360 mg; 19 mg Pantothenic Acid, Copper 12 mg; 60mg Iron, Iodine 0.8 mg, Manganese 70 mg, Selenium 0.54 mg and Zinc 70 mg.

Treatments

The hens were distributed in three experimental groups with similar body weight and uniformity (average of 3.62 kg and uniformity of 90%). The hens were acquired from a commercial poultry company to be used in the trial (22 weeks old) and they were submitted to different treatments from 28 weeks of age. The hens were fed with the same basal diet (corn and soybean) from 22 to 60 weeks of age. Each one of experimental treatments was randomly assigned in 5 replicates, each one with 22 hens and 3 roosters per pen. Three feeding schedules were tested: a single feeding at 8:00 AM, twice daily feeding (50% at 8:00 AM and 50% at 3:00 PM), and a single feeding at 3:00 PM. From 40 to 60 weeks of age, the hens' performance was evaluated.

Experimental responses measured

The egg production per hen housed was calculated weekly. The egg production was determined by the mathematical relationship of number of laid eggs, hens, and production days multiplied by 100. Laying rate was determined by 6 daily collections over the experimental period. Fifteen eggs/treatment (5 replicates of 3 eggs each) were used to determine egg weight, eggshell weight and eggshell thickness. The egg specific gravity was determined through the immersion of the eggs in saline solutions with densities of 1070, 1075, 1080, 1085, 1090, 1095, and 1100 g/cm³ using Archimede's principle as described by Peebles and McDaniel (2004). Egg weight and eggshell weight were determined through a precision scale (0.001 g). The



eggshell was weighted after being dried in ambient temperature for 3 days (Rodríguez-Navarro *et al.*, 2002). Shell thickness was measured with the Electronic Outside Micrometer 0.001 mm at three equatorial points on each egg (Lin *et al.*, 2004).

Analysis of calcium and phosphorus level in blood

At 55 weeks of age, blood samples were collected from 3 hens per replicate immediately after oviposition time (0 h). The hens were marked with dye and 21 h after oviposition, blood samples were collected once again (Miles *et al.*, 1984; Junqueira *et al.*, 2002). Blood samples from each treatment were placed in tubes and packed in ice. Subsequently, they were centrifuged at 3000 rpm for a period of time up to 15 min to separate the plasma. The Ca and P measurements of the samples were analyzed by the Clinical Laboratory Veterinary (LACVET/UFSM), in Santa Maria (RS), Brazil.

Analysis of bone quality

At 60 weeks of age, 3 hens per pen were used to evaluate the right tibia. The tibia was cooked, the fibula and adherent tissues were removed and dried, and after this, their length, weight, and Seedor index were measured. Tibia weight was determined through a precision scale (0.001 g). Bone density was measured by the Seedor index (mg/mm) and obtained by the value of weight (mg) and the length (mm) of the bone assessed (Seedor, 1993).

Experimental design and statistical analysis

The experimental design was completely randomized, with three treatments (a single feeding at 8:00 AM, twice daily feeding (50% at 8:00 AM and 50% at 3:00 PM), and a single feeding at 3:00 PM) with 5 replicates each. All the data were subject to Analysis of Variance. When significant differences were observed at 10% in the variance average, the Turkey test was applied for comparison among treatments. Statistical procedures were performed using the SAS software, 2013.

Results

In the egg production, only in the 44 weeks of age group did production decrease significantly ($P = 0.0319$) when the hens were fed at 3:00 PM compared to hens fed 8:00 AM (Table 2). The egg specific gravity ($P = 0.0219$) and eggshell thickness ($P = 0.0419$) were significantly higher in hens fed at 3:00 PM than the broilers breeder fed once in the morning. Hens fed at 3:00 PM produced egg ($P = 0.0105$) and eggshells ($P = 0.0066$) that were heavier than the other groups (Table 3). The Ca and P plasma levels were not affected by feeding schedules used at the time of oviposition (0 h), but at 21 h after oviposition, the hens fed at 3:00 PM showed higher levels than hens fed at 8:00 AM (Table 4). Hens fed at 8:00 AM had higher tibia weight than hens fed at 8:00 AM and 3:00 PM. The feeding schedules used in broiler breeders did not affect the tibia length and Seedor index (Table 4).

Table 2. Laying rate of broiler breeders submitted to the different treatments¹.

Breeder age (weeks)	Feeding time (h)			SEM*	P values
	8:00 AM	8:00 AM and 3:00 PM	3:00 PM		
	Egg production (%)				
40	77.14	73.85	73.73	5.13	0.5119
41	77.08	74.21	73.36	5.00	0.4893
42	79.09	72.08	73.24	6.21	0.2027
43	77.46	76.49	71.81	4.42	0.1396
44	76.23 ^a	75.71 ^{ab}	71.54 ^b	2.66	0.0319
45	74.15	69.74	69.74	3.82	0.1518
46	72.08	70.77	68.70	3.94	0.4198
47	74.28	72.07	71.81	4.72	0.6719
48	69.97	73.24	68.05	4.20	0.1853
49	70.13	70.26	64.82	5.69	0.2659
50	70.00	70.78	66.75	2.89	0.1053
51	66.10	69.74	66.10	4.33	0.3428
52	67.40	69.35	65.19	3.41	0.1992
53	67.27	69.35	64.80	4.35	0.2916
54	65.32	67.53	63.77	3.26	0.2282
55	63.11	64.80	60.52	3.98	0.2693
56	62.34	65.71	63.24	4.50	0.4928
57	62.73	63.22	61.17	3.95	0.6995
58	60.00	61.67	60.65	5.04	0.8716
59	58.96	64.12	58.31	4.48	0.1215
60	58.05	57.84	57.18	4.29	0.9463
Average (40-60 weeks)	68.99	68.95	66.25	2.63	0.2112

¹Data represent means from 5 replicates (i.e., pens) per treatment. ^{a,b}Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$). *Pooled SEM, $n = 5$.

Table 3. Specific gravity (SG), egg weight (EW), eggshell weight (SW) and eggshell thickness (ET) evaluated in broiler breeder hens from 40 to 60 weeks of age¹.

	Feeding time (h)			SEM*	P values
	8:00 AM	8:00 AM and 3:00 PM	3:00 PM		
SG (g/cm ³)	1084.36 ^b	1084.89 ^{ab}	1085.93 ^a	0.77	0.0219
EW (g)	72.17 ^b	71.98 ^b	73.63 ^a	0.77	0.0105
SW (g)	6.70 ^b	6.72 ^b	7.00 ^a	0.13	0.0066
ET (mm)	0.435 ^b	0.437 ^{ab}	0.445 ^a	0.00	0.0419

¹Data represent means from 5 replicates (i.e., pens) per treatment. ^{a,b}Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$). *Pooled SEM, $n = 5$.

Table 4. Calcium and phosphorus in plasma at the time of egg laying (0 hours) and 21 hours after laying¹

Treatments	8:00 am	8:00 am and 3:00 pm	3:00 pm	SEM*	P values
Calcium (mg/dl)	23.24	27.1	28.32	4.60	0.2307
Phosphorus (mg/dl)	6.3	7.12	6.73	1.09	0.5097
21 hours					
Calcium (mg/dl)	23.35 ^b	27.04 ^{ab}	28.12 ^a	3.11	0.0758
Phosphorus (mg/dl)	4.93 ^b	6.17 ^{ab}	6.79 ^a	1.19	0.0811

¹Data represent means from 5 replicates (i.e., pens) per treatment. ^{a,b}Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$). *Pooled SEM, $n = 5$.

Discussion

The total egg production and the weekly egg production are shown in Table 2. The total egg production in the experimental period was not affected. At 44 weeks of age, the hens fed once at 8:00 AM had better egg production than hens fed once at 3:00 PM ($P = 0.0319$). However, in the other weeks evaluated, the different schedule feeding did not influence egg production. In the same way, Avila *et al.* (2003a) did not observe a difference in the total egg production through 66 weeks of age between broiler breeders fed once or twice a day. Moreover, the egg production results were reported by Harms (1991), which changed the time of feeding in broiler breeder hens from 8:00 AM to 4:00 PM, verifying a significant reduction in their egg production. Broiler breeder hens fed once a day in the afternoon had more egg production than those fed once a day in the morning (Farmer *et al.*, 1983a; Brake and Peebles, 1986; Wilson and Keeling, 1991).

Spradley *et al.* (2008) reported that broiler breeders fed twice a day laid more and heavier eggs through 42 weeks of age than those fed once a day. They believed that increased egg weight was related to providing feed later in the day (3:00 PM). Moradi *et al.* (2013) evaluated hens fed once a day at 6:15 AM and a twice a day feeding (at 6:15 AM and 12:15 PM) and Taherkhani *et al.* (2010) evaluated hens fed once a day at 7:30 AM and twice a day feeding (at 7:30 AM and 11:30 AM), both authors reported that broiler breeders fed twice a day laid more and heavier eggs than those fed once a day. The influence of increasing feeding frequency may be related to a reduction in the fasting period during the day-light cycle (Moradi *et al.*, 2013). The results of this study were according to the results

reported by Cave (1981), Bootwalla *et al.* (1983) and Samara *et al.* (1996), who reported that different feeding schedules had no effect on egg production. Backhouse and Gous (2005) evaluated different feeding schedules for broiler breeders (57 week old), at 07:30 AM, 10:00 AM, 1:00 PM and 3:30 PM and other treatments, in which hens were daily half fed at 07:30 AM and at 3:30 PM (half-feeding) and it was observed that feeding time had no significant effect on total egg production from 26 to 32 weeks of age.

The provision of a limited daily allowance of feed in the morning may not supply the nutrients to coincide with the broiler breeder hen's needs (Cave, 1981). This is particularly important with Ca, because it is an essential component of eggshell formation. It normally begins in the afternoon or evening, and the utilization of dietary Ca in morning-fed broiler breeders is poor due to the inability of these hens to maintain Ca from the crop to the lower digestive tract at a uniform rate (Farmer *et al.*, 1983a). Feeding broiler breeders later in the day supplies dietary Ca at times that correspond more closely to periods of shell deposition (Farmer *et al.*, 1983a), resulting in a better Ca utilization (Farmer *et al.* 1983b; Roland and Farmer, 1984), which is usually manifested as an increase in egg specific gravity, eggshell weight and shell thickness (Backhouse and Gous, 2006). In this study, the highest egg specific gravity was observed in hens fed at 3:00 PM (Table 3). Backhouse and Gous (2005) showed that split feeding could improve the eggshell quality. Harms (1991) also observed that egg specific gravity is increased when the broiler breeder hens were fed daily at 4:00 PM, the improvement was very small compared with the result at 8:00 AM.

The egg weight increase observed in this study is in agreement with the study by Cave (1981), which



described that egg weight was better for hens fed two or three times per day than hens fed once a day (Table 3). Spradley *et al.* (2008) compared hens that received once-a-day feeding treatment at 6:30 AM, whereas the hens under the twice-a-day feeding treatment received 60% of their total daily feed allotment at 06:30 AM and 40% at 3:00 PM, and they concluded that the average egg weight for the entire egg production period was better in hens fed twice a day than once-a-day. Moreover, Samara *et al.* (1996) and Harms (1991) reported that egg weight was not affected by feeding time. In addition, Backhouse and Gous (2005) report that egg weight was not significantly affected by the feeding time, suggesting that differences in shell thickness and oviposition times were not due to increased oviducal transit times.

The weight of eggshells was significantly higher in hens fed at 3:00 PM than broiler breeders fed once in the morning or twice a day. Lewis and Perry (1988) reported an increase in eggshell thickness and eggshell weight when broiler breeders were given half the daily feed allocation twice daily compared to a single allocation of feed provided in the morning. In this study, the eggshell thickness was significantly higher in hens fed once in the afternoon than broiler breeders fed once in the morning. Thus, the delayed feeding improved shell thickness by allowing more Ca to bypass the bone and depositing Ca directly on the egg via blood (Roland and Farmer, 1984).

Feinberg *et al.* (1937) was a pioneer in registering a slight fluctuation in Ca and marked fluctuation of serum P during the egg formation. Likewise, Miller *et al.* (1977) also observed cyclical variation blood phosphorus in the laying hen, with the lowest P level about 2 h before oviposition, remaining constant up to 4 h after oviposition. The average values were 5.29 mg/dl, and then kept increasing, reaching a peak 21-22 h after oviposition, corresponding with the active period of eggshell formation and bone demineralization. Taylor and Stringer (1965) have suggested that the period where the phosphorus plasma level is minimum represents the process pause of the eggshell formation, the return of the P to the bone and the kidney filtration of the element.

Mongin and Sauveur (1979) confirmed the cyclical nature of the phosphorus plasma, reporting that the phosphorus declining of hens, 10 h after the oviposition, is due solely to eggshell formation and it is independent of the light program that is imposed on them or the amount of feed ingested. The blood P values found by the authors were minimal during oviposition, continuing up to 10 h after the oviposition. Then, an increase was observed reaching a peak of 18 to 20 h following oviposition and returning to normal levels before the next oviposition (Junqueira *et al.* 2002).

In this study, different feeding schedules (8:00 AM, 8:00 AM and 3:00 PM, and 3:00 PM) did not affect P levels at the time of oviposition (0 h) (6.3, 7.12,

6.73 mg/dl). But after 21 h of oviposition, there was a difference among the feeding schedules on P levels (4.93, 6.17, 6.79 mg/dl; $P = 0.0811$; Table 4. Miles *et al.* (1984), after performing experiments, reported that the average of P content in plasma at the time of oviposition for hens fed in the morning or afternoon was 3.95 and 4.10 mg/dl, respectively. On the other hand, higher values from 4.91 to 5.90 mg/dl were obtained from the plasma of hens which blood was collected 21 h after oviposition. In relation to the plasmatic calcium, it was observed that there was no fluctuation at the time of oviposition (0 h; 23.24, 27.1, 28.32 mg/dl), along the process of egg formation, when the hens were fed at different times. But, after 21 h of oviposition, there was difference among the feeding schedules on Ca levels (23.35, 27.04, 28.12 mg/dl; $P = 0.0758$). Junqueira *et al.* (2002), evaluating hen feed restriction, reports a small increase in plasmatic calcium levels 21 h after oviposition and this increase can be related to the fact that there is less Ca elimination through the renal tract, or also, because there is an increase in the mobilization of the bones, as an attempt to maintain normal levels in the hen.

Calcium metabolism in laying hens is dominated by the uterus for shell formation. As a result, inadequate Ca in diet leads to disruption of ovulation and cessation of lay until their meager Ca reserves are replenished. Under normal conditions, when a high Ca diet is being applied, the medullary bone is resorbed when supplies of Ca from the gut are not sufficient to provide it for the shell gland demands. This occurs in the early morning hours, when most of the previous day's feeding has been absorbed (Taylor and Dacke, 1984). It is suggested that reabsorption of medullary bone is induced by an increase in the circulating concentration of the parathyroid hormone (PTH) secretion, which is stimulated by the decline in Ca^{2+} concentration that occurs during shell formation (Taylor and Dacke, 1984).

No treatment effect was observed on the tibia length, but the tibia weight was higher in hens fed at 8:00 AM than hens fed 8:00 AM and 3:00 PM ($P = 0.0697$). However Ávila *et al.* (2003b) evaluated different feeding schedules for broiler breeders: 06:30 AM, 50% feeding at 06:30 AM and 50% feeding at 3:30 PM, 11:30 AM, or 3:30 PM, and reported that there was an effect of treatment for tibia weight of hens fed at 11:00 AM compared to those with other feeding schedules, and related this result to the low mobilization of Ca and P to the plasma required for eggshell synthesis. Bootwalla *et al.* (1989) fed female broiler breeders in different periods of the day and found that Ca and P plasma levels were influenced by feeding, stage of egg formation, and the moment of oviposition. In this study, bone quality measured by the Seedor index was not affected (Table 5).

In conclusion, the present study suggests that the breeding of broilers submitted to different feeding times (40 to 60 weeks of age) showed no difference in



the egg production. Hens fed once in the afternoon had better egg specific gravity and eggshell thickness than hens fed once in the morning. Hens fed later showed better egg weight and eggshell than others. Ca and P

levels were higher in hens fed at 8:00 AM than hens fed at 3:00 PM after 21 h of oviposition. The tibia weight was higher in hens fed at 8:00 AM than hens fed at 8:00 AM and 3:00 PM.

Table 5. Effect of time of feeding on bone quality in broiler breeder hens from 40 to 60 weeks of age¹.

	Feeding time (h)			SEM*	P values
	8:00 AM	8:00 AM and 3:00 PM	3:00 PM		
Weight (mg)	15999 ^a	15061 ^b	15210 ^{ab}	615.56	0.0697
Length (mm)	121.10	120.30	118.50	1.86	0.1260
Seedor index (mg/mm)	132.23	125.15	128.40	5.17	0.1378

¹Data represent means from 5 replicates (i.e., pens) per treatment. ^{a,b} Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$). *Pooled SEM, n=5.

Acknowledgments

The location offered by UFSM.

References

- Avila VS, Penz Jr AM, Brum PAR, Rosa PS, Guidoni AL, Figueiredo ÉAP.** 2003a. Performance of female broiler breeders submitted to different feeding schedules. *Braz J Poult Sci*, 5:197-201.
- Avila VS, Penz Jr AM, Rosa PS, Brum PAR, Guidoni AL, Ledur MC.** 2003b. Influence of feeding time on sexual maturity and carcass composition in female broiler breeders. *Braz J Poult Sci*, 5:189-196.
- Backhouse D, Gous R.M.** 2005. The effect of feeding time on shell quality and oviposition time in broiler breeders. *Br Poult Sci*, 46:255-259.
- Backhouse D, Gous RM.** 2006. Responses of adult broiler breeders to feeding time. *World's Poult Sci, J*, 62:269-281.
- Bootwalla SM, Wilson HR, Harms RH.** 1983. Performance of broiler breeders on different feeding systems. *Poult Sci*, 62:2321-2325.
- Bootwalla SM, Wilson HR, Harms RH.** 1989. Plasma calcium and phosphorus levels of broiler breeders with different feeding schedules. *Poult Sci*, 39:391-398.
- Brake J, Peebles ED.** 1986. Effect of strain and time of day of feeding on reproductive performance and shell quality of broiler breeders. *Poult Sci*, 65:156.
- Calderon C.** 1994. Efectos nutricionales sobre la calidad de la cáscara. In: Conferência APINCO 1994 de Ciência e Tecnologia Avícolas, 1994, Santos, SP. Santos, SP: FACTA. pp. 35-66.
- Cave NA.** 1981. Effect of diurnal programs of nutrient intake on the performance of broiler breeder hens. *Poult Sci*, 60:287-1292.
- Chen SE, McMurtry JP, Walzem RL.** 2006. Overfeeding-induced ovarian dysfunction in broiler breeder hens is associated with lipotoxicity. *Poult Sci*, 85:70-81.
- Farmer M, Roland DA Sr, Brake J, Eckman MK.** 1983a. Calcium metabolism in broiler breeder hens. 1. Calcium status of the digestive tract of broiler breeders throughout a 24 hour period. *Poult Sci*, 62:459-464.
- Farmer M, Roland DA Sr, Eckman MK.** 1983b. Calcium metabolism in broiler breeder hens. 2. The influence of the time of feeding on calcium status of the digestive system and egg shell quality in broiler breeders. *Poult Sci*, 62:465-471.
- Feinberg ,JG, Hughes JS, Scott HM.** 1937. Fluctuations of calcium and inorganic phosphorus in the blood of the laying hen during the cycle of on egg. *Poult Sci*, 16:132-134.
- Halls A.** Egg formation and eggshell quality in layers. 2005. Nutrifax, Nutrition News and Information Update. Available: <http://www.nutrecocanada.com/docs/shur-gain--poultry/egg-formation-and-eggshell-quality-in-layers.pdf>. Accessed on: Febr 20th, 2015.
- Harms RH.** 1991. The influence of changing time of feeding on performance of broiler breeder hens. *Poult Sci*, 70:1695-1698.
- Junqueira OM, Andreottil MO, Rodrigues EA, Faria DE, Casartelli EM.** 2002. Influência da restrição alimentar sobre alguns constituintes plasmáticos e qualidade da casca dos ovos de poedeiras comerciais. *Acta Sci Maringá*, 24:1021-1025.
- Lewis PD, Perry GC.** 1988. Effect of a single or double daily allocation of food on shell weight and oviposition time of broiler breeder hens. In: Proceedings of the Fourth International Poultry Breeders Conference, 1988, Ayr, Scotland. Ayr: The West of Scotland College. pp. 72-78.
- Lin H, Mertens K, Kemps B, Govaerts T, De Ketelaere B, De Baerdemaeker J, Decuyper E, Buyse J.** 2004. New approach of testing the effect of heat stress on eggshell quality: mechanical and material properties of eggshell and membrane. *Br Poult Sci*, 45:476-482.
- McCormack HA, Fleming R, McTeir L, Whitehead CC.** 2001. Bone development up to 6 weeks of age in feed-restricted broiler breeders fed on diets supplemented with different concentrations of ascorbic acid. *Br Poult Sci*, 42(suppl):91-92.
- Miles RD, Junqueira OM, Harms RH.** 1984. Plasma phosphorus at 0, 6, and 21 hours postoviposition in hens laying in the morning or afternoon. *Poult Sci*, 63:354-359.
- Miller ER, Harms RH, Wilson HR.** 1977. Cyclic



- changes in serum phosphorus of laying hens. *Poult Sci*, 56:586-589.
- Mongin P, Sauveur B.** 1979. Plasma inorganic phosphorus concentration during egg-shell formation. Effect of the physical form of the dietary calcium. *Br Poult Sci*, 20:401-412.
- Moradi S, Zaghari M, Shivazad M, Osfoori R, Mardi M.** 2013. The effect of increasing feeding frequency on performance, plasma hormones and metabolites, and hepatic lipid metabolism of broiler breeder hens. *Poult Sci*, 92:1227-1237.
- Peebles ED, McDaniel CD.** 2004. *A Practical Manual for Understanding the Shell Structure of Broiler Hatching Eggs and Measurements of their Quality*. Mississippi State, MS: Mississippi Agricultural and Forestry Experimental Station, 16 pp. (Bulletin 1139).
- Renema RA, Robinson FE.** 2004. Defining normal: comparison of feed restriction and full feeding of female broiler breeders. *World's Poult Sci J*, 60:508-522.
- Rodriguez-Navarro A, Kalin O, Nys Y, Garcia-Ruiz JM.** 2002. Influence of the microstructure on the shell strength of eggs laid by hens of different ages. *Br Poult Sci*, 43:395-403.
- Roland DA Sr, Farmer M.** 1984. Egg shell quality II: importance of time of calcium intake with emphasis on broiler breeders. *World's Poult Sci J*, 40:255-260.
- Rostagno HS, Albino LFT, Donzele JL.** 2011. *Tabelas Brasileiras para Aves e Suínos: composição de alimentos e exigências nutricionais*. 3.ed. Viçosa, MG: Universidade Federal de Viçosa. 252 pp.
- Samara MH, Robbins KR, Smith MO.** 1996. Interaction of feeding time and temperature and their relationship to performance of the broiler breeder hen. *Poult Sci*, 75:34-41.
- Seedor JG.** 1993. The bisphosphonate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. *J Bone Miner Res*, 4:265-270.
- Spradley J M, Freeman M E, Wilson J L, Davis A J.** 2008. The influence of a twice-a-day feeding regimen after photostimulation on the reproductive performance of broiler breeder hens. *Poult Sci*, 87:561-568.
- Taherkhani R, Zaghari M, Shivazad M, Zare Shahneh A.** 2010. A twice-a-day feeding regimen optimizes performance in broiler breeder hens. *Poult Sci*, 89:169-1702.
- Taylor TG, Stringer DA.** 1965. Eggshell formation and skeletal metabolism. In: Sturke PD (Ed.). *Avian Physiology*. New York: Cornell University Press. pp. 485-514.
- Taylor TG, Dacke CG.** 1984. Calcium metabolism and its regulation. In: Freeman BM (Ed.). *Physiology and Biochemistry of the Domestic Fowl*. London, UK: Academic Press., vol. 5, pp. 127-164.
- Wilson HR, Keeling LJ.** 1991. Effect of time of feeding on oviposition time and production parameters in broiler breeders. *Poult Sci*, 70:254-259.
-