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Replacement of corn and soybean meal with corn gluten meal on rooster's diet

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Abstract: Using alternative feedstuffs to replace corn and soybean in domestic poultry diets, as agroindustry by-products, may reduce production costs. Corn gluten meal is a by-product resulting of corn processing to obtain oil and starch. Aiming to evaluate the use of this ingredient in rooster's diet and its effects on sperm characteristics, 40 roosters with 68 to 72 weeks of age, randomly distributed in individual boxes, were tested. Two treatments were used: treatment 1 (control) – with corn and soybean meal, and treatment 2 – with 10% of corn gluten meal in the diet. Semen collection and body weight verification were performed during 4 weeks. Semen variables evaluated were volume, sperm concentration, motility, membrane integrity, acrosome integrity, and DNA integrity. Results showed that there was no significant difference between treatments, which allows the use of 10% of corn gluten meal in rooster's diet. Therefore, corn gluten meal can be an alternative diet's input, especially when there is corn or soybean market restriction.

Key words: Alternative feed, *Gallus gallus*, reproductive parameters, seminal quality

1. Introduction

Total number of poultry housed (laying hens, broiler and broiler breeders) has been growing in recent years and, in order to support animal welfare, there is an increased demand of raw material for animal feed[1] .

Corn, the main energy source, and soybean meal, the main protein source, are the basic ingredients of poultry diets, accounting for about 70% of total production costs [1]. Supply of these inputs in Brazilian intern market for use in animal feed has been compromised due to the increased use of grains for ethanol (corn) and biodiesel (soy) production, besides their use in human feed. These factors make prices high, especially off-season and in unfavourable climate, and consequently production costs are increased [2]. These circumstances make companies, nutritionists and researchers to seek alternative raw material, with the purpose of partial or total replacement of these grains. However, use of alternative feed or industry by-products in diets must be efficient, both on productive and on economic side, and should provide similar performance to that obtained by the feed replaced [3].

Due to its wide possibility of use, corn is the most produced cereal in Brazil and in the world. Corn gluten meal (CGM-21) is a by-product of corn wet processing, consisting of the fibrous part of the grain (outside), part of the germ (after oil extraction), and part of the gluten, together with reduced amounts of starch and soluble protein fractions. CGM-21 presents about 21% of crude protein and 1813 Kcal/kg of metabolisable energy [4], turning it a good alternative for animal feed. However, it should not be used as the main protein source of nonruminant diet, since it has a high crude fiber content (7.62%). In a study using CGM-21 to partially replace corn and soybean meal, Freitas et al. [5] concluded that it is possible to use up to 15% of CGM-21 without reducing broiler´s performance.

Several factors affect flock fertility, among them nutrition, that may alter animal physiological aspects and

[1] Associação Brasileira de Proteína Animal (ABPA) (2016). Relatório Anual ABPA [online]. Website http://abpa-br.com.br/setores/avicultura [accessed 17 February 2017].

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its reproductive performance [6]. In view that industry's final product is the chick produced by bird; changes in fertility impacts economic gain.

In poultry industry, breeder selection is usually made by physical characteristics of birds such as body weight, leg length and appearance, and comb and barb size and color [7]. This selection is easy to perform, however birds discarded from the flock often have better reproductive characteristics than those selected, since physical characteristics do not always have a positive correlation with fertility [8]. Therefore, aiming a more assertive selection, some ejaculate characteristics (as volume, motility, and sperm concentration), which can be evaluated with simple equipment, should also be considered.

Males are the main responsible for flock reproductive performance, and a good feeding management may directly influence the number of chicks hatched [9]. Changes in semen quality can cause decrease, both in number and in quality of chicks produced, thus it is necessary to test every food provided to the roosters. Therefore, the aim of this study was to evaluate the use of CGM-21 on roosters' diet and its effects on semen characteristics (volume, sperm concentration, motility, and membrane, acrosome, and DNA integrity) and on body weight.

2. Materials and methods

Experimental period comprised the months of December 2015 and January 2016, and trial was approved by the Animal Experimentation Ethics Committee of the Federal University of Pelotas (number 9088).

Forty great-grandparents of commercial Rhode Island Red males, 68 to 72 weeks of age were used. Birds were housed in the Poultry Sector of the Central Biotério of the Federal University of Pelotas, *campus* Capão do Leão, in south of Brazil. They were allocated in individual boxes equipped with a nipple type drinker and a trough type feeder. Water was supplied ad libitum and feed was controlled to 160 g per animal per day, always provided in the morning.

Treatments consisted of 2 experimental diets: treatment 1 (control) – with corn and soybean meal, and treatment 2 (CGM-21) – with 10% of corn gluten meal replacing 10% of control diet's corn. Diets (Table 1) were formulated to meet rooster's nutritional requirements [8] and feed analysis was carried out at the Laboratory of Animal Nutrition (LNA, Department of Animal Science, UFPel). Animals underwent a period of physiological adaptation to experimental diets, which lasted 30 days. During semen collection period, roosters were weighed weekly, using a digital scale (Elgin® DP15, Brazil).

Semen was collected by dorsal-abdominal massage [10] in 15 mL Falcon tubes. Semen analyses were carried out in the Poultry Reproduction Biotechniques Laboratory (LABRA, Department of Physiology and Pharmacology, Institute of Biology, UFPel), and at the Center for Teaching and Research in Animal Reproduction (ReproPEL, Veterinary School, UFPel). Seminal parameters evaluated were volume, concentration, motility, and integrity of membrane, acrosome, and DNA.

Volume was measured in the collection tube, with 0.1 mL graduation, after sedimentation. Sperm concentration was analyzed using spectrophotometer at a wavelength of 450 nm (Micronal B542®, Brazil). Semen was diluted 1:1000 (sperm:diluent) in 2.9% sodium citrate with 0.4% glutaraldehyde, transmittance values were converted to billions of sperm per milliliter.

Sperm motility was observed at microscope (BX 41, Olympus America, Inc., São Paulo, SP, Brazil), under 400´ magnification. Semen was diluted 1:1 in 0.9% sodium chloride (NaCl), placed in a glass slide, covered with

Table 1. Composition of experimental diets for semiheavy roosters aged 68–72 weeks.

Ingredients (%)	Control	CGM-21
Corn	59.87	50.26
Soybean meal	23.60	20.91
Corn gluten meal	0.00	10.00
Limestone	9.27	9.27
Dicalcium phosphate	1.50	1.44
Mineral and vitamin premix[1]	4.00	4.00
Soy oil	1.13	3.50
Salt	0.50	0.50
Methionine	0.13	0.13
Total	100.00	100.00
Calculated nutritional levels		
Gross energy Kcal/Kg	2700	2700
Crude protein (%)	15.88	15.95
Crude fiber (%)	2.42	2.87
Calcium (%)	3.94	3.93
Avaliable phosphorus	0.37	0.36
Met + cist (%)	0.65	0.66
Total lysine (%)	0.81	0.75
Linoleic acid (%)	2.78	3.86

[1]Warranty levels per kilogram of product: Vitamin A 2,500,000 IU, Vitamin D3 500,000 IU, Vitamin E 1750 mg, Vitamin K3 375 mg, Vitamin B1 400 mg, Vitamin B2 1100 mg, Vitamin B6 750 mg, Vitamin B12 3000 mcg, Niacin 6500 mg , Folic acid 175 mg, Pantothenic acid 2500 mg, Methionine 300 g, Hill 90 g, Manganese 17500 mg, Zinc 12500 mg, Iron 15000 mg, Copper 2500 mg, Iodine 90 mg, Selenium 76 mg; Met + cist = methionine + cystine.

a coverslip, and evaluated on a scale of 0–100% (0% representing all immotile and 100% all motile cells). Evaluation was always made in duplicate, by the same technician, and the final value is the arithmetic mean of the 2 observations [11].

Evaluation of membrane, acrosome, and DNA integrity was done using fluorescence probes, in an epifluorescence microscope (BX 51, Olympus America, Inc., São Paulo, SP, Brazil), under 1000´ magnification. In each slide, 100 cells were counted, and the values were expressed as percentage of intact membranes, whole acrosomes, and normal DNA, respectively.

To evaluate membrane integrity, cells were incubated with SYBR-14 and PI (live/dead sperm viability), cells with green fluorescence (SYBR-14) were considered intact, and those with red fluorescence (PI) or double-stained (SYBR-14+PI) were considered damaged [12].

Acrosome integrity was evaluated using FITC and Arachis hypogeae (PNA) lecithin conjugate [13]. Only acrosomes emitting green fluorescence, without roughness or vacuoles were considered intact; all the others were classified as damaged.

DNA integrity was assessed using acridine orange probe [14]. Cells with normal (double stranded) DNA showed green fluorescence, and with denatured (single stranded) DNA showed red or yellowish coloration.

Ambient temperature was recorded daily with a thermometer (Wellmix®Wx4143, Brazil) throughout the experimental period. Maximum, minimum, and time of collection temperatures were recorded. In order to evaluate possible cumulative effects of temperature on seminal characteristics, 2 variables were generated: mean minimum (Tmin) and mean maximum (Tmax) temperature of the collection week, Tmin corresponding to the mean of the minimal temperatures recorded in 4 consecutive days prior to collection, and Tmax to the mean of the maximal temperatures recorded also in 4 consecutive days prior to collection.

Experimental design was completely randomized with 20 animals per treatment; each rooster represented an experimental unit. Data normal distribution was checked using Shapiro-Wilk test; when necessary they were transformed using arc-sine's square root. For interpretation purposes, all data are presented in their original scale. Repeated measures analyses of variance was used, and means comparisons were made by Tukey test considering ($P < 0.05$). Pearson correlations among variables were also performed. All analyses were done in statistics software Statistix 9.0 (Analytical Software, Tallahassee, FL, USA).

3. Results

Results of volume, concentration, and sperm motility are observed in Table 2. There was no significant difference ($P > 0.05$) between treatments for any of these parameters. However, for sperm concentration, a significant difference ($P < 0.05$) was observed between means of collections 1 and 3. In collection 1, concentration was lower (1.85 billion/mL) than in collection 3 (3.34 billion/mL).

Table 3 shows the data of membrane, acrosome, and DNA integrity. Although no significant differences ($P >$

Table 2. Means and standard errors of ejaculated volume (Vol), sperm concentration (Conc), and sperm motility (Mot) of semiheavy roosters fed control diet (corn and soybean meal) or diet containing 10% corn gluten meal (CGM-21) in different collection periods.

Diets		Variables		
	N[1]	Vol (mL)	Conc (bilions/mL)	Mot (%)
Control	65	0.41 ± 0.03^{ns}	2.55 ± 0.17^{ns}	73.5 ± 2.33^{ns}
CGM-21	65	0.49 ± 0.03^{ns}	2.96 ± 0.18^{ns}	71.4 ± 2.98^{ns}
P-value		0.1122	0.0601	0.7272
Collection				
1	19	0.32 ± 0.04^{ns}	1.85 ± 0.22^{a}	51.9 ± 4.28^{ns}
2	36	0.38 ± 0.04^{ns}	2.47 ± 0.19^{ab}	79.7 ± 2.34^{ns}
3	39	0.52 ± 0.04^{ns}	3.34 ± 0.26^{b}	76.7 ± 3.60^{ns}
4	36	0.50 ± 0.04^{ns}	2.88 ± 0.23^{ab}	71.2 ± 3.58^{ns}
P-value		0.0920	0.0416	0.0553

[ab]Means in the same column with distinct letters differ by Tukey test ($P < 0.05$).
[ns]There was no significant difference between means ($P > 0.05$).
[1] Number of repetitions.

0.05) were found between diets for any of these seminal parameters, a significant difference was observed between collection weeks for acrosome integrity.

There was a higher number of sperm cells with intact acrosome when the mean minimum temperatures were lower (weeks 1 and 3, with temperatures of 20.5 °C and 21.6 °C, and acrosome integrity of 88.9 and 87.6%, respectively), and greater acrosome damage at higher temperatures (weeks 2 and 4, with mean minimum temperatures of 24 °C and 23.9 °C, and acrosome integrity of 82.1 and 83.8%, respectively).

Results of body weight are presented in Table 4. There was no difference between treatments (P > 0.05).

4. Discussion

Semen volumes observed are within the standard preconized for semiheavy roosters [15]. A decrease in volume and concentration is considered normal with advancing age, as well as a decline in fertility, which occurs as a consequence of decreased sperm production after 40 weeks of age [8]. However, this was not observed in the present work for 68–72 week old roosters.

For artificial insemination, ejaculated volume and sperm concentration are important variables, because they determine the number of doses that can be produced [16]. In a research with Ross roosters, Surai et al. [17] observed that volume and concentration had a direct relationship, which was also found in the current study, where these variables showed a positive correlation of 97% (P = 0.0331).

Motility means observed in the present study are slightly below the recommended by the Brazilian College of Animal Reproduction [18], which indicates, for a good quality sample, values above 80% of motility for poultry semen. However, Tonini [19], studying the same roosters at younger ages (41, 42, 43, 44, 45, and 46 weeks), observed mean motilities of 72.4%, 81.4%, 85.0%, 75.0%, 75.0%, and 71.0%, respectively, demonstrating that this may be a breed's characteristic. Values below the recommended may also be explained by high temperatures recorded during the experimental period, which may have caused bird's heat stress. A strong and significant correlation was observed between motility and mean maximum (Tmax 99%, P = 0.0133) and mean minimum (Tmin 99%, P = 0.0072) temperatures recorded in the days preceding collection.

Bongalhardo [20] observed that lipid profile of sperm membranes can be altered according to the diet fed to birds. Diet containing CGM-21 had a higher amount of linoleic acid (3.86%) in its composition when compared to control (2.78%), which could increase membrane fluidity and affect its integrity, but this was not observed in the present experiment with fresh semen. However, addition of CGM-21 may have a significant effect on semen subjected to cryopreservation, improving freezing.

In this work, inclusion of 10% CGM-21 in the diet did not alter acrosome integrity. This result can be attributed to ambient temperature in the days prior to collection, since a negative correlation (97%, P = 0.0303) between acrosome integrity and the mean minimum temperature (Tmin) was observed (Figure). Fertilization physicochemical process is initiated by the connection of acrosomal membrane with egg's perivitelline membrane, thus the quantification of intact acrosomes, able to perform this reaction, is an important property to be analyzed [21].

Temperature factor has a strong influence on birds' performance and their extremes increase losses in productivity. Very high temperatures have a greater impact, since birds have a low efficiency in heat dissipation, causing a metabolism imbalance that leads to respiratory alkalosis [22]. Although it is possible to observe normal seminal parameters, such as volume, motility, and concentration, in animals submitted to high environment temperature, the main damages occur at cellular level, such as membrane, acrosome and DNA [23].

Bird's sperm is rich in polyunsaturated fatty acids, making them susceptible to oxidation by free radicals.

Table 4. Means and standard errors of body weight of semiheavy roosters fed with control diet (corn and soybean meal) or diet containing 10% corn gluten meal (CGM - 21) in different collection periods.

Diets	Weight (g)			
	Collection			
	1	2	3	4
Control	2850 ± 0.04^{ns}	2885 ± 0.03^{ns}	3005 ±0.05^{ns}	2965 ± 0.05^{ns}
CGM-21	2840 ± 0.05^{ns}	2855 ± 0.04^{ns}	2960 ± 0.05^{ns}	2940 ± 0.05^{ns}
P-value	0.9050	0.6117	0.5417	0.7331

^{ns}There was no significant difference between means (P > 0.05).

Table 3. Means and standard errors of membrane integrity (MI), acrosome integrity (AI), and DNA integrity (DNA) of semen of semiheavy roosters fed with control diet (corn and soybean meal) or diet containing 10% corn gluten meal (CGM-21) in different collection periods.

Diets	N[1]	MI (%)	AI (%)	DNA (%)
Control	65	82.9 ± 1.56[ns]	85.1 ± 0.55[ns]	98.8 ± 0.38[ns]
CGM - 21	65	85.9 ± 1.56[ns]	85.2 ± 0.55[ns]	99.0 ± 0.38[ns]
P-value		0.3014	0.9716	0.8051
Collection				
1	19	80.0 ± 2.94[ns]	88.9 ± 1.05[a]	99.5 ± 0.73[ns]
2	36	82.1 ± 2.11[ns]	82.1 ± 0.75[b]	98.2 ± 0.52[ns]
3	39	84.5 ± 2.00[ns]	86.9 ± 0.71[a]	98.8 ± 0.49[ns]
4	36	90.9 ± 2.08[ns]	83.7 ± 0.74[ab]	99.0 ± 0.51[ns]
P-value		0.0946	0.0276	0.7833

[ab]Means in the same column with distinct letters differ by Tukey test (P < 0.05).
[ns]There was no significant difference between means (P > 0.05).
[1] Number of repetitions.

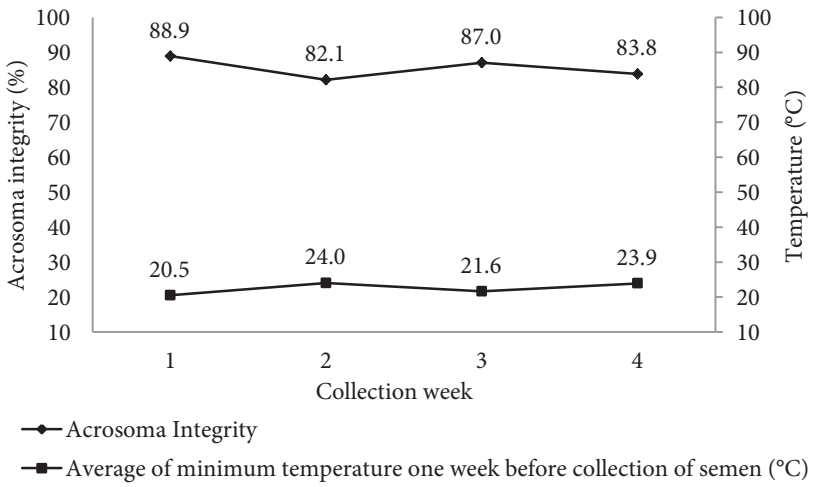

Figure. Correlation (97%, P = 0.0303) between semiheavy rooster's acrosome integrity and mean minimum temperature in the week prior to semen collection in different collection periods.

Elevated temperatures cause even more oxidative stress, resulting in irreversible sperm damage. In order to obtain the best reproductive performance, thermal comfort range for birds is from 18 °C to 22 °C. Above 26 °C oxidative stress can be observed, and fertilization potential decline [24]. In this work, it was observed that when minimum temperatures were above 24°C, there was a decrease in acrosome integrity, which may reflect in lower fertilization rates.

In ruminants, corn gluten meal has been investigated as a possible source of protein and energy. Canchim and Nelore beef cattle, with 14 months of age, submitted to a diet containing 33% of this corn by-product did not present reduction in food efficiency [25]. In nonruminant nutrition, corn gluten meal is still little explored, especially in poultry farming. Seyedi and Hosseinkhani [26] observed higher weight at slaughter, lower feed intake and better feed conversion ratio in Ross 308 broilers that received diet containing 12% corn gluten meal. In this study, although CGM-21 treatment had a slightly higher fiber content (2.87%) than control (2.42%), this difference did not cause weight loss.

In view of the results presented, where there was no diet's influence in any of the studied characteristics, it can be stated that up to 10% corn gluten meal may be included in the diet of semiheavy roosters, aged 68 to 72 weeks, without losses in semen quality or body weight. Therefore, corn gluten meal can be an alternative diet's input, especially when there is corn or soybean market restriction. Independent of diet, when mean minimal environmental temperatures are above 24 °C, sperm concentration and acrosome integrity are negatively affected.

Conflict of interest

The authors have declared that no conflict of interests exist.

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