

EFFECT OF OKARA SUPPLEMENTATION ON THE SEMEN QUALITY OF DARAG PHILIPPINE NATIVE CHICKEN

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ABSTRACT

Avian sperm can suffer oxidative damage, affecting male fertility due to their high polyunsaturated fatty acid content. This study investigated the effect of a diet supplemented with dried soybean pulp (okara) on the semen quality of the Darag Philippine native chicken. Twenty-eight (28) roosters, blocked by five age groups, were randomly assigned to two treatments: (1) fed only 65 grams of commercial feed, and (2) fed with 65 grams containing 80% commercial feed and 20% dried okara daily for two months. The initial and final body weights and daily feed intake were recorded. Semen quality was evaluated monthly using a computer-assisted semen analyzer (CASA). Fertility of roosters was assessed based on the fertile eggs produced after artificial insemination of hens. Results showed no significant differences in the final body weight, average feed intake, semen morphology, motility, and fertility. Interestingly, okara-fed roosters had a higher frequency of creamy semen ($P=0.0123$) and with no incidence of watery semen, had higher semen volume ($P=0.0048$) and sperm concentration ($P=0.0140$) on the second month of feeding and higher overall semen concentration ($P=0.0048$). Further, replacing 20% of the diet with dried okara reduced feed costs by 10%. In conclusion, 20% okara supplementation proves to be cost-effective and shows potential to enhance semen quality in Darag Philippine native chicken without affecting body weight and feed intake.

Keywords: CASA, Darag native chicken, okara, soybean pulp, semen quality

INTRODUCTION

Despite the rising popularity of commercial chicken hybrids, the Philippine native chickens continue to be a significant food resource, providing additional income for rural farmers. The hardiness and resistance of these chickens are well known, and they can live and thrive even under the most unfavorable environmental and management conditions (Lambio, 2024). Darag Philippine native chicken is one of the most popular breeds in the country due to the popularity of its meat in local dishes. Inconsistent supply of native chicken breeders in other native chicken-producing regions and high prices of production inputs are problems faced by the Philippine native chicken industry.

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In poultry production, the reproductive performance of roosters plays a crucial role in the dissemination of desired genetic material. In the chicken industry, artificial insemination (AI) technology has made it feasible to quickly transfer the desired genetic material from a limited number of chosen males to more female breeder birds.

To ensure the desired level of fertility in hens, high-quality semen should be used in this procedure (Mohan *et al.*, 2018). The quality of healthy rooster sperm depends on low sperm cell damage and death rates. Reactive oxygen species (ROS) and enhanced lipid peroxidation are both caused by increased oxidative stress. Problems with the roosters' reproductive system, semen characteristics, and fertility can be mitigated by nutritional factors. The birds' diet may partially compensate for genetic flaws, but they may also cause nutritional deficits that lower breeding systems' effectiveness. As such, the goal is to keep rooster fertility high even when they are fed diets that satisfy female nutritional needs (Gonçalves *et al.*, 2015).

Reactive oxygen species can be lessened through the addition of substances with antioxidant properties to the diet. Utilizing foods that can regenerate and recycle antioxidants, preventing oxidative damage to semen, and preserving the fluidity and membrane integrity of the sperm can lead to improved fertility rate (Fouad *et al.*, 2020). In the animal industry, soybeans are an important feed ingredient. Isoflavones, which act as antioxidants, are found in soybeans (Nakai *et al.*, 2020). However, the co-product generated during the production of tofu or soy milk, known as okara, is often overlooked. This nutrient-rich material contains approximately 50% dietary fiber, 25% protein, and 10% lipids, making it a valuable feed resource. Despite its nutritional potential, the large quantities of okara produced annually are discarded, posing significant environmental and waste management challenges (Li *et al.*, 2012). Therefore, exploring effective utilization strategies for okara is essential to mitigate concerns about its disposal and capitalize on its inherent nutritional potential.

The objective of this study is to evaluate the effects of soybean pulp supplementation on the semen quality of the Darag Philippine native chicken. Specifically, this study aims to compare the semen characteristics (color and consistency, motility, viability, % normal and abnormal sperm), fertility rate of eggs from AI, and feed cost with or without okara.

MATERIALS AND METHODS

The study was conducted from March 2023 until June 2023. Semen was collected from the Darag Philippine native chickens (*Gallus gallus domesticus*) housed at the University Native Animal Farm in Brgy. Putho Tuntungin, Los Banos, Laguna (14.145485903561903, 121.25209186479026) with protocol approved by the UPLB Institutional Animal Care and Use Committee (Approval Reference No: UPLB-2023-037). Artificial insemination was also conducted at the University Native Animal Farm, while the semen analysis and incubation were done at the physiology laboratory at the Institute of Animal Science (IAS), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños, Laguna, Philippines. Thirty Darag Philippine native roosters from two different generations were placed in individual cages. The roosters were blocked according to their age (i.e., 32 weeks, 40 weeks, 60 weeks, 75 weeks), and random sampling was conducted in each block. There were 3 replications per treatment in each block. The birds were labeled according to their block, treatment, and replication. Roosters in treatment 1 (average live weight of 1.63±0.15kg) were fed 65g of commercial layer mash (control), and roosters in treatment

2 (average live weight of 1.67 ± 0.29 kg) were fed 52g of feed and 13g of dried okara. The okara was oven-dried at 70 °C until constant weight was obtained and ground to about 2mm particle size. Clean water was provided *ad libitum*. The feeding lasted for two (2) months. The calculated nutrient composition of the experimental diet is presented in Table 1.

Table 1. Calculated nutrient composition of treatments with and without okara.

Nutrient	Commercial Layer Mash (CLM)	CLM + 20% Okara
Moisture, %	5.13	4.99
Ave. Ash, %	7.31	6.37
Ave. Crude Protein, %	10.71	10.33
Ave. Crude Fat, %	2.98	3.31
Ave. Crude Fiber, %	1.86	3.99
Ave. Calcium, %	1.94	1.59
Total Phosphorus, %	0.30	0.28

Body weight (kg) before and after two (2) months of the feeding trial was measured. Average daily feed intake was computed based on feed refusal. At the end of each month, semen was collected from all roosters by the abdominal massage method. The average volume was recorded per bird. Collected semen from all roosters was characterized to determine its color and consistency. The ejaculated semen from the same block and treatment was pooled, then diluted into a 1:40 ratio and analyzed using the default profile for *Gallus gallus* species of the Computer Assisted Semen Analyzer (CASA). Three (3) slides per treatment for each block were subjected to CASA. Data was collected at 5 random fields of view on each slide. Total concentration, percentage for motile, progressive, slow, static, bent tail, coiled tail, distal mid-piece reflex (DMR), distal droplet, and proximal droplet sperm cells were recorded. Darag hens belonging to different age groups were used to assess fertility rate. Semen samples were collected from all roosters and were pooled according to block and treatment. The collected semen was diluted using the commercially available Ringer's solution at a 1:10 ratio and was inseminated into hens twice a week at the end of each month. The eggs were collected three days after insemination for one week and were artificially incubated for 10 days. On the 10th day of incubation, the fertility of the eggs was checked through manual candling.

The final weight of the roosters was subjected to analysis of covariance (ANCOVA) with initial weight as covariate. Analysis of variance (ANOVA) was performed on the data for fertility rate and average daily feed intake. For the frequency of semen color and consistency, the chi-square test of independence was used. Semen quality parameters were subjected to ANOVA following Randomized Complete Block Design (RCBD) at $P = 0.05$.

RESULTS AND DISCUSSION

Results showed that incorporating okara into the diet did not negatively impact the birds' body weight ($P=0.3050$; Table 2), similar to previous findings that supplementation of

okara had no detrimental effects on growth and feed intake of broiler (Motawe *et al.*, 2012; El-Nagmy *et al.*, 2004; Ibrahim, 2006). Additionally, Table 1 shows that although the crude fiber content of the diet with okara (3.99%) was twice as high as that of the commercial feed (1.86%), the average feed intake of the roosters was not significantly affected ($P = 0.1075$). This provides evidence that native chickens can utilize fibrous materials.

Table 2. Average ($\pm$ SD) production performance of Darag native roosters fed with and without okara.

TREATMENT	PARAMETER	
	Final Body Weight	Average Daily Feed Intake
Without Okara	1.80 $\pm$ 0.16	64.21 $\pm$ 2.08
With Okara	1.76 $\pm$ 0.27	61.94 $\pm$ 4.87
<i>p-value</i>	0.3050	0.1075

Table 3 presents a summary of the number of roosters that produced different semen consistencies after two months of feeding. Some of the roosters were not able to give semen samples, which is why the total number of roosters varied between treatments. The significant effect of okara supplementation on semen color and consistency ($P=0.0123$) in both treatments can be associated with semen concentration. Semen color is typically a good measure of ejaculate density. The color of the semen may vary according to the kind of bird used, but in general, creamy semen correlates to high sperm concentration and fertilizing capability (Khoso *et al.*, 2024).

Table 3. Summary of frequency (count) of semen consistency of Darag native roosters fed with and without okara.

TREATMENT	PARAMETERS			<i>p-value</i>
	Creamy	Milky	Watery	
Without Okara	17% (3)	67% (12)	16% (3)	0.0123
With Okara	50% (11)	50% (11)	0% (0)	

The frequency of roosters that produced creamy semen is higher (11) in those fed with okara than in the control (3) ($P=0.0123$). This is consistent with the result of the semen analysis, where the total concentration of semen from okara-fed roosters is significantly higher than semen produced by roosters fed the control diet ($P=0.0140$). Watery semen was also observed in some roosters from the control diet. Nutritional deficiencies and heat stress may cause differences in semen concentration (Leão *et al.*, 2021; Brugaletta *et al.*, 2022). However, additional antioxidants were likely provided through the supplementation of okara in the diet, and this may have contributed to the reduction of oxidative damage. Okara is rich in antioxidants, including flavonoids, isoflavones, and phenolic compounds (Pešić *et*

al., 2023). These antioxidants play a role in neutralizing free radicals, thereby minimizing oxidative stress within the reproductive system. By safeguarding sperm cells from harm, these compounds can help enhance sperm concentration and promote a denser and creamier semen consistency (Leão *et al.*, 2021).

The results presented in Tables 4 and 5 show that okara supplementation can significantly improve semen volume ($P=0.0048$) and total concentration ($P=0.0140$) after two months of feeding. The results are consistent with a previous report where immature roosters (10 weeks of age) fed with soybean isoflavones for 9 weeks increased the diameter of seminiferous tubules and germ cell layers, increasing the relative weight of the testes (Heng *et al.*, 2017). The antioxidant properties of soybean isoflavones likely contributed to the increase in sperm concentration by improving spermatogenesis, sperm motility, and membrane integrity. Similarly, the enhanced semen volume and sperm concentration of Lohmann Brown breeder roosters fed with a Vitamin E supplemented diet had higher semen volume and sperm cell concentration (Danikowski *et al.*, 2002). Because the sperm membrane of avian sperm cells has a high long-chain polyunsaturated fatty acid content, it is prone to lipid peroxidation. This can affect sperm membrane function and sperm motility, thereby lowering the reproductive potential of older roosters. Such problems on the roosters' reproductive system, semen characteristics, and fertility can be mitigated by nutritional factors (Gonçalves *et al.*, 2015). Since preserving the quality of semen depends critically on the antioxidant defense, the production of these reactive oxygen species is offset by enhancing the animal's antioxidant mechanism through the addition of antioxidant-rich ingredients in the diet such as okara (Surai *et al.*, 2001).

Table 4. Summary of monthly semen analysis of *Darag* native roosters fed with and without okara.

PARAMETERS	MONTH 1			MONTH 2		
	Without Okara	With Okara	<i>p- value</i>	Without Okara	With Okara	<i>p-value</i>
Volume	0.08	0.09	0.4134	0.10	0.12	0.0048
Total Concentration	68.42	74.83	0.1483	66.48	78.55	0.0140
Static	36.47	39.00	0.5950	38.47	35.79	0.4445
Progressive	16.48	14.79	0.3144	11.55	10.83	0.6824
Motile	63.51	61.00	0.5950	61.53	64.21	0.4457
Slow	34.95	36.30	0.6729	41.75	46.40	0.0792
Bent Tail	0.19	0.10	0.3196	1.12	0.88	0.5017
Coiled Tail	0.01	0.01	1.0000	0.07	0.02	0.0816
DMR	0.45	0.40	0.5355	0.55	0.70	0.2141
Distal Droplet	0.02	0.04	0.1850	0.21	0.28	0.3508
Proximal Droplet	0	0	na	0	0	na

Table 5. Summary of the overall semen quality of Darag native roosters fed with and without Okara.

PARAMETERS	TREATMENTS		<i>p- value</i>
	Without Okara	With Okara	
Volume (mL)	0.09	0.11	0.0512
Total Concentration (M/mL)	67.45	76.69	0.0048
Static (%)	37.48	37.39	0.9766
Progressive (%)	14.01	12.81	0.4248
Motile (%)	62.52	62.60	0.9775
Slow (%)	38.35	41.35	0.2281
Bent Tail (%)	0.66	0.49	0.4925
Coiled Tail (%)	0.04	0.02	0.1906
DMR (%)	0.50	0.55	0.5395
Distal Droplet (%)	0.12	0.16	0.4332
Proximal Droplet (%)	0	0	na

Okara contains 0.02 to 0.12 g/100 g solids of isoflavones, which represents ~12-30% of the soybean isoflavones retained in okara throughout the production of soy milk (Jackson *et al.*, 2002). Generally, the study has shown that okara supplementation at 20% of the diet does not negatively impact the semen quality of Darag Philippine native roosters. This may be due to the higher concentration of flavonoids in their diet, which helped mitigate the detrimental effects of reactive oxygen species (ROS) on semen quality. The incorporation of antioxidants in a well-balanced chicken ration has been widely documented to promote reproductive activities in avian species, including sperm cell concentration, motility, semen volume, viability, and integrity (Bréque *et al.*, 2003; Cerolini *et al.*, 2006; Partyka *et al.*, 2012). Further, as fed basis, the crude protein (CP) content of okara is 13.52% (data not shown). Bean curd, okara, and soybean milk all have a similar ratio of essential amino acids to total amino acids (Waliszewski *et al.* 2002; Rahman *et al.*, 2021). Positive impact of amino acid supplementation, particularly L-Arginine, on sperm concentration, motility, and volume, and reduction in sperm abnormalities were reported (Youssef *et al.*, 2015). The isoflavones and protein content of okara likely contributed to the favorable sperm quality seen in the study.

Results in Table 6 showed that there was no significant difference between the number of fertile eggs produced using semen of roosters from the two treatments ($P=0.1664$). This is parallel with the results on sperm motility and morphology (Tables 4 and 5), indicating that the fertilizing capacity of the sperm is not different between treatments. Progressive motility is one of the main parameters of spermatozoa contributing highly to a successful fertilization (Nguyen, 2019). Additionally, the characteristics of the sperm storage tubules (SSTs) in hens may have impacted their ability to store sperm in the oviduct. A sperm dose of 150×10^6 is adequate for fertilization and inseminating three times a week is the optimal frequency for maintaining a high sperm density in the SSTs of older Thai native hens (Kheawkanha *et al.* 2024). Insemination of hens in the present study was done only once a week.

As presented in the economic analysis in Table 7, the diet with dried okara reduced feed costs by approximately 10%. This is assuming that fresh okara (with 75% moisture content) is sold at P5.00 per kilogram.

Table 6. Fertility rate of eggs collected from hens inseminated with semen of *Darag* rooster fed with and without okara.

TREATMENT	FERTILITY
Without Okara	76.60 ± 42.80
With Okara	63.41 ± 48.77
p-value	0.1664

Table 7. Economic analysis of supplementing the *Darag* native rooster with okara in the diet for two months.

PARAMETERS	TREATMENT	
	Without Okara	With 20% dried Okara
Total Feed consumed (kg/bird)	3.92	3.78
Price of feeds (Peso per kg)	35.00	32.60*
Total feed cost (Peso/bird)	137.20	123.23
% change	-	-10.18%

In conclusion, the semen color and consistency were better, and the semen volume and sperm concentration were higher in *Darag* roosters supplemented with 20% okara in the diet. No significant differences were found in sperm motility, the percentage of abnormal sperm cells, and percent fertility of eggs. Similarly, the body weight and average feed intake of the roosters were unaffected by the inclusion of okara, indicating that this supplementation does not compromise overall health or growth. These findings demonstrated that 20% okara supplementation does not negatively affect growth and semen quality and may even lead to a reduction of feed cost. Incorporating okara into poultry diets at 20% offers an alternative and cost-effective feed resource for farmers.

Based on these findings, it is recommended to conduct further research on the impact of okara supplementation on egg fertility and hatchability, as these aspects were not fully addressed in this study. Future investigations could explore the effects of more frequent insemination to better understand the relationship between increased semen volume and consistency and fertility outcomes. Additionally, studying the impact of okara supplementation on semen quality in other native chicken breeds could provide further insights. A comparative study between synthetic antioxidants, such as Vitamin E, and okara supplementation may also be valuable for optimizing poultry breeding practices.

ACKNOWLEDGMENT

The authors would like to thank the Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD) of the Department of Science and Technology (DOST) for the financial support through the project “*Genome-wide association study for growth and egg production traits of Darag native chicken*”.

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