Theriogenology 89 (2017) 178–182

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Research article

Effects of guanidinoacetic acid diet supplementation on semen quality and fertility of broiler breeder roosters

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ARTICLE INFO

Article history:
Received 13 August 2016
Received in revised form
13 November 2016
Accepted 13 November 2016
Available online 16 November 2016

Keywords:
Aging
Fertility
Artificial insemination
Chicken
Sperm

ABSTRACT

Decreased semen quality and fertility rate is a common feature in broiler breeder roosters. This decrease is associated with dysfunction of Sertoli cells and defective spermatogenesis. Guanidinoacetic acid (GAA), as a precursor of creatine, plays an important role in the proper functioning of Sertoli cells and energy metabolism in sperm. Twenty, 29-wk-old broiler breeder roosters (Ross 308) were randomly allotted to 4 treatment groups and fed diets supplemented with different levels of GAA, including 0 (GAA-0), 600 (GAA-600), 1200 (GAA-1200), and 1800 (GAA-1800) mg GAA/kg of diet for 26 successive weeks. During a 24-wk period, the seminal characteristics were weekly evaluated. At the end of experiment, sperm penetration and fertility rates were determined, using 68 artificially inseminated age-matched broiler breeder hens of the same strain (for 2 weeks). Semen concentration ($P = 0.003$), total sperm number ($P = 0.005$) and sperm forward motility ($P = 0.01$) were increased by GAA-1200 group. Also, sperm plasma membrane functionality was marginally affected ($P = 0.06$) in roosters received all levels of GAA. Sperm abnormality and plasma membrane integrity were not affected by dietary GAA. The highest number of sperm penetration holes was recorded for the GAA-1200 group ($P = 0.08$). Interestingly, fertility rate was increased by the feeding of all levels of GAA ($P = 0.01$). In conclusion, dietary GAA was associated with improvement in most of the rooster's seminal characteristics and fertility rate, suggesting a potential for using GAA to attenuate the age-related sub-fertility in commercial broiler breeder roosters.

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1. Introduction

Collected data from numerous farms indicated a decrease in fertility of broiler breeder flocks from 45 weeks of age onwards [1]. It has been well defined that sub-fertility is most likely a reflection of problems with rooster rather than hen [2]. Maximum fertility of rooster is occurred at 32 weeks of age and declines at about 45 weeks of age [3]. It has been distinguished that decreased availability of ATP result in decreased sperm motility [4]. However, it seems that further researches are required to introduce efficient available nutritional energy sources for improving reproductive performance in male broiler breeders.

Guanidinoacetic acid is synthesized from glycine and arginine by l-arginine:glycine amidinotransferase (AGAT) in kidney and liver of avians [5]. Subsequently, guanidinoacetate N-methyltransferase (GAMT) catalyzes the formation of creatine through methyl group transfer from S-adenosylmethionine to GAA [6]. Creatine, phosphocreatine, and creatine kinase reaction are known components of the high-energy phosphate metabolism of cells and tissues with high or rapidly changing energy demand. In a study, the role of creatine synthesis in male reproductive tract has been shown [7]. Adenosine triphosphate (ATP) is fuel for sperm motility whose content in semen and sperm is used to predict male fertility [8]. Mitochondria are responsible for ATP production in sperm and the energy is delivered to the sperm tail *via* phosphorylated creatine [9]. Creatine phosphate can transform adenosine diphosphate (ADP) to ATP, and consequently supports sperm motility [8]. However, low ATP content or ATP/ADP ratios can be causal to decreased sperm function [8]. In addition to energy-related effects of creatine, it also has anti-apoptotic and anti-oxidation effects on the cells [10,11]. Therefore, the aim of this study was to evaluate the effects of dietary GAA on semen quality and fertility rate of broiler breeder roosters.

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http://dx.doi.org/10.1016/j.theriogenology.2016.11.012
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2. Materials and methods

The experimental design and procedures used in the current study were approved by the Animal Welfare Committee of the Department of Animal Science, University of Tehran.

2.1. Birds and dietary treatments

A total of 20 Ross 308 breeder roosters (27-wk old) were selected from a commercial flock, housed in single cages under a controlled environment, and reared under at a 14L:10D photoperiod and 21 °C ambient temperature. The birds were habituated to abdominal massage 2 weeks. Thereafter, they were randomly allotted to 4 treatment groups (n = 5 birds/group) and fed a diet supplemented with graduated levels of GAA [0 (GAA-0), 600 (GAA-600), 1200 (GAA-1200) and 1800 (GAA-1800) mg GAA/kg of diet] for 26 weeks (29–54 weeks of age). Semen characteristics were weekly evaluated for 24 weeks (29–52 weeks of age). The semen samples for last 2 weeks (53–54 weeks of age) were used for artificial insemination. The diets were formulated according to recommendations of Ross 308 male parent stock nutrient specifications (Table 1). Guanidinoacetic acid was added to corresponding experimental diets as a replacement of sand. A total of 68 Ross 308 breeder hens aged 54 weeks (17 hens/group) were used to testify the sperm penetration and fertility rates. After 3 weeks of sexual resting, the hens were inseminated. The hens were fed a diet with 2750 kcal ME/kg, 13.8% crude protein, 3.2% calcium, and 0.32% available phosphorus and were housed in floor pens bedded with wood shavings.

Table 1
Ingredients and composition of experimental diets supplemented with 0 (GAA-0), 600 (GAA-600), 1200 (GAA-1200) and 1800 (GAA-1800) mg guanidinoacetic acid (GAA)/kg of diet.

Ingredient (%)	Experimental diet[a]			
	GAA-0	GAA-600	GAA-1200	GAA-1800
Corn	69.00	69.00	69.00	69.00
Soybean meal	8.5	8.5	8.5	8.5
Wheat bran	19.19	19.19	19.19	19.19
Dicalcium phosphate	1.14	1.14	1.14	1.14
CaCO3	0.80	0.80	0.80	0.80
Sodium chloride	0.32	0.32	0.32	0.32
Vitamin premix[b]	0.25	0.25	0.25	0.25
Trace-mineral premix[c]	0.25	0.25	0.25	0.25
DL-Met	0.11	0.11	0.11	0.11
Sand[d]	0.18	0.12	0.06	–
GAA	–	0.06	0.12	0.18
Total	100	100	100	100
Composition				
ME (kcal/kg)	2754	2754	2754	2754
CP (%)	11.99	11.99	11.99	11.99
Ca (%)	0.70	0.70	0.70	0.70
P (%)	0.35	0.35	0.35	0.35
L-Lys (%)	0.46	0.46	0.46	0.46
DL-Met (%)	0.30	0.30	0.30	0.30
L-Thr (%)	0.38	0.38	0.38	0.38

[a] The birds were fed by experimental diets containing 0 (GAA-0), 600 (GAA-600), 1200 (GAA-1200), or 1800 (GAA-1800) mg GAA/kg of diet for 26 successive weeks (29–54 week of age).

[b] Provides (per kg of diet): vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3500 IU; vitamin E (DL-α tocopheryl acetate), 100 IU; vitamin K, 5.0 mg; thiamin, 3.0 mg; riboflavin, 12 mg; D-pantothenic acid, 13 mg; niacin, 50 mg; pyridoxine, 6 mg; biotin, 0.66 mg; folic acid, 2 mg; vitamin B12, 0.03 mg.

[c] Provides (per kg of diet): copper ($CuSO_4 \cdot 5H_2O$), 10 mg; iodine (KI), 2 mg; iron ($FeSO_4 \cdot 7H_2O$), 50 mg; manganese ($MnSO_4 \cdot H_2O$), 120 mg; selenium (Na_2SeO_3), 0.3 mg, Zn (ZnO), 110 mg.

[d] 0.18% of the diet was considered as the neutral part (sand), of which a certain proportion was replaced by GAA for corresponding treatment.

2.2. Semen characteristics

2.2.1. Gross assessment

Semen samples were weekly collected and evaluated for 24 weeks (29–52 weeks of age). The ejaculates obtained from each rooster in each treatment were considered as a single sample. Seminal volume was measured in graduated collecting tubes. Diluted semen (1:200 in 0.9% NaCl) was used for subjectively evaluation of sperm forward motility using a phase contrast microscope (Labomed, Lx 400, USA; magnification: X 400). A portion of diluted semen was placed on a slide and covered with a coverslip on a warm stage (37 °C), and then 10 microscopic fields were observed to evaluate sperm forward motility by one person. Motility was expressed as a percentage of spermatozoa progressively exhibiting moderate to rapid movement [12]. Sperm viability and abnormal forms were evaluated (200 spermatozoa/slide) following eosin-nigrosin staining by light microscopy (Labomed, Lx 400, USA).

2.2.2. Plasma membrane functionality

Hypo-osmotic swelling (HOS) test was done to assay sperm plasma membrane functionality [13]. Briefly, mixed samples of semen (5 μL) and HOS solution (200 μL) were incubated (37 °C) for 30 min. A sample was prepared on a slide covered with a cover slip and the percentage of HOS positive spermatozoa was determined using phase contrast microscopy (Labomed, Lx 400, USA; magnification: X 1000).

2.3. Artificial insemination and fertility

Semen samples in each treatment group were pooled and diluted in a modified Beltsville extender [14]. The hens (n = 17/group) were inseminated (200×10^6 spermatozoa/hen) for 2 times at 1400 h on the last 2 d of wk 24 (52 weeks of age) and once at 1400 h on the last day of wk 25 (53 weeks of age). The eggs were collected over 2 weeks, starting from the second day after the first insemination through 7 d after the second insemination [12,15]. The fertility rate was visually assessed by observing the development of the blastoderm immediately after egg collection with no incubation period [16]. A total of 441 eggs (all groups) were evaluated for this test. The fertility rate was calculated as the number of eggs with clearly developed blastoderm/the total number of eggs × 100.

2.4. Sperm penetration (SP) assay

Collected eggs on days 2 and 3 after the 2nd insemination of wk 24 and insemination of wk 25 were used for SP assay [15]. Sperm penetration was determined by using the method described by Bramwell et al. [17]. Briefly, the eggs were broken and the yolk was separated from the albumen. The yolks were washed in a 1% (wt/vol) NaCl solution for at least 10 minutes to completely remove the chalaziferous layer and any remaining albumen residue. The yolk was then placed in a Petri dish with the germinal disc facing upward. The IPL was then transferred on a slide and several drops of neutral phosphate-buffered formalin (15%) were added on the IPL and immediately decanted. The IPL was stained with Schiff's reagent and air-dried. The holes were counted using light microscopy (Labomed, Lx 400, USA; magnification: × 40). The average number of sperm holes in one visual field (0.5 mm^2) was determined by counting the SP sites.

2.5. Statistical analysis

Repeated data were analyzed by the MIXED procedure of SAS 9.1

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