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Original Article

The Potential Benefits of Legumes Germ and Cereal Grains on Roosters' Reproductive Performance

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Abstract

Vit E is known as one of the most important antioxidant. It has been previously approved that cereal grains and leafy plants are considered as the main source for α-tocopherol (Vit E). One of the recommended therapies for male infertility would be the Vit E therapy. Following Vit E consumption the semen parameters such as sperm concentration, ejaculation volume, sperm progressive motility, and in vitro function (zone binding assay) have been significantly improved. Therefore, present study was designed to investigate the effects of oral administration of cereal grain and seeds on reproductive performance of local cocks. During a period of 6۳ weeks, 100 local (Iraqi breed) rooster chicks were randomly divided into the five groups (n=20). Animals in group 1 served as control group and had not received any supplementations in their diet. The animals in the Groups 2-5 received diets which were fortified with 100, 200, 300, and 400 g of cereal grain and legume seeds pure germs. The results of the current study showed that the total number of spermatozoa and percentages of abnormal sperm were decreased by adding more amount of germ of cereal grain and seeds *(P<0.01)*. Increased germ of cereal grain and seeds was not associated with pH volume, colour, consistency and motility of the sperm compared to corresponding rates in control group. Phospholipids content and thiobarbituric acid reactive substance of semen sample as well as density of ejaculate (sperm/µl) were decreased by adding increasing germ of cereal grain and seeds in diet of rosters. Weight of testis decreased by increasing levels of cereal grains and legume seeds germ in the diets (P<0.05).

Keywords: Germ of cereal grain, Roster, phospholipids, Parameters semen

1. Introduction

Vit E is known as one of the most important antioxidant. It has been previously approved that cereal grains and leafy plants are considered as the main source for α-tocopherol (Vit E). In fact their Vit E contents significantly higher than the animals' sources (1). The living body antioxidant contents have great influence on the scavenging the free radicals and reactive oxygen species (ROS). One of the adverse effects of ROS and oxidative elements in the animals' body would be the reduction in their reproductive performance. In the previously published study conducted by Bansal and Bilaspuri (2) it was revealed that human sub-fertility/infertility is associated with the ROS content which may affect acrosome reaction, and the ability of sperm–oocyte fusion (3). Vit E supplementation lead to improvement in the diets antioxidant capacity, therefore, sperm quality and concentration improved significantly (4).

Previous studies showed that semen quality, spermatozoa concentration, viability and progressive motility, significantly improved due to the Vit E supplementation in poultries (5). Vitamin E is known as lipid-soluble vitamin mainly resides within the cell membrane and maintains structural and functional characteristics of living cells' membrane via antioxidant activities (5). In a study conducted by Therond, Auger (6) the results showed that the total amount of α-tocopherol in seminal plasma is significantly related or the percentage of motile spermatozoa.

One of the recommended therapies for male infertility would be the Vit E therapy. Following Vit E consumption the semen parameters such as sperm concentration, ejaculation volume, sperm progressive motility, and in vitro function (zone binding assay) have been significantly improved (7).

Therefore, present study was designed to investigate the effects of oral administration of cereal grain and seeds on reproductive performance of local cocks.

2. Materials and Methods

2.1. Animals, Study Design

The cereal grains and Clover, Barley, White Bean, Red Bean, Alfalfa, Pea, Wheat, and Lentil (legume seeds) were laid on moisturized cotton cloth and regularly hydrated till the germs grew up to 1-1.5 cm. Thereafter, using micro scissor the germs were dissected from the seeds and air dried at room temperature. After that the germs were grinded to produce fine powder.

It was important to measure Vit E contents in different periods of 3rd to 7th days before leaf rising. Cereal grain and legume seeds germs (6 days' old), with maximum content of Vit E, were supplemented into the diets of local (Iraqi) 2 days old rooster chicks accordingly up to 63 weeks of age. Clover seed and wheat grains were the richest source of vitamin E accordingly (370 and 329 mg/1000g) at the 7th day of measurements with increasing trends from the 3rd to the 7th days of measurements (P=0.001 for both trends).

* Trends, linearity (chi-squared)

100 local (Iraqi breed) rooster chicks were randomly divided into the five groups (n=20). Animals in group 1 served as control group and had not received any supplementations in their diet. The animals in the Groups 2-5 received diets which were fortified with 100, 200, 300, and 400 g of cereal grain and legume seeds pure germs (Table 1). At the end of week 43, the biochemical, morphological, and physiological parameters of semen samples was investigated. At the end of week 63 the last day of experiment the roosters' testicles were dissected and weighted.

2.2. Semen Collection and Examination of Ejaculates

After completion of sexual maturation at week 43, the semen samples were collected by abdominal massage method described previously by Leão, de Souza (8) twice a week (3 rosters were selected randomly each group) throughout the 63 weeks of the experiment. Using an equal volume of Ringer solution the collected semen samples were diluted and frozen at -79 ºC.

Following semen collection, semen parameters such as ejaculate volume by using a graduated tube; colour, consistency and contaminations (blood or excrement) by visual examination; pH value by pH paperwere measured for each dietary group were measured. Sperm motility was determined on a hotplate at 37˚C at a magnification of 400× with an optical microscope. The sperm total motility was determined according to the method previously described by Rodríguez-Gil and Rigau (9). In brief 100 (cells) spermatozoa even with a slight movement counts on the two squares of hemocytometer slide and the rate is expressed as percentages of sperm total motility.

The assessment of the percentage of some abnormalities in spermatozoa was determined by evaluating 400 cells of the semen samples after mixing an aliquot of 5µl of the ejaculate with 300µl of formol citrate.

During the experimental period the biochemical examinations were carried out once a month. The level of α-tocopherol in the whole semen samples was determined as follows: the ejaculate was saponified and the solvent extracted by a modified method previously described by Danikowski, Sallmann (10). For measurement of Vit E a method previously described by Freitas and Moretti (1) using a U.V. spectrophotometer was used with a minor modification.

Thiobarbituric acid-reactive substances (TBARS) were measured according to a method previously described by Battisti, Maders (11). The fatty acid esters were measured by gas chromatography method.

2.3. Statistical Analysis

Temporal relations were excluded by regression analysis as far as the test data of the pooled semen samples of each group were concerned. The Statistical Analyses System (SAS, 2001) was used for all analyses. All data were tested for Gaussian distribution and submitted to one-way ANOVA.

Table 1. Composition of experimental diets

	Control
Corn (8.5 % protein)	61.5kg
Soya bean meal (48 % protein)	30.40kg
Calcium carbonate (38 % calcium)	1.35kg
De-calcium phosphate (20 % phosphate)	1.5kg
Animal fat	3.5kg
Salt	0.4g
Premix [1]	1.0g
Dl-methionine	0.1g
Lysine- HCL	0.25g
Germ of cereal grain and seeds (g) [2]	0

[1] Each kg premix contains: Vitamin A 4000000 IU, Vitamin D3 100000IU,
Vitamin E 2000 mg, Vitamin K3 750 mg, Vitamin B1 600 mg,
Vitamin B2 2000 mg, Vitamin B6 600 mg, Vitamin B 12 10000 mcg,
Vitamin C 2000 mg, L- Lysine 10000 mg, DL- Methionine 25000 mg,
Copper Carbonate 2500 mg, Cobalt Carbonate 550 mg,
Ferrous Carbonate 4000 mg, Manhanese Carbonate 50000 mg,
Zinc Carbonate 5000 mg, potassium Iodide 150 mg,
Choline Chloridi 110000 mg, Nicotinic Acid 9000 mg,
Folic Acid 225 mg, Calcium pantothenate 3500 mg, B.H.T 125 mg.
[2] for enrichment groups, germ of cereal grains and legume seeds were added to the basal (control) diet at the levels of 100, 200, 300 and 400 grams

3. Results and Discussion

Roosters fertility in a breeder poultry flock has attract greater attention compared with female's fertility. This could be explained as the rooster roles for fertilizing 8-10 hens for production of fertilized eggs. The recorded data showed that the pooled semen samples in all the groups had milky color with creamy consistency. Results revealed that ejaculate volume, pH, and total sperm motility were not significantly differed between the treated groups.

The recorded data showed that the lowest mean semen volume was found in the control group. The results of pH measurements showed that the mean pH values for all groups ranged from 7.0 to 7.4 (Table 2).

Table 2. Semen parameters of pooled semen samples (mean ± SD)

Treatments	Volume (ml)	Density (million/µl)	TS (billion/ml)	pH	TM (%)	MDS (%)
0 (control)	4.1 ± 0.8^{b}	3.1 ± 0.9^{a}	13.3 ± 3.5^{b}	7.4 ± 0.4^{a}	70.1 ± 6.2^{b}	9.0 ± 2.1^{c}
100	5.2 ± 0.9^{a}	3.0 ± 0.8^{a}	16.7 ± 3.6^{a}	7.1 ± 0.1^{b}	79.2 ± 2.2^{a}	7.0 ± 1.4^{c}
200	4.3 ± 0.8^{b}	2.9 ± 0.4^{b}	11.8 ± 2.9^{c}	7.0 ± 0.2^{a}	$64.4 \pm 2.1^{a, b}$	12.4 ± 3.8^{a}
300	5.2 ± 0.9^{a}	2.2 ± 0.5^{b}	14.3 ± 4.0^{b}	7.0 ± 0.3^{b}	$63.1 \pm 1.3^{a, b}$	$11.0 \pm 1.3^{a, b}$
400	4.3 ± 0.6^{b}	2.0 ± 0.0^{b}	10.9 ± 2.5^{c}	7.3 ± 0.2^{a}	69.2 ± 3.3^{b}	10.5 ± 3.2^{b}

[a-c] Values with no common superscript within columns differ significantly ($P<0.05$)
TS: Total Spermatoza
TM: Total Motion
MDS: Morphologically Deformed Spermatozoa
SD: Standard deviation

The recorded data in case of total motility showed that supplementation of 100 g of cereal grain and legume seeds pure germs to the normal diet formulation (Group 2) lead to the significant increase in the percentage of total motility (79.2 %) ($P<0.05$) compared with control (70.1 %) and other treated group 3-5 (64.4, 63.1, 69.2 %) (Table 2). The highest sperm concentration was recorded in the control group compared with group 3-5 (Table 2). The recorded data showed that 100g of cereal grain and legume seeds pure germs to the normal diet formulation lead to the same sperm concentration compared with control group ($P<0.01$), and stand at the highest sperm concentration between treated groups (Table 2). In case of semen abnormalities the semen samples obtained from Groups 2 showed the lowest value of morphologically abnormal spermatozoa with the following defects: deformed head, broken or bent neck or tail or rolled up sperm). The recorded data showed that the percentages of abnormal spermatozoa in groups 3, 4 and 5 were higher than of the control group (Table 2). The negative association of the high vitamin E dosage with the total amount of spermatozoa is more important than density, because density varies with the changing the ejaculation volume. The recorded reduction in total amount of spermatozoa might be related to the decreased weight of the testes (12). As previously mentioned by Yakubu, Akanji (13) the concentration of spermatozoa is dependent upon testes weight. In contrast the results of a study conducted by Jenkins and Mitchell (14) reported no influence of vitamin E supplementation on testes in rats feeding with Vit E deficient diets. While the results of a study conducted by Aburto and Britton (15) in chickens with Vit E deficiency revealed the disrupted spermatogenesis by decreasing in glutamyl transpeptidase activity in the testicles and the reduction in the leydig cell count with degenerative changes in the germinal epithelium (16). Vit E supplementation leads to an elevation in the concentration of Vit E in the pooled ejaculates, therefore, the prooxidative effects must be considered (10). The mentioned metabolism procedure, PO-E, consequently leads to an elevation in the levels of free radicals which causing cell destruction. Therefore, the reduction in the weight of the testicles could be explained as the consequence of the alterations in the expected germinal epithelium. The results of the current study which showed that the levels of TBARS in pooled ejaculates reduced while the Vit E levels in the diets increased were in accordance with a previous study conducted by Danikowski, Sallmann (10). Increased morphological abnormalities in spermatozoa in groups 3-5 showed and confirmed the importance of diet composition in the male's reproductive performance. The spermatozoa morphological alterations occurred in three different steps as follows: 1) Primary abnormalities of spermatozoa revealed disrupted spermatogenesis, 2) the secondary

morphological abnormalities occur during the passage of spermatozoa through the epididymis, and 3) tertiary alterations occur during or after ejaculation.

The recorded data on the testicles weigh showed that the testes in group 5 had significantly lower weight compared with other groups. While the results revealed that there were no significant differences among testicles weight between other groups (Table 3).

The results of biochemical analysis of the semen samples showed that the concentration of Vit E was linearly linked to the amount of germ of cereal of grains and legume seeds supplementation as a substitute source of dietary vitamin E (Table 4).

Table 3. Body and testicles weight

Amount of germ (g)	Body weight (g)	Weight of testes(g)	Weight ratio of testes (g)
0 control	2410 ± 111	23.01 ± 2.20[a]	0.8043
100	2302 ± 171	19.20 ± 3.20[a]	0.7133
200	2011 ± 152	18.10 ± 0.42[a]	0.7952
300	2013 ± 162	19.70 ± 3.40[a]	0.8526
400	2214 ± 231	16.30 ± 1.70[b]	0.6201

Values with no common superscript within columns differ significantly (*P<0.05*)
SD: Standard deviation

Table 4. Biochemical analysis of semen samples

Amount of germ (g)	Vitamin E (µl/ml)	TBARS (nmol/ml)	Phospholipids (ng/ml)
0 (control)	0.41 ± 0.30[c]	3.11 ± 1.01[a]	2941.1 ± 461.0[a]
100	0.91 ± 0.21[c]	1.14 ± 0.98[a]	2421.3 ± 421.1[a]
200	2.45 ± 1.98[c]	1.93 ± 0.36[b]	2313.6 ± 321.0[b]
300	8.91 ± 4.66[b]	1.98 ± 0.32[b]	2402.0 ± 411.8[a]
400	15.12 ± 9.12[d]	1.62 ± 0.31[b]	1921.3 ± 210.1[b]

[a-c] Values with no common superscript within columns differ significantly (*p<0.05*)
SD: Standard deviation
Mean ± SD

The recorded data of the current study showed that the Thiobarbituric acid-reactive substances (TBARS) production in whole semen was not reduced significantly in the treated groups compared with the control group. In fact the level of TBARS was significantly higher in control group compared with the treated group. However, the levels of produced TBARS did not change when germ cereal grains supplementation increased from 200 g to 300 g *(P<0.05)*.

The recorded data, in the case of semen samples' phospholipids concentration, showed a significant reduction when the amount of administered germ of cereal grains and seeds increased in the normal diet formulation. In group 5 total phospholipids concentration in ejaculation was reduced significantly compared with other groups with the exception of this concentration recorded in group 3. The recorded data showed that the total concentration of semen ejaculate significantly decreased when the amount of germ cereal grains and seed raised from 0 to 400 g. Roosters' spermatozoa are identified by the high levels of long chain polyunsaturated fatty acids. It is well documented that long chain polyunsaturated fatty acids are highly sensitive to lipid peroxidation (17). Results of the previously published research showed that the

spermatozoa membrane consistency significantly increased consumption of Vit E deficient diets in roosters (18). Since phospholipids are generally placed in the spermatozoa membrane instead of seminal plasma, therefore, reduced spermatozoa count whit increased abnormalities in spermatozoa are anticipated (19).

In conclusion, the total content of Vit E in the ejaculation increased significantly in the rooster semen with high-level supplementation of Vit E in the standard rooster diets. Vit E supplementation in the roosters' diet has not any positive effects on ejaculation volume and sperm total progressive motility.

Authors' Contribution

Study concept and design: E. M. M. A. and R. A.
Acquisition of data: S. K. H. and A. J. K.
Analysis and interpretation of data: S. H. A. and A. H. A.
Drafting of the manuscript: M. J. A. and F. A. E.
Critical revision of the manuscript for important intellectual content: M. J. A., A. J. O. and Y. K. A.
Statistical analysis: H. T. A. and G. S. B.
Administrative, technical, and material support: M. J. A.

Ethics

All the procedures in this study was approved and inspected by the university ethics committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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