

## SOME FACTORS AFFECTING REPRODUCTIVE PERFORMANCE IN NORFA CHICKENS

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### SUMMARY

This work was carried out at the Farm of Poultry Production, Faculty of Agriculture, Minufiya University, Shibin El- Kom. Four trials were conducted as follows: **Trial (1)**: To study the effect of both body weight and months of the year on physical characteristics of semen. Thirty cocks were divided into equal two groups depending on body weight; (HW) and (LW). Semen was individually collected for 9 months, from November to July. **Trial (2)**: Semen was collected over 3 months from 60 cocks in 3 equal groups; once, twice and three times weekly to study the effect of frequency of semen collection on physical characteristics of semen. **Trial (3)**: To study the relationship between body weight (heavy VS light) and some seminal plasma constituents. Semen collected in pooling of each group. Blood samples were collected individually from (HW) and (LW) cocks to study the relationship of some blood constituents with semen production. **Trial (4)**: Eggs were collected from 480 hens to study the effect of frequency of semen collection, semen dose, types of diluters and dilution rate on fertility and hatchability.

The results revealed that, sperm motility (SM) and abnormal sperm (SA) were significantly higher ( $P \leq 0.05$ ) in (LW) than (HW) cocks. The opposite was true for the live sperm (SV). The ejaculate volume (EV), sperm concentration (SC) and semen pH were similar in both (HW) and (LW) cocks. All semen physical characteristics significantly ( $P \leq 0.01$ ) differed among months. As the frequency of ejaculation increased the (EV) significantly ( $P \leq 0.01$ ) decreased, (SC) increased and (SM) fluctuated.

The seminal total protein, globulin, A/G ratio, cholesterol, inorganic phosphorus, alkaline and acid phosphatases, GPT and GOT did not significantly differ between (HW) and (LW) cocks, meanwhile, that albumin, calcium and Ca/IP ratio were significant ( $P \leq 0.05$ ).

The blood total protein, albumin and globulin of high semen production cocks were significantly ( $P \leq 0.01$ ) higher than that of low semen production ones. However, the blood cholesterol, inorganic phosphorus, alkaline and acid phosphatase activities were not significant different between high and low semen production cocks.

There were no significant differences between insemination doses and frequency of semen collection used on fertility and hatchability. The effect of dilution ratio and type of diluents was not significantly on egg fertility and hatchability. Vaginal pH of high fertility hens was significantly ( $P \leq 0.05$ ) lower than that of low fertility ones.

**Keywords:** Semen characteristics, artificial insemination, fertility, hatchability

### INTRODUCTION

Economic consideration continues to stimulate interest in application of artificial insemination in poultry farms. However, artificial insemination in chickens has been mainly limited to genetic and physiological studies, maintaining control and the development of new lines. In contrast, this technique is already used by the majority of turkey breeders.

Artificial insemination is considered as a valuable technique in poultry industry and research. One of advantages of its application over natural mating is the efficient use of males. Poultry semen must be extended before its efficient use, which allows the increase in numbers of inseminations per ejaculate. This in turn, decreases the cost of AI directly by reducing the number of males needed (Benoff *et al.*, 1981 and Bootwalla and Froman, 1988). On the other hand, the modern housing system for poultry industry in individual cages, makes such a procedure to be the unique applicable one to produce fertile eggs. It is also considered as an important tool to solve some of the practical problems of crossbreeding in ducks (Kalamah, 1990). However, the ideal semen extender and dilution rate are not yet available.

Limited information are available concerning the physical and chemical characteristics of Norfa cocks semen. There are several factors could affect these traits. Therefore, the present study was carried

out in order to investigate the effect of body weight, frequency of semen collection and month of year changes on the characteristics of Norfa cocks semen. Also the effect of semen extender, dilution rate and insemination dose on fertility and hatchability in Norfa hens were studied. This work can give helpful informations about factors influencing semen production of Norfa cocks as a new breed and also may help in application of artificial insemination in poultry flocks raised in ARE.

## MATERIALS AND METHODS

This work was carried out at the Farm of Poultry Production, Faculty of Agriculture, Minufiya University, Shibin El-Kom. The study was run in four trails:

### Trial (1)

To study the effect of cock body weight and months of the year, on physical characteristics of semen. 30 Norfa cocks after sexual maturity were divided into two equal groups (i.e. heavy weighed 2037 g VS light weighed 1744 g). In this trial semen was individually collected from 30 cocks twice weekly over 9 months (from November to July).

### Trial (2)

To investigate the effect of frequency of semen collection on physical characteristics of semen. Semen was collected over 3 months from 60 cocks in 3 equal groups which were subjected to 3 system of semen collection frequency, (i.e. once, twice and three times weekly). The cocks were individually raised in wire cages under good ventilation and illumination. Semen was collected using the massage method and, the ejaculate volume to the nearest 0.01 ml. using 1.00 ml tuberculine syringe., mass motility according to Nagae *et al.* (1987), initial semen pH by comparative pH paper, percentage of live and abnormal sperms after staining with iosine and nigrosine and sperm concentration using Thomas - Zeis haemocytometer were measured.

### Trial (3)

Blood and semen samples were collected from 10 cocks of heavy weight (HW) (1920 g) and other 10 light cocks (LW) (1570g). Total plasma proteins, albumin, calcium, inorganic phosphorus, cholesterol, activity of plasma alkaline and acid phosphatases were determined in both the blood and semen, and activity of GOT and GPT in the semen only according to Gornall *et al.* (1949), Rodkey (1965), Krawczynski and Osinski (1962), Bratkowski (1975), Btaszczyzyn (1976), Kind and King (1954) and Reitman and Frankel (1957), respectively.

### Trials (4)

To study:

#### 1- Effect of semen dosage and frequency of semen collection on egg fertility and hatchability :-

Semen was collected in pooling from 90 adult Norfa cocks on the same day of insemination to inseminate hens. 310 Norfa hens were divided into three groups which were inseminated with semen collected with once, two and three / week. Every group contained semen dose (0.05, 0.10 and 0.15 ml.) of three subgroups.

#### 2 - Effect of diluters and dilution rate on egg fertility and hatchability:-

Semen was collected in pooling from 60 adult Norfa cocks on the same day of insemination to inseminate hens twice weekly. The effect of two selected diluents namely Na-glutamic-Na Citrate glucose (GCG) and Tris with two dilution ratios (1 : 3 and 1 : 5 semen:diluent) were tested. 170 hens were divided into 5 groups; 1 and 2 groups inseminated by GCG diluter with dilution ratio 1:3 and 1:5. The tris diluter (1:3 and 1:5) were used to inseminated groups 3 and 4. A control (group 5) of hens was also included, which was artificially inseminated with undiluted semen. All hens were managed alike in individually wire cages. Eggs produced by hens were used to estimate fertility and hatchability. Fertility was calculated as percentage of total eggs set in the incubation. Hatchability was calculated as percentage of hatched eggs to fertile eggs. pH of vaginal hens was measured using comparative pH paper over 3 months.

### Statistical analysis

Data obtained were statistically analyzed according to Snedecor and Cochran (1973) and Gill (1978). Duncan's multiple range test was used for the multiple comparisons of means. Values expressed in percentages (i.e. fertility and hatchability) were obtained by retransformation from Arcsin to original a scales prior statistical analysis.

## RESULTS AND DISCUSSION

### Semen physical characteristics

#### 1 - Effect of body weight

The sperm motility (SM) and abnormality (SA) of semen samples produced by the light weighed cocks (LW) were significantly greater ( $P \leq 0.05$ ) than those produced by heavy ones (HW), (Table 1). The reverse was true for percentage of live sperms (SV), meanwhile, the ejaculate volume (EV), sperm concentration (SC) and semen pH were not significantly affected by body weight of cocks. The total spermatogenic output, (SA) and (SV) per ejaculate were considerably greater in the semen samples produced by the (LW) cocks as compared to those produced by the (HW) ones.

El-Hammady *et al.* (1995) reported that (EV) and (SM) were quite similar in both (HW) and (LW) Dandarawi cocks. They added that (SC) and (SV) were higher and semen pH was lower in (HW) than that in (LW) Dandarawi cocks. Semen volume and pH showed an opposite trend in Fayoumi cocks. Moreover, Holcman *et al.* (1993) found that the (EV), (SM), (SC) and (SV) were higher in heavy cocks than light ones. An opposite trend was true for (SA) and semen pH. In this respect, Ramamurthy *et al.* (1989) reported that (EV) of semen, pH and (SA) showed significant positive correlation with body weights at 25 and 35 weeks old in White Cornish, meanwhile, (SC), (SM) and (SV) showed significant negative correlation. However, Kunev and Manolov (1988) found that body weight was correlated positively with either (SC) or (SM) and negatively with (SA) in New Hampshire cocks. The differences between these results may be due to the differences of breeds used.

**Table 1. Effect of body weight on semen physical characteristics (mean  $\pm$  S.E.)**

Traits	Body weight (B.W.)		Significance
	Light (1744 g)	Heavy (2037 g)	
Ejaculate volume (ml.)	0.23 $\pm$ 0.004	0.22 $\pm$ 0.004	N.S
Sperm concentration $\times 10^9$ /ml	2.57 $\pm$ 0.086	2.61 $\pm$ 0.091	N.S.
Total sperm/ejaculate $\times 10^9$	0.59	0.57	
Sperm motility	3.00 $\pm$ 0.051	2.84 $\pm$ 0.050	P<0.05
Semen pH	7.84 $\pm$ 0.015	7.84 $\pm$ 0.015	N.S.
Abnormal sperm (%)	22.06 $\pm$ 0.605	20.56 $\pm$ 0.508	P<0.05
Total abnormal sperm/ejaculate $\times 10^9$	0.13	0.11	
Live sperm (%)	76.95 $\pm$ 0.887	78.87 $\pm$ 0.772	P<0.05
Total live sperm /ejaculate $\times 10^9$	0.45	0.44	

N.S.: Not significant

#### 2- Effect of month of the year on semen production

All semen physical characteristics were affected significantly ( $P \leq 0.01$ ) by month of the year from November to July (Table 2). The (EV) decreased from November to April. The (EV) in November and December was greater than that in other months. The reverse trend was true for semen collected during March and April. The (SC), total sperms per ejaculate and (SM) declined gradually nearly from December until July. In April and May, pH of semen was significantly higher than that in other of months, while, in November and December lower values of semen pH were detected. The (SA) was lower value from December to March, while it was higher value from May to July. The (SV) was greater from December to February, but it was lower from May to June. The total (SA) and total (SV) decreased gradually from December to July. The interaction between body weight and months was significant ( $P \leq 0.01$ ) for (EV) and ( $P \leq 0.05$ ) for either (SC) or (SM).

Kamar *et al.* (1979) found that (EV) in Fayoumi cocks showed a decline from a highest value during February to the lowest value during September, meanwhile, the Rhode Island Red, the highest was obtained during July and the lowest value in February and March. They also reported that the (SC), total sperms per ejaculate, (SA) and (SV) in Fayoumi cocks attained it's maximum value in March followed by a gradually decline till September. Moreover, El-Sharkawy (1981) and Attia *et al.* (1984) reported that there were significant effects season on (EV), (SC), (SM), semen pH and (SA), while, there were no significant differences in (SV) due to season for Fayoumi cocks. Saied and Al-Soudi (1975) found that the highest (EV) produced from New Hampshire cocks was in autumn and

spring, while the lowest value was noticed during winter. They also reported that the (SV) in spring was higher than that in summer. Hafez (1968) showed that the avian sperms were motile over a wide range of temperatures from a low of 2 °C to a high of 43 °C showing that the movement increased with the temperature increasing. The present study suggest that increase semen pH in April and May may be due to decrease in both (EV) and (SC) that causes a corresponding decrease in lactic acid production in semen, that results the increase in semen pH . The decrease of ( EV ), ( SC ), ( SM ) and ( SV ) in summer months may be due to high temperature ( Kalamah, 1990 ).

### 3- Effect of frequency of semen collection

Table 3 shows that, the ( EV ) declined significantly ( $P \leq 0.01$ ) when the frequency of ejaculation increased. An opposite trend was true for ( SC ). The ( SM ) was significantly ( $P \leq 0.01$ ) greater for semen collection once weekly than that for both twice and three times weekly. This may be due to larger ( EV ) for semen collection once weekly than others. However, semen pH, ( SA ) and ( SV ) were not significantly affected by semen collection frequency. The total sperm, abnormal and live sperms per ejaculate decreased with increasing times of semen collection.

Results of ( EV ) and ( SC ) of chicken were reported with McDaniel and Sexton (1977) agree with present study. McDaniel and Sexton (1977) found that ( EV ) of semen collected 3 times weekly was significantly greater than that of semen collected 5 times weekly in both White Leghorn and broiler cocks. They also indicated the sperm cells per ejaculate was significantly greater of broiler breeder type males ejaculated three times weekly than those ejaculated once or twice daily. They added that Leghorn males that ejaculated three times weekly produced significantly greater sperm cells per ejaculate than that of five times weekly, while once weekly had (SC) equal to three times weekly and had significantly more than five times weekly.

In turkey, as the frequency of ejaculation increased, the (EV)), (SC) and (SV) decreased (Buckland *et al.*, 1980 and Ansah *et al.*, 1984). In ducks, Kalamah (1990) reported that the (SM) , (SV), (SA) for semen collected once weekly were higher than that for twice weekly, while pH of semen did not affect the semen frequency.

### Effect of body weight on seminal plasma constituents

The total proteins, globulins and A/G ratio in seminal plasma was not significant between (HW) and (LW) cocks, (Table 4). However, the albumin was significantly ( $P \leq 0.05$ ) higher in ( HW ) than in (LW) cocks. The results of Blesbois and Caffin (1992) indicated that seminal plasma albumin may be one of the motility stimulating factors in seminal plasma.

The seminal plasma cholesterol of (LW) was insignificantly greater than that of (HW) cocks. However, Resseque and Hughes (1984) reported that the molar ratio of cholesterol to phospholipid and the mole percent of cholesterol remained essentially constant for the various time intervals. Differences that were detected had no apparent pattern and were probably indicative of procedural or chance variation rather than membrane changes. The ratios for spermatozoa membrane cholesterol to spermatozoa membrane phospholipid obtained were 0.22 to 0.38.

The average of seminal plasma calcium of (HW) was significantly ( $P \leq 0.05$ ) lower than that of (LW) cocks. They were  $7.25 \pm 0.30$  and  $8.92 \pm 0.09$  mg /100 ml., respectively (Table 4). In this respect, Hammond *et al.* (1964) found that the seminal plasma calcium from White Plymouth Rock males was  $7.03 \pm 0.40$  mg / 100 ml. The seminal plasma inorganic phosphorus was nearly the same in heavy and light cocks ( $5.17 \pm 0.11$  and  $5.25 \pm 0.16$  mg /100 ml., respectively). El-Sharkawy (1981) and Attia *et al.* (1984) reported that total mean of seminal plasma inorganic phosphorus ranged from 6.95 to 7.50 mg /100 ml of Fayoumi cocks. The seminal plasma Ca/IP ratio was significantly greater ( $P \leq 0.05$ ) in (LW) than (HW) cocks.

The seminal plasma alkaline and acid phosphatases , GPT and GOT activities in (HW) and ( LW ) cocks were nearly similar. From Table 4 it is clear that acid phosphatase was higher than alkaline phosphatase in both heavy and light cocks. These results agree with that reported by Hammond *et al.* (1964). Bilgili *et al.* (1985) stated that in seminal plasma of White Leghorn, enzyme of (GOT) activity showed a significant positive relationship with increasing concentration of dead spermatozoa.

### Effect of some blood plasma constituents on semen production

The total plasma protein, albumin and globulin of high semen production cocks were significantly higher than that of low semen production ones .While , A/G ratio was similar (Table 5). El-Zarkouny (1994) showed that when semen was collected from New Zealand White rabbits male, the ejaculate volume was  $0.64 \pm 0.03$  ml semen, serum total protein 7.51 g / 100 ml , serum albumin 3.52 g / 100 ml and serum globulin 4.05 g / 100 ml.

Table 2. Monthly changes in semen physical characteristics of Norfa cocks (Mean  $\pm$  S. E.)

Months	Semen Physical Characteristics								
	Ejaculate Volume (ml)	Sperm Concentration ( $\times 10^9$ /ml)	Total sperm/ejaculate $\times 10^9$	Motility	Semen pH	Abnormal Sperm (%)	Total abnormal sperm/ejaculate $\times 10^9$	Live Sperm (%)	Total live sperm/ejaculate $\times 10^9$
November	0.28 $\pm$ 0.022 <sup>a</sup>	4.14 $\pm$ 0.112 <sup>a</sup>	1.10	4.09 $\pm$ 0.337 <sup>b</sup>	7.60 $\pm$ 0.070 <sup>d</sup>	17.09 $\pm$ 0.820 <sup>c</sup>	0.19	86.12 $\pm$ 1.312 <sup>a</sup>	0.95
December	0.26 $\pm$ 0.007 <sup>ab</sup>	3.24 $\pm$ 0.155 <sup>b</sup>	0.75	4.62 $\pm$ 0.092 <sup>a</sup>	7.71 $\pm$ 0.024 <sup>c</sup>	16.98 $\pm$ 1.145 <sup>c</sup>	0.13	84.93 $\pm$ 1.134 <sup>a</sup>	0.64
January	0.23 $\pm$ 0.008 <sup>c</sup>	3.23 $\pm$ 0.171 <sup>b</sup>	0.71	4.54 $\pm$ 0.130 <sup>c</sup>	7.79 $\pm$ 0.030 <sup>c</sup>	16.96 $\pm$ 0.695 <sup>c</sup>	0.12	86.13 $\pm$ 1.396 <sup>a</sup>	0.61
February	0.22 $\pm$ 0.011 <sup>cd</sup>	3.04 $\pm$ 0.177 <sup>c</sup>	0.58	3.50 $\pm$ 0.177 <sup>cd</sup>	7.86 $\pm$ 0.043 <sup>bc</sup>	17.40 $\pm$ 0.721 <sup>bc</sup>	0.10	81.20 $\pm$ 1.392 <sup>b</sup>	0.47
March	0.19 $\pm$ 0.008 <sup>f</sup>	2.10 $\pm$ 0.100 <sup>d</sup>	0.42	3.54 $\pm$ 0.157 <sup>c</sup>	7.83 $\pm$ 0.033 <sup>bc</sup>	21.67 $\pm$ 0.267 <sup>b</sup>	0.09	76.15 $\pm$ 1.918 <sup>c</sup>	0.32
April	0.20 $\pm$ 0.007 <sup>ef</sup>	1.84 $\pm$ 0.085 <sup>ef</sup>	0.39	2.09 $\pm$ 0.093 <sup>f</sup>	7.96 $\pm$ 0.027 <sup>a</sup>	24.68 $\pm$ 0.574 <sup>a</sup>	0.10	72.13 $\pm$ 0.983 <sup>d</sup>	0.28
May	0.21 $\pm$ 0.006 <sup>de</sup>	1.77 $\pm$ 0.072 <sup>f</sup>	0.42	1.74 $\pm$ 0.095 <sup>f</sup>	7.95 $\pm$ 0.022 <sup>a</sup>	26.56 $\pm$ 0.661 <sup>a</sup>	0.11	71.08 $\pm$ 1.067 <sup>d</sup>	0.30
June	0.24 $\pm$ 0.006 <sup>bc</sup>	1.95 $\pm$ 0.151 <sup>de</sup>	0.41	1.47 $\pm$ 0.080 <sup>h</sup>	7.88 $\pm$ 0.025 <sup>ab</sup>	25.20 $\pm$ 0.829 <sup>a</sup>	0.10	73.13 $\pm$ 1.437 <sup>d</sup>	0.30
July	0.21 $\pm$ 0.009 <sup>de</sup>	2.66 $\pm$ 0.062	0.41	1.70 $\pm$ 0.149 <sup>g</sup>	7.85 $\pm$ 0.033 <sup>bc</sup>	21.31 $\pm$ 0.397	0.10	77.92 $\pm$ 0.589	
Overall average	0.23 $\pm$ 0.003			3.03 $\pm$ 0.036	7.82 $\pm$ 0.010	21.31 $\pm$ 0.397		77.92 $\pm$ 0.589	
Significant	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	

Means in the same column followed by different letters are significantly different at 5% level as shown by Duncan's test.

Table 3. Effect of semen collection frequency on semen physical characteristics (Mean  $\pm$  S.E.)

Semen characteristics	Times of frequency			Significance
	1/weekly	2/weekly	3/weekly	
Ejaculate volume (ml)	0.28 $\pm$ 0.004 <sup>a</sup>	0.24 $\pm$ 0.004 <sup>b</sup>	0.21 $\pm$ 0.003 <sup>c</sup>	P < 0.01
Sperm concentration $\times 10^9$ /ml	3.39 $\pm$ 0.072 <sup>b</sup>	3.47 $\pm$ 0.085 <sup>b</sup>	3.80 $\pm$ 0.083 <sup>a</sup>	P < 0.01
Total sperm/ejaculate $\times 10^9$	0.95	0.83	0.80	
Sperm motility	4.42 $\pm$ 0.045 <sup>a</sup>	4.05 $\pm$ 0.045 <sup>c</sup>	4.24 $\pm$ 0.036 <sup>b</sup>	P < 0.01
Semen pH	7.75 $\pm$ 0.016	7.75 $\pm$ 0.015	7.73 $\pm$ 0.012	N. S.
Abnormal sperm (%)	16.91 $\pm$ 0.349	17.05 $\pm$ 0.517	16.74 $\pm$ 0.382	N. S.
Total abnormal sperm/ejaculate $\times 10^9$	0.16	0.14	0.13	
Live sperm (%)	86.93 $\pm$ 0.732	84.61 $\pm$ 0.664	85.70 $\pm$ 0.672	N. S.
Total live sperm/ejaculate $\times 10^9$	0.83	0.70	0.69	

Means in the same row followed by different letters are significantly different at 5% level as shown by Duncan's test.

The plasma cholesterol of high and low semen production cocks was not significant ( $129.57 \pm 3.16$  and  $138.37 \pm 3.81$  mg / 100 ml , respectively, Table 5). However, El-Zarkouny (1994) reported that serum cholesterol concentration was 55.25 mg / 100 ml with ejaculate volume of semen  $0.64 \pm 0.03$  ml of New Zealand White rabbit.

The average of plasma calcium, inorganic phosphorus, Ca/IP ratio, alkaline and acid phosphatases of high and low semen production cocks were nearly similar. Abaza *et al.* (1995) reported that plasma calcium concentration was 3.63 mmol / L with ejaculate volume of semen ( 0.805 ml ) of Godollo New Hampshire males.

**Table 4. Effect of body weight on seminal plasma constituents (Mean  $\pm$  S.E)**

Seminal plasma const.	Body weight		Significance
	Light	Heavy	
Total protein (g %)	4.21 $\pm$ 0.35	4.28 $\pm$ 0.23	N.S.
Albumin (A) (g %)	1.40 $\pm$ 0.04	1.49 $\pm$ 0.06	P<0.05
Globulin (G) (g %)	2.80 $\pm$ 0.26	2.79 $\pm$ 0.22	N.S.
A/G ratio	0.52 $\pm$ 0.04	0.59 $\pm$ 0.06	N.S.
Cholesterol (mg %)	100.5 $\pm$ 1.66	98.27 $\pm$ 2.86	N.S.
Calcium (Ca) (mg %)	8.92 $\pm$ 0.09	7.25 $\pm$ 0.30	P<0.05
Inorganic Phosphorus (IP) (mg %)	5.25 $\pm$ 0.16	5.17 $\pm$ 0.11	N.S.
Ca/IP ratio	1.70 $\pm$ 0.05	1.40 $\pm$ 0.07	P<0.05
Alkaline phosphatase (U/100ml)	3.96 $\pm$ 0.07	4.05 $\pm$ 0.13	N.S.
Acid phosphatase (U/100ml)	10.10 $\pm$ 0.24	10.51 $\pm$ 0.37	N.S.
GPT (U/I)	14.29 $\pm$ 0.42	13.86 $\pm$ 0.34	N.S.
GOT (U/I)	20.86 $\pm$ 0.86	20.00 $\pm$ 0.66	N.S.

N.S. : not significant

**Table 5. Effect of some blood plasma constituents on semen production (Mean  $\pm$  S.E.)**

Blood plasma const.	Semen production		Significance
	High semen Production cocks	Low semen production cocks	
Total protein (g %)	5.06 $\pm$ 0.14	4.33 $\pm$ 0.15	P<0.01
Albumin (A) (g %)	1.51 $\pm$ 0.03	1.35 $\pm$ 0.03	P<0.01
Globulin (G) (g %)	3.54 $\pm$ 0.12	3.08 $\pm$ 0.16	P<0.05
A/G ratio	0.44 $\pm$ 0.02	0.47 $\pm$ 0.03	N.S.
Cholesterol (mg %)	129.57 $\pm$ 3.16	138.37 $\pm$ 3.81	N.S.
Calcium (Ca) (mg %)	16.67 $\pm$ 1.20	15.33 $\pm$ 1.24	N.S.
Inorganic Phosphorus (IP) (mg%)	9.68 $\pm$ 0.40	8.51 $\pm$ 0.39	N.S.
Ca/IP ratio	1.72 $\pm$ 0.08	1.79 $\pm$ 0.08	N.S.
Alkaline phosphatase (U/100ml)	72.25 $\pm$ 0.36	70.35 $\pm$ 0.40	N.S.
Acid phosphatase (U/100ml)	3.36 $\pm$ 0.26	3.86 $\pm$ 0.28	N.S.

N.S. : not significant

### Fertility and hatchability

#### I - Effect of semen dosage and semen collection frequency on egg fertility and hatchability

Table 6 shows that the percentage of the fertile eggs produced by hens inseminated with 0.15 ml. semen was insignificantly higher than that with either 0.05 or 0.10 ml. undiluted semen. The fertility percent of hens inseminated by semen collected once weekly was insignificantly higher than that by both twice and three times. Both insemination dosage and frequency of semen collection did not effect significantly on hatchability of eggs (Table 7). McCartney (1976) reported that the best fertility of commercial broiler breeder pullets was obtained by inseminating once weekly with 0.10 ml. undiluted semen. He found also that the hatchability percentages were 89.2, 88.5 and 87.5 when the hens were inseminated twice weekly with 0.10, 0.05 and 0.025 ml. undiluted semen, respectively. In this respect, McDaniel and Sexton (1977) reported that collection frequency of semen from the broiler- type males daily, twice daily and three times weekly, and from White Leghorn males weekly, three times weekly and five times weekly had no effect on fertility.

**Table 6. The effect of insemination dosage with undiluted semen and semen collection frequency on fertility % of Norfa hens**

Insemination dosage/ml	Semen collection frequency			Overall Average	Significance
	3/weekly	2/weekly	1/weekly		
	Fertility .....				
0.05	86.2	89.5	85.6	87.10	
0.10	83.8	84.1	90.4	86.10	
0.15	90.9	84.96	91.2	89.02	N.S.
Overall average	86.97	86.19	89.07	87.41	
Significance	N.S.....				

N.S., Not significant.

**Table 7. The effect of insemination dosage with undiluted semen and semen collection frequency on hatchability % of Norfa hens**

Insemination dosage/ml	Semen collection frequency			Overall Average	Significance
	3/weekly	2/weekly	1/weekly		
	hatchability %.....				
0.05	82.98	76.47	75.24	78.23	
0.10	78.50	82.10	80.85	80.48	
0.15	76.36	75.00	75.81	78.72	N.S.
Overall average	79.28	77.86	77.30	78.15	
Significance	N.S.....				

N.S., Not significant.

**II - Effect of diluents and dilution rate on fertility and hatchability**

Data in Tables 8 and 9 indicate that the effect of both type of diluents and dilution ratio were not significantly on egg fertility and on hatchability. The fertility percentage of undiluted semen (control) and semen diluted with tris were higher than that of semen diluted with GCG. Regardless the diluter, the percentage of fertility of both 1 : 3 and 1 : 5 were quite similar. The dilution rate (1 : 3) of GCG was higher than (1 : 5) in fertility percentage. An opposite trend was noticed for Tris. It can be recommend that, using GCG diluter with dilution rate 1 : 3 but using Tris diluter with dilution rate 1 : 5 for insemination of Norfa hens. The hatchability percent of GCG diluter was higher than that either undiluted semen or Tris diluter, followed by undiluted semen (control). However, the differences in this respect were not significant. The dilution rate (1 : 5) of GCG was higher than (1 : 3) in hatchability percentage. The reverse was noticed for Tris. Kalamah (1993) found that in Norfa chickens the effect of dilution ratios (1 : 1, 1 : 3 and 1 : 5) and type of diluents (0.9 % NaCl, 0.65 % NaCl and Ringer's solution) were significantly on hatchability. Regardless the extenders, the percentage of fertility was reduced by increasing the dilution rate, the difference between the dilution ratios 1 : 1 and 1 : 3 was not significant, however, both significantly ( $P \leq 0.01$ ) differed from that diluted with 1 : 5. He added that hatchability percentage showed a reduction after using the highest dilution rate (1 : 5). Therefore, Sexton (1980) found that the fertility percentages were 62, 76, 83 and 59 for semen dilution rate 1 : 1, 1 : 3, 1 : 5 and 1 : 7, respectively.

**Table 8. The effect of diluters and dilution rate of semen on fertility % of Norfa eggs**

Dilution rate	Diluters		Overall Average	Control (undiluted semen)	Significance
	GCG	Tris			
	Fertility .....				
1 : 3	79.75	76.16	77.96	78.71	
1 : 5	73.83	81.76	77.80	78.85	N.S.
Overall average	76.79	78.96	77.88	78.78	
Significance	N.S.....				

N.S. not significant.

**Table 9. The effect of diluters and dilution rate of semen on hatchability % of Norfa eggs**

Dilution rate	Diluters		Overall Average	Control (undiluted semen)	Significance
	GCG	Tris			
.....Hatchability %.....					
1 : 3	76.15	84.61	80.38	82.17	
1 : 5	93.64	75.15	84.40	82.11	N.S.
Overall average	84.90	79.88	82.39	82.14	
Significance	.....N.S.....				

N.S. Not significant.

#### Effect of vaginal pH on egg fertility

The obtained results indicated that the vaginal pH of high fertility hens was lower than that of low fertility ones, it was  $8.50 \pm 0.023$  and  $8.87 \pm 0.020$  of high and low fertility hens, respectively. The differences in this respect, were significant ( $P \leq 0.05$ ). It means that the fertility was increased significantly with the decreased vaginal pH. The present study suggests that the vaginal pH may be effect on spermatozoa activity in reproductive tract of hens. Kalamah (1990) found that there was significantly negative correlation between fertility and vaginal pH ( $-0.40$ ) in duck.

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