

Effects of dietary supplementation of *Thymus vulgaris* (*Lamiaceae*) on reproductive parameters in local Kabir rooster of Cameroon

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Abstract:

Background: Feed additives are a group of nutrient and non-nutrient compounds which helps farmers in improving the efficiency of feed utilization and thus reducing the high cost of feed. The current study aimed to determine some reproductive performances of local Kabir rooster fed with diets supplemented with thyme (*Thymus vulgaris*, *Lamiaceae*) powder.

Materials and Methods: A total of 40 kabir chicks were divided into four groups on the basis of the diet. One group (T_0) received a basal diet (control), two others received a basal diet supplemented with 0.5% (T_5) and 1% (T_{10}) of thyme powder while the fourth (T_{Oxy}) received a commercial antibiotic, Oxykel 80 WP (0.5g/L H_2O). From the 24th week old, six roosters of each group were bred with females and eggs were collected and incubated to evaluate fertility and hatchability. At 28th week, the chickens were weighed; their comb and wattle size measured and they were slaughtered thereafter. Blood was collected for hormonal assessment. Collected sperm was characterized and testes were weighted, their size and volume evaluated.

Results: Comb height and comb length as well as wattle length were not affected by the thyme supplementation of the diet. However, roosters fed on thyme, especially at 1% significantly increase ($P < 0.05$) the serum level of LH (5.51 ± 0.39 mUI/mL) and testosterone (1.21 ± 0.27 mUI/mL). These treatments increased sperm volume and the sperm mass motility (76.67 ± 8.16 %), sperm density (3.93 ± 0.12 %) the percentage of live sperm (94.33 ± 0.52 %) compared to the control diet. the spermatozoa abnormality rate was significantly high in T_{Oxy} group (3.75 ± 0.76 %) and lowest in T_{10} (1.08 ± 0.49 %). The same observation was recorded with the type of abnormality. Addition of 1% thyme in bird diet increased testes relative weight and volume. Thyme positively affected testes length, width and right testicular shape index. This supplement also significantly increased the fertility rate. Eggs fertilized by semen from roosters fed diet containing 0.5 and 1% of Thyme showed the highest fertility rate.

Conclusion: It could be concluded from this study that *Thymus vulgaris* could be used in poultry as additive diet to improve reproductive hormone level, semen quality, testes characteristics, fertility and eggs hatchability.

Key words: *Thymus vulgaris*, feed supplementation, reproductive parameters, Kabir rooster.

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I. Introduction

Increasing local poultry production in order to solve the high demand of its tasty meat and eggs is a real challenge for farmers in developing countries (Mengesha, 2012; Padhi, 2016 and Mingoas *et al.*, 2017). To improve

feed efficiency, growth rate, high potency and fertility, farmers refer to antibiotics, hormone and synthetic antioxidants (Castanon, 2007 and Ndzi *et al.*, 2016). However, antibiotic has shown its limits. Its prolonged use may cause a significant risk of toxicity and induce resistance in some bacteria strains (Lu *et al.*, 2006). Nowadays, antibiotics have been prohibited in livestock and poultry (Hajati and Rezaei, 2010). In developing country therefore, scientists are looking for alternative as natural plants (Lee *et al.* 2005; Javed *et al.*, 2009 and Tellez and Latorre, 2017).

Herbs are advised for their efficiency, availability, accessibility, low toxicity and low cost (Molla *et al.*, 2012). They are known for their various properties as antioxidant, anti-infectious, anti-inflammatory, anti-protozoal, hepato-protectives, growth promoter (Kale *et al.*, 2003; Chen and Yen 2007; Chowdhury *et al.*, 2009 and Tauer *et al.*, 2019). They can be administered as extracts, essential oil or as feed supplementation. Supplementation remains the easiest form of administration, characterized by its low cost, rapid extension and applicable in the farm. Karangiya *et al.* (2016) reported that supplementation of feed with garlic and ginger significantly increases growth performance of commercial broilers. Administration of alcoholic extract of *Nigella sativa* at the dose of 1.5g/kg BW via drinking water improved body and testis weight, sperm density, motility, grade activity and viability and decreased the percentage of abnormal spermatozoa. It also increased testosterone and LH levels and improve testis structures in rats (Al-Sa *et al.*, 2009).

Thymus vulgaris (L) has been used for many purposes, in the food sector, traditional medicine, pharmaceutical and cosmetic industries (Adwan *et al.*, 2006 and Jordán *et al.*, 2009). Its essential oil has antibacterial (Yakhlef *et al.*, 2011), antifungal (Lee *et al.*, 2007), (Katooli *et al.*, 2012), anti-coccidian (Abou-elkhair *et al.*, 2014), antiviral and antioxidants properties (Edris, 2007 and Amarti *et al.*, 2011). According to Shanon and Jassim, (2012), its aqueous extracts improve some reproductive parameters as ejaculate volume, sperm concentration and testes weight in broilers. It also considerably decreases sperm abnormality. On the other hand, *Thymus vulgaris* has been identified as a natural additive in poultry diets (Khan *et al.*, 2012). Its powder has varied properties as growth promoters in local chicken production with better feed conversion, immune stimulating effects and defence enhancing ability (Ndzi *et al.*, 2016). Its supplementation in the broiler diet (5 g/kg) improve the immune status hence, their productive performance (Hassan and Awad, 2017). *Thymus vulgaris* also improves the quality of egg production in laying hens (Mansoub, 2011). Despite these results, few studies have been done on the beneficial effects of supplemented thyme powder on reproductive performance in local rooster. The objective of this study was to evaluate the effects of feed supplementation of thyme on some reproductive performance (testes, semen, fertility and hormonal characteristics) in local Kabir rooster.

II. Materials And Methods

Study site

This research was carried out at the Buea's Agri-Science Action and Development Poultry Research, South-West Region of Cameroon (4° 12,773' to 4° 4,25' N and 9° 19,425' to 9° 9,20' E) and at the Life Sciences Laboratory of the Faculty of Science, University of Buea and the Laboratory of Animal Physiology and Health, FASA, University of Dschang.

Preparation of plant's powder

The whole *Thymus vulgaris* plant used was purchased from the local market. Dried samples (roots, stem and leaves) were grinded to powder, conserved into separate and labelled plastic bags, sealed and stored in a cool dry place until use.

Experimental Animals

Local Kabir chicks were provided by the Association of Farmers and Breeders of Kevin Foto Dschang (AEKDS), Cameroon. Chicks were transferred to study site after sexing at 6 weeks old. They were reared in 1 metre square wood cages (density of 5 animals/m²) equipped with drinkers and feeders. Cages were cleaned with water and disinfected with virunet® solution two weeks before their arrival. Chicks were identified by tags and allowed for acclimatization for a week, then placed in the cages. Males were assigned to four equal groups, receiving different treatments, while mature females were used for assessment of male fertility characteristics.

Diets and experimental design

Chicks received water and feed *ad libitum*. Male were fed on basal diet (Table 1) according to their stage of development: growth diet from 8 to 21 weeks then reproductive diet from 22 weeks and above (Tadondjou *et al.* 2014).

Table1: Ingredient and chemical composition of rooster growth and reproductive diets

Feed ingredient (kg)	Growth	Reproduction
Maize	49.0	52.0
Wheat bran	22.0	25.5
Cotton seed cake	8.0	3.0
Soybean cake	7.0	3.0
Fish meal	6.5	3.0
Bone meal	1.5	7.0
Shellfishpowder	5.0	1.0
Premix 5% (*)	1.0	5.5(1)
Total	100.0	100.0
Crudeprotein (%)	20.70	15.38
ME (kcal/kg)	3013.51	2723.78
Calcium (%)	1.51	3.45
Phosphor (%)	0.73	0.69
Lysine (%)	1.10	0.72
Met (%)	0.40	0.29

*Premix 5%: crude protein = 40%; Metabolizable energy = 2078 kcal/kg; Calcium = 8%; Phosphor = 2.05%; Lysine = 3.30%; Met (Methionine) = 2.40%; ME = Metabolizable energy.

Forty Kabir roosters were randomly distributed in 4 groups of 5 chickens each (repeated once) corresponding to treatments. They were fed on experimental diets formulated (from basal diet) to contain graded levels of *Thymus vulgaris* (0.5% and 1.0%) powders against two control diets: basal diet with a commercial antibiotic and without a plant powder nor commercial antibiotic (Oxykel 80WP). Addition of plant powders to basal diet was done daily while the antibiotic was added to water following manufacturer’s instruction (0.5g/L of water for 3 continuous days weekly) (Ndzi *et al.*, 2016) (Table 2).

Tableau 2: Additive feed allocated to the experimental animals

Treatments	Additive	Basal diet (g)	Total feed composition (g) 1000
T ₀	/	1000	1000
T ₅	5 g <i>Thymus vulgaris</i>	995	1000
T ₁₀	10g <i>Thymus vulgaris</i>	990	1000
T _{Oxy}	0.5g oxykel 80 WP/1L H ₂ O	1000	1000

Fertility assessment

From the 24th week, six roosters of each treatment were randomly selected and bred with females (1 male and 4 females) for fertility characteristics assessment. Eggs were collected at the 25th, 26th and 27th weeks and incubated. On the 8th day of incubation, eggs fertility was determined by candling. After 21 days of incubation, non-hatched eggs were broken-out and observed to evaluate the embryonic mortality rate. Percentage fertility was then calculated by dividing the number of fertile eggs by the total number of eggs incubated (Sun *et al.*, 2017). At the 28th week, roosters cohabitating with females were removed and weighed. Comb and wattle sizes were assessed and then they were slaughtered by decapitation. Serum, sperm and testes were collected for analysis.

Comb and wattle sizes

Comb and wattle sizes were measured using digital calipers (accuracy = 0.01 mm). The height of the comb and wattle from where the comb met the head to the top of the highest spike were measured as well as the length of the comb from end to end (Navara *et al.*, 2012).

Measurement of Follicle Stimulating Hormone, Luteinizing Hormone and testosterone level

Serum levels of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were determined by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using commercial kits (DIASOURCE LH ELISA, Diasourceimmuno Assays SA, KAPD1289) and (Accubind ELISA MICROWELLS, INC, 425-300 Monobind Lake forest, CA 92630) respectively. Testosterone was evaluated by a competitive enzyme-linked immunosorbent assay (ELISA) (Omega Diagnostics LTD, Pathozyme Testosterone, Ref OD497).

Analysis of semen characteristics

After sperm collection from spermiductus (Tadondjou *et al.* 2013), semen volume was assessed by drawing the sample into a 1mL syringe (0.02 mL of accuracy) and colour was recorded by visual examination. The collected semen was maintained at 38°C in a water bath for sperm motility assessment and sperm density. The semen characteristics (concentration, sperm motility percentage, sperm grade activity, forward progressive movement, abnormal sperm morphology and sperm viability) were determined using the methods described by Tadondjou *et al.* (2014).

Testes measurements and volume

The testes size (shape index) was noted. Their weight was measured then divided by the animal weight, and multiplied by 100 to determine in percentage the testis relative weight. The testis volume was determined by water displacement method. Thus, the testis was immersed into a measuring cylinder half-filled with normal saline, and the volume displaced by the testicle was recorded (Lin *et al.*, 2009).

Statistical analyses

Data collected were subjected to analysis of variance (ANOVA) at $P < 0.05$. When differences were significant, Duncan multiple range tests were used to separate means. All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, IBM version 21.0).

III. Results

Effects of supplementation of *Thyme vulgaris* on kabir rooster comb and wattles

As shown in table 3, neither comb nor wattles development were consistently influenced by treatment ($P > 0.05$).

Table 3: Comb and wattles measurements of roosters treated by thyme

Parameters (mm)	T ₀ (n=6)	T ₅ (n=6)	T ₁₀ (n=6)	T _{Oxy} (n=6)	p
Combheight	59.13±15.26	64.17±13.98	65.52±16.30	60.03±8.04	0.82
Comblength	74.82±22.19	92.95±11.65	100.53±18.77	91.65±16.29	0.37
Wattlelength	54.64±13.79	62.29±14.70	66.30±19.38	60.79±20.27	0.71

T₀, T₅, T₁₀ and T_{Oxy} are groups which received feed supplementation respectively with *Thymus vulgaris* 0, 0.5 and 1 % or Oxykel 0.5g/L; n: number of roosters.

Effects of supplementation of *Thymus vulgaris* on serum levels of testosterone, Luteinizing Hormone and Follicle Stimulating Hormone

The effects of thyme on the blood concentration of testosterone, LH and FSH are presented in table 4. It comes out from this table that the serum levels of testosterone and LH were increased significantly ($P < 0.05$) in kabir rooster fed diet containing 1% *T. vulgaris* supplemented dietas compared to values recorded with other treatments. However, there was not significant variation of FSH concentration between treatments ($P > 0.05$).

Table 4: Effects of *Thymus vulgaris* on testosterone, LH and FSH levels of local kabir roosters

Parameters	T ₀ (n=6)	T ₅ (n=6)	T ₁₀ (n=6)	T _{Oxy} (n=6)	p
FSH (mUI/ml)	3.38±0.76 ^a	3.74±0.66 ^a	3.61±0.51 ^a	3.67±0.74 ^a	0.825
LH (mUI/ml)	4.19±1.18 ^{ab}	3.58±1.11 ^b	5.51±0.39 ^a	3.58±1.12 ^b	0.048
Testosterone (ng/ml)	0.21±0.07 ^b	0.23±0.08 ^b	1.21±0.27 ^a	0.24±0.08 ^b	0.000

^{a,b}Means with the same letters within the lines are not significantly different ($P > 0.05$). T₀, T₅, T₁₀ and T_{Oxy} are groups which received feed supplementation respectively with *Thymus vulgaris* 0, 0.5 and 1 % or Oxykel 0.5g/L; n: number of roosters.

Effects of dietary *Thymus vulgaris* supplementation on semen characteristics in Kabir roosters

Supplemented feed with 1% of *Thyme vulgaris* powder (T₁₀) significantly ($P < 0.05$) increased the semen volume, sperm motility, sperm density and viability (Table 5). The lowest values of these parameters were found in roosters receiving oxykel (T_{Oxy}), and their sperm morphological abnormalities values were significantly ($P > 0.05$) higher compared to those fed on supplemented feed. On the other hand, sperm color was not affected by treatments ($P > 0.05$).

Table 5: Effects of *Thymus vulgaris* on semen characteristics of Kabir roosters

Parameters	T ₀ (n=6)	T ₅ (n=6)	T ₁₀ (n=6)	T _{Oxy} (n=6)	p
colour (% white Milk)	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	1.00
Volume (ml)	0.35±0.05 ^b	0.36±0.04 ^b	0.47±0.14 ^a	0.27±0.04 ^b	0.00
Mass Motility (%)	66.67±10.33 ^{ab}	73.33±10.33 ^a	76.67±8.16 ^a	60.00±0.00 ^b	0.01
IndividualMotility (%)	70.00±10.95 ^a	73.33±10.33 ^a	76.67±8.16 ^a	66.67±10.33 ^a	0.37
Spermdensity (10 ⁹ /mL)	3.65±0.11 ^{bc}	3.84±0.16 ^{ab}	3.93±0.12 ^a	3.47±0.32 ^c	0.00
Live sperm (%)	92.75±1.21 ^b	93.08±0.38 ^b	94.33±0.52 ^a	90.58±1.39 ^c	0.00
Semen abnormality (%)	2.67±0.61 ^b	2.08±0.73 ^b	1.08±0.49 ^c	4.0±1.05 ^a	0.00
Head abnormality (%)	0.67±0.41 ^b	0.42±0.20 ^{bc}	0.17±0.26 ^c	1.42±0.58 ^a	0.00
Mid-piece abnormality (%)	0.92±0.37 ^{ab}	0.75±0.41 ^{ab}	0.42±0.37 ^b	1.25±0.52 ^a	0.00
Tailabnormality (%)	1.08±0.49 ^{ab}	0.92±0.49 ^{ab}	0.50±0.46 ^b	1.33±0.87 ^a	0.00

^{a,b} Means in the same line with different letters are significantly different (P < 0.05). T₀, T₅, T₁₀ and T_{Oxy} are groups which received feed supplementation respectively with *Thymus vulgaris* 0, 0.5 and 1 % or Oxykel 0.5g/L; n: number of roosters.

Effects of dietary supplementation of *Thymus vulgaris* on testes characteristics in Kabir roosters

Thyme supplementation did not have any significant effects of testicular weight and shape index of left testes (Table 5). However, testes volume, left testicular length, right testes width and shape index significantly (P<0.05) increased in roosters fed thyme supplemented diet. Testes relative weight significantly (P<0.05) decreased in roosters which received oxykel.

Table 5: Effects of *Thymus vulgaris* on the variation of testicular measurement

Parameters	T ₀ (n=6)	T ₅ (n=6)	T ₁₀ (n=6)	T _{Oxy} (n=6)	p
Body weigh	3633.50±91.43 ^c	4315.67±83.21 ^b	4707.33±88.61 ^a	4284.67±127.75 ^b	0.00
Pared testes relative weight (%)	0.81±0.02 ^a	0.85±0.11 ^a	0.87±0.02 ^a	0.65±0.04 ^b	0.00
Left testes relative weight (%)	0.42±0.02 ^a	0.44±0.06 ^a	0.45±0.04 ^a	0.32±0.02 ^b	0.00
Right testes relative weight (%)	0.40±0.01 ^a	0.41±0.06 ^a	0.42±0.05 ^a	0.32±0.02 ^b	0.00
Testicular weight ratio	1.05±0.05 ^a	1.07±0.09 ^a	1.09±0.21 ^a	1.01±0.02 ^a	0.74
Lefttesticularlength (mm)	40.27±8.11 ^b	44.76±2.94 ^a	55.12±7.30 ^a	47.27±10.72 ^{ab}	0.03
Right testicularlength (mm)	47.25±0.32 ^{ab}	44.74±1.66 ^b	51.03±5.20 ^a	46.92±4.95 ^{ab}	0.04
Left testes width (mm)	24.00±0.31 ^b	34.15±0.60 ^a	35.89±7.97 ^a	28.02±4.47 ^b	0.00
Right testes width (mm)	25.33±0.81 ^b	33.01±0.79 ^a	34.70±7.63 ^a	27.63±2.94 ^b	0.00
Lefttesticularshape index	0.62±0.14 ^a	0.77±0.05 ^a	0.65±0.09 ^a	0.62±0.17 ^a	0.15
Right testicular shape index	0.54±0.01 ^c	0.74±0.02 ^a	0.67±0.10 ^b	0.59±0.02 ^c	0.00
Volume (ml)	14.67±0.20 ^c	16.42±1.36 ^b	19.00±2.02 ^a	13.17±1.17 ^c	0.00

^{a,b} Means in the same line with different letters are significantly different (P < 0.05).

T₀, T₅, T₁₀ and T_{Oxy} are groups which received feed supplementation respectively with *Thymus vulgaris* 0, 0.5 and 1% or Oxykel 0.5g/L; n: number of roosters.

Effect of dietary *Thymus vulgaris* supplementation on fertility and hatchability rates in Kabir roosters

Results of the fertility and hatchability assessment are shown in figure 1 and 2 respectively. The fertility rate increased in roosters fed on thyme powder. However, the increase was significant (P<0.05) only with treatment T₁₀ (figure 1, a) compared to the values recorded in roosters receiving basal diet (T₀) and antibiotic (T_{Oxy}). As presented in figure 1 (b), hatchability rate increased significantly (P<0.01) in eggs fertilized by rooster fed on 1% and 0.5% *Thymus vulgaris*. The lowest values were recorded in animals treated with T_{Oxy} (84.65 ± 4.75%).

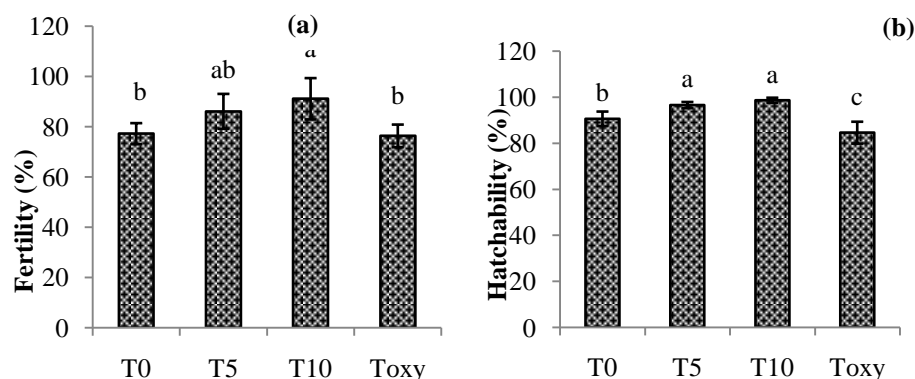


Figure 1: Effects of *Thymus vulgaris* on fertility (a) and eggs hatchability (b) of kabir rooster. T₀, T₅, T₁₀ and T_{oxy} are groups which received feed supplementation respectively with *Thymus vulgaris* 0, 0.5 and 1% or Oxykel 0.5 g/L. ^{a,b} Means in the same line with different letters are significantly different (P < 0.05).

IV. Discussion

This work was carried out to verify the scientific claim of the use of *Thymus vulgaris* as feed additive to boost reproductive performances in local kabir rooster. *Thymus vulgaris* supplementation in Kabir roosters accelerated the comb growth and wattle which resulted in large size. Their important size may be an evidence of their healthy (Zuk *et al.*, 1990a). Moreover, rooster's comb is an indicator of male's state (Navara *et al.*, 2012). So, roosters with larger combs and wattles seem to be more preferred by hens for mate (Zuk *et al.*, 1990b). It was also reported that broiler breeder roosters with wider combs produce sperm that were better able to reach and hydrolyse the perivitelline membrane of the ovum (McGary *et al.*, 2003). This would thus positively affect the fertility, fecundity and consequently hatchability.

Supplemented feed with *Thymus vulgaris* significantly increased bird's body weight. This increase in bird's body weight is dose dependent. So, roosters which received feed with highest dose T₁₀, had the highest weight gain. This is in agreement with Toghyani *et al.* (2010), who reported that feed incorporated with thyme powder at 5 g/kg significantly increased body weight gain. Furthermore, the gradual incorporation of increasing thyme powder at 0.5, 1 and 2% significantly increased body weight and daily weight gain (El-Ghousein and Al-Beitawi, 2009 and Saki *et al.*, 2014). Feed supplemented with *Thymus vulgaris* also affected testicular development in Kabir roosters. In fact, testicular development characteristics (testes relative weight, testicular volume and shape) was higher in treatments where birds were fed with diet supplemented with 1% (T₁₀) or 0.5% (T₅) compared to those fed on basal diet (T₀). This result supported the findings of Tadondjouet *et al.* (2013) who reported that high body weight gain during growth period seems to increase testes. The effect of thyme on testicular growth may then be related to its growth promoter properties.

Roosters fed on diet supplemented with 1% of *Thymus vulgaris* powder presented the best semen characteristics and sex hormone profile. According to Taborsky *et al.*, *Thymus vulgaris* contain several chemical compounds among which thymoquinone which concentration could be about 300 mg/kg of dry weight material (Taborsky *et al.*, 2012). Its administration at 5 mg/kg body weight could improve sperm parameters and increase the thickness of the germinal epithelium. Gökçe *et al.* (2011) reported that, administrated at 10 mg/kg, thymoquinone improved semen quality and the structure of testes. The best semen characteristics and sex hormone profile observed in this study are in agreement with the finding of Parandin *et al.* (2012) who reported that, administrated during 60 days at 200 and 400 mg/kg body weight, thymoquinone could increase fertility, gonadotropin and testosterone levels (Parandin *et al.*, 2012 and Haseena *et al.*, 2015). Furthermore, he reported that even after the treatment period, an increase of genital weight, sperm motility, sperm viability, and sperm count was observed (Parandin *et al.*, 2012).

Thyme supplementation globally increased the fertility. These results are similar with the findings of Radwan *et al.* (2008) who found that fertility were improved in hens fed with diet containing 0.5 % and 1% thyme. Ali *et al.* (2007) also reported that addition of 0.25 % thyme in diet of hens tended to improve their fertility. The benefit effects of thyme on fertility may be attributed to its antioxidant activities (Edris, 2007; Radwan *et al.*, 2008; Amarti *et al.*, 2011) or/and its ability to improve semen characteristics (Parandin *et al.*, 2012; Radwan *et al.*, 2008).

Addition of thyme in rooster's diets increased hatchability. According to Ali *et al.* (2007), improvement in hatchability by thyme may be the result of its antioxidant constituent's activities. In fact, it has been demonstrated that thyme contents antioxidant compounds which may reduce oxidative stress by inhibiting lipids oxidation and hence decrease the sources of radicals passing to egg (Ali *et al.*, 2007 and Radwan *et al.*, 2008).

V. Conclusion

This work attempts to evaluate the effects of dietary supplementation of *Thymus vulgaris* on reproductive parameters in Kabir rooster. From the obtained results, it could be concluded that supplemented feed with *Thymus vulgaris* powder improve semen characteristics, LH and testosterone production and consequently fertility and hatchability of Kabir rooster.

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