



**COMPARATIVE STUDIES OF SOME NATURAL AND COMMERCIAL FEED ADDITIVES TO IMPROVE  
REPRODUCTIVE PERFORMANCE OF TURKEY TOMS**

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**Abstract**

The present study was carried out to investigate the effect of supplementation of turkey toms with basil, thyme, kemzyme either alone or in combination on the testicular performance. This study was carried on 36 male turkey toms three months old and the experiment extended for ten weeks. The toms were randomly divided into six groups (six birds each), the first group was fed the basal ration and considered as control group (C), the second group (B) fed on basal ration to which basil powder was added at a dose 3 g/ kg ration, (T) group fed on basal ration to which thyme powder was added at a dose 2 g/kg ration, (BT) group was fed basal ration to which basil and thyme powder were added with the same previous doses, group (K) was supplied with 0.5 g/kg ration kemzyme and the last group (BTK) was supplemented with a mix of basil, thyme and kemzyme with the same previous doses. Serum samples were collected to measure some biochemical parameters, the testicular hormones (Estradiol 17  $\beta$  and testosterone). At the same time testicular tissues were collected and prepared for morphological and molecular studies. Results of this study revealed that tom fed with either (B), (T) or (BT) had significantly higher serum lipid level than both (C) and (k) groups. Supplementation with (k) significantly increases the estradiol 17  $\beta$  and testosterone levels of turkey toms which accompanied with the lower level of serum lipid. Concerning the antioxidant total activities, in (B), (T), (BT) and (BTK) revealed a significant increase than its level in control group. Studies of intestinal morphology and integrity indicated that the group supplemented with (BTK) had a higher intestinal villous /crypts ratio plus increase in the goblet cell numbers. Moreover, the testicular tissue recorded an increase in both DNA concentration indicating increase cell size and accomplished with the increase of DNA/Protein which means increase of testicular cell mitosis. Thus the aim of the present study is to compare the effect of supplementing enzyme (Kemzyme), as commercial feed additives, to basil or thyme as natural feed additives and their combinations on some testicular performance.

**Keywords:** Turkey Toms, Basil, Thyme, Kemzyme

**INTRODUCTION**

In the recent times there has been a great concern about the increasing trend of male infertility in domesticated (Multigner et al.,2000) and wild animals (Donohoe et al., 2000). The causative factors are essentially environmental, occupational and /or dietary (Hussien and Brasel 2001 and Sarin et al., 2001). Herbs and plant extracts used as feed additives include many bioactive ingredients such as alkaloids, bitters, flavonoids, glucocides, mucilage, saponin and taninis (Wenk, 2000 and Zheng and Wang 2001).

The main constituents of essential oils extracted from thyme were barneal, thymol, carvacrol methyl ether, camphene,  $\alpha$ -Humulene and carvacrol (Bounatirou et al.,2007 and Amaraweiz et al.,2008). Thymol and Carvacrol displayed a concentration dependent antioxidant capacity (Underger et al.2009)

Anti oxidant properties of herbs such as thyme and oregano contain large amounts of monoterpenes, thymol and carvacrols (Ciftci et al., 2010, Rahim et al., 2011 and Hashemipour et al.2013). Plants rich in flavinoid such as basil and green tea have been described as natural anti-oxidant and testicular enhancer (Hafez ,2010, Hemayatkhah et al, 2011, and Hossein et al 2013). The use of the enzyme in the feed of animals particularly poultry has become more common (Wang et al, 2005) looking forward, (Saleh et. al. 2006) have observed that kemzyme in turkey toms improved the performance and fertility capability of such species either through reducing the viscosity of digesta and improving nutrient utilization, specially lipids or stimulating the synthesis and secretion of hormone involved estroidogenesis or spermatogenesis. Therefore the present study aimed to investigate the benefits of adding commonly used commercial kemzyme

preparation (multienzyme) and some herbal feed additives (basil and thyme) to the diet of turkey

### Materials and Methods

Thirty six male turkey toms of Big six breed three months old, with an average body weight 5.5 kg were divided into six groups of six toms each, they were treated as follow: the control group (C) was fed a basal diet without supplementation, group (B) was supplemented with Basil (3g/kg diet), group (T) was fed Thyme (2g/kg diet), group (BT) was fed a mixture of basil and thyme (3g basil+2g thyme/kg diet), group(K) was supplemented with Kemzyme (0.5g/kg diet) and group (BTK) was fed a mixture of Basil, Thyme and Kemzyme. Each group was kept in individual cage with lighting regimen 23 hours light daily (Prescott et al., 2003).

### Feed additives

**Basil:** Natural feed additive, its main constituents are methyl chavicol, eugenol,

**Table (1):** Composition percentage and calculated nutrients profile of the basal diets:

Ingredients %	8-12 Weeks of age	13-17 Weeks of age	18-21 Weeks of age	22-24 Weeks of age
Yellow corn	49.336			
gluten meal	5.657	59.262	68.766	75.353
Soybean meal (44% CP)	38.426	4.358	3.227	2.467
Soy oil	2.176	29.598	21.917	16.758
Dicalcium phosphate	2.273	2.326	2.10	1.169
Limestone	1.162	2.074	2.153	2.21
Common salt	0.298	1.383	0.788	0.795
DL Methionine	0.142	0.30	0.30	0.30
L-Lysin		0.148	0.196	0.229
Vitamin& mineralpremix*	0.230	0.251	0.253	0.419
	0.30	0.30	0.30	0.30

\*Per kg premix: 10 000 000 IU vit. A, 1 000 000 IU vit. D<sub>3</sub>, 50 000 mg vit. E, 7 000 mg vit. K<sub>3</sub>, 2000 mg vit. B<sub>1</sub>, 6000 mg vit. B<sub>2</sub>, 2000 mg vit. B<sub>6</sub>, 25 mg vit. B<sub>12</sub>, 50000 mg niacin, 220 mg biotin, 15000 mg folic acid, 400000 mg choline, 2000 mg pantothenic acid, 400000 mg magnesium, 70000 mg zinc, 30000 mg manganese, 75000 mg iron, 5000 mg copper, 750 mg iodine and 250 mg cobalt.

### Measured Parameters

#### a. Serum Biochemical Parameter:

- **Serum Total Lipids:** estimated using kits purchased from Spectrum Company, Dokki, Giza, Egypt. according to (Zollner & Kirsch, 1962),
- **Estradiol 17-β:** is estimated by in vitro quantitative determination immunoassay in serum and plasma using Cobas kit, according to Kronenberg et al., (2008).

toms to evaluate some testicular parameters.

linalool, camphor and methyl cinnamate. It is obtained from **Haraz spice shop Egypt**.

**Thyme:** Natural feed additives, its main constituents are thymol, carvacrol, linalool and Caffeic acid. It is obtained from **Haraz spice shop Egypt**.

**Kemzyme:** A multienzyme feed additive containing protease, α-amylase, β-glucanase, cellulose, amylase and lipase produced by Kemin industry and provided as a gift from United Biomed Company-Egypt.

**Feed:** Basal ration were formulated to cover the nutrient requirements of growing turkey according to National Research Center NRC (1994).

The composition of the formulated diet is shown in Table (1), the additives were added according to the group

- **Testosterone:** is estimated by in vitro quantitative determination immunoassay in serum and plasma using Cobas kit, according to Rosner et al. (2007).
- b. Serum Antioxidant Parameters:** Antioxidant parameters such as Malonaldehyde (MDA), Serum Oxide Dismutase (SOD) were performed using kits purchased from Biodiagnostic Company, Dokki, Egypt according to method by Ohkawa et. al. (1979), (Nishikimi et al., 1972) respectively and Total Antioxidant

Capacity (TAC) according to (Koracevic et al., 2001).

#### c. Intestinal Morphometry:

At the end of experiment samples from duodenum taken for measuring the length of intestinal villous, depth of crypts and number of goblet cells using light microscope according to (Brancroft et al., 1996).

#### d. Testicular Integrity:

DNA and RNA purification from tissues (QIA amp DNA, RNA min kit) according to (Fisher & Suttle 2011).

e. **Statistical Analysis:** data are represented as means and analyzed by one-way ANOVA using Microsoft office excel computer program excel version 2007 according to (Snedecor & Cochran, 1980). The groups were compared by pooled standard error (SE pooled) at P value  $\leq 0.05$  (Bret Larget, 2003).

### Results and Discussion

Natural herbs and their products have many biological properties (Hosseinimehr 2014). Basil and Thyme are rich sources of flavinoids which posses an antioxidant activities (Zhang et al., 2009). Moreover Herbs extract protect testes from toxicity by restoration of histological structure and increase the number of the germ cells (Khaki et al., 2011). Enzymes as commercial feed additives have been shown to improve performance and nutrient digestion when added to poultry diets (Odetallah et al., 2002).

The results obtained in table (2) demonstrated that turkey toms supplemented with kemzyme showed a significant decrease in serum total lipids than control while the other groups recorded significant increase in the serum total lipids than control. Moreover, it is clear that addition of kemzyme with basil and thyme (BT) showed highly significant decrease in total lipids than the turkeys supplemented with basil and thyme (BT).

The reduction of serum total lipids in group (K) may be attributed to a reduction in digesta viscosity leading to a subsequent increase in nutrient digestion especially lipids (Saleh et al., 2010), this is in agreement with Danicke et al.(1999) who reported depression in fat digestion due to non-starch poly saccharides (NSPs).

These findings strongly coincide with morphological findings in table (6) which demonstrate that Kemzyme supplementation

restored the absorptive capacity of the intestinal mucosa through increasing intestinal villous size (Mathlouthi et al.,2002). However the other groups supplemented with (B), (T), (BT) and (BTK) showed an increase in V/C ratio than the (C) and (K) group. (BTK) group assured that the addition of Kemzyme was essential in lowering intestinal viscosity, increasing V/C ratio and lowering level of serum lipid level – up taken by the gonads- which may be the proper cause of increasing the gonadal hormones promoting the action of B, T as testicular enhancers. This was cleared by the results in table (3 & 4) where it is clear that the serum testosterone and Estradiol 17 $\beta$  levels were significantly higher in kemzyme treated turkeys compared to other groups. This strongly suggests that kemzyme supplementation aids in supplying testicular tissue with its optimum needs for essential phospholipids and fatty acids necessary for spermatogenesis, further more this coincides with the histomorphological findings demonstrated in both kemzyme (K) and thyme (T) supplemented groups (Fig. 1a) which is proud with finding of (Saleh et al.2006). The former suggestion recently assured by Shanoon and Mahd (2012) who found that thyme supplementation to diet improved androgenic activity as a result of antioxidant effect of thyme. At the same time Ahmed et al. (2015) pointed that thyme supplementation resulted in significant increase of both testosterone and estradiol 17  $\beta$  levels compared to control.

The morphological study, Toms supplemented with basil showed normal morphological structures of testes without any changes compared to control (fig. 1a) which is in agreement with (Nashwa et al.2015).

Concerning the results of serum antioxidant activity in table (5) at the end of experimental period revealed that there was a significant decrease in the activity of Malonaldehyde (MDA) in groups supplemented with (T), (B), (BT), (K) and (BTK). Mean while the level of MDA recorded no change versus control and this contradict the results obtained by Hamada et al (2015). Moreover, Hashemipour et al. (2012) found that chicken supplemented with thymol caused an increase in SOD activity while MDA was reduced in the serum. The former study is in agreement with the results of MDA while antagonize the results of Superoxide dismutase

(SOD) level. In the same table total antioxidant capacity (TAC) which measure the overall antioxidant capacity (Sharma and Kaur, 2015). Assured the antioxidant properties of all used feed additives (B), (T), (BT), (K) and (BTK) by the obtained higher value of TAC.

In conclusion of the former results either testicular performance and the measure of antioxidant capacity are proud with the former study of Sikka (1996) who recorded that production of ROS in various organs including the testes is a normal physiological event, the alteration of their synthesis stimulate oxidation and DNA damage of the cells. Testicular tissue DNA, RNA and protein

concentration together with the morphological study of the testes provided novel knowledge about the testicular development and integrity such as DNA/RNA ratio, DNA/protein ratio the DNA concentration of the tissue of testes reflects its mitosis in the cell population and this is cleared with the results present in table (7) for groups supplemented with (BT) and (K) compared to other groups. The Protein /DNA ratio indicates the cell size. The recorded results illustrate increase in the cell size of all treatments versus control. The former obtained results go hand by hand with the previous suggestion of Jin et al. (1998) and Iji et al. (1999).

**Table (2):** Effect of Basil, Thyme, (Basil +Thyme), Kemzyme and (Basil +Thyme + Kemzyme) on Serum Total Lipid

Group	(C)	(B)	(T)	(BT)	(K)	(BTK)	SE pooled
Parameters							
Total lipid(mg/dl)	454	480	545	686	419	582	5.6

Data indicate mean, n=3/group. Control (C), Basil (B), Thyme (T), Basil + Thyme (BT), Kemzyme (K), Basil + Thyme + kemzyme (BTK), pooled standard error (SE pooled).

**Table (3):** The effect of Basil, Thyme, Basil +Thyme, Kemzyme and Basil+ Thyme +Kemzyme on Serum Estradiol 17 β level (pg/ml):

(C)	(B)	(T)	(BT)	(K)	(BTK)	SE Pooled
38.23	23.23	34.14	28.50	52.53	23.10	10.6

Data indicate mean ± standard error at p≤ 0.05, n=3/group. C (control), B (Basil), T (Thyme), BT (Basil+ Thyme), K (Kemzme) and BTK (Basil+ Thyme+ kemzyme), pooled standard error (SE pooled).

**Table (4):** The effect of Basil, Thyme, Basil +Thyme, Kemzyme and Basil+ Thyme +Kemzyme on serum Testosterone level (ng/ml):

(C)	(B)	(T)	(BT)	(K)	(BTK)	SE Pooled
0.42	0.33	0.59	0.38	0.65	0.54	0.1

Data indicate mean ± standard error at p≤ 0.05, n=3/group. C (control), B (Basil), T (Thyme), BT (Basil+Thyme),K (Kemzyme), BTK (Basil+Thyme+ kemzyme), pooled standard error (SE pooled).

**Table (5):** The effect of Basil, Thyme, Basil +Thyme, Kemzyme and Basil+ Thyme +Kemzyme on Serum Anti Oxidants:

Group	(C)	(B)	(T)	(BT)	(K)	(BTK)	SE pooled
Anti-Oxidants							
MDA(nmol/ml)	34	30	39	47	37	39	3.0
SOD(U/ml)	200.8	202.1	202.1	203.4	201.7	200.9	2.1
TAC(mM/L)	0.6	1.6	0.8	2.1	0.9	1.5	0.1

Data indicate mean ± standard error at p≤ 0.05, n=3/group. C (control), B (Basil), T (Thyme), BT (Basil+Thyme), K (Kemzme) and BTK (Basil+Thyme+ kemzyme). pooled standard error (SE pooled).

**Table (6):** Effect of Basil, Thyme, (Basil +Thyme), Kemzyme, (Basil +Thyme + kemzyme) on Intestinal Villus length to Crypt depth ratio and Number of Goblet Cells/Villus in turkey duodenum:

Group	(C)	(B)	(T)	(BT)	(K)	(BTK)	SE Pooled
Parameter							
V/C Ratio	3.00	8.00	6.00	7.00	5.00	8.60	1.0
N.G.C (mean)	74	121	79	106	69	179	8.6

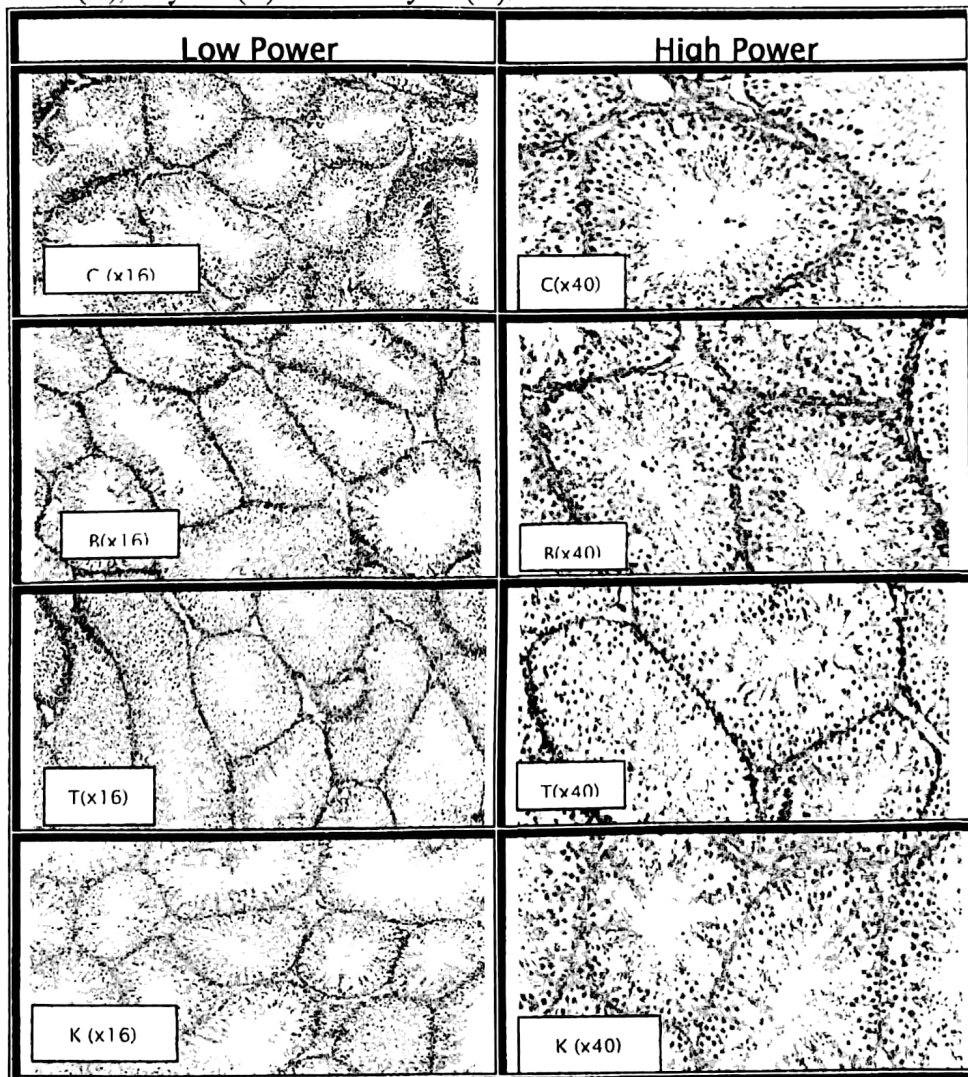
Data indicate mean, n=4/group. Control (C), Basil (B), Thyme (T), Basil + Thyme (BT), Kemzyme (K), Basil + Thyme + kemzyme (BTK), villus length :crypts depth ratio( V/ C) Ratio, number of goblet cells (N.G.C). pooled standard error (SE pooled).

**Table (7):** The effect of Basil, Thyme, Basil +Thyme, Kemzyme and Basil+ Thyme +Kemzyme on Testicular Tissue DNA, RNA, Protein and their ratios:

	(C)	(B)	(T)	(BT)	(K)	(BTK)
DNA	0.97	0.95	0.94	1.48	1.50	1.48
RNA	4852	443	215	546	397	707
RNA/ DNA	9.84	2.18	0.94	1.4	1.15	1.48
RNA/ Protein	5002.1	466.3	228.7	366.7	264.7	477.7
DNA/ Protein	0.002	0.005	0.004	0.004	0.044	0.003

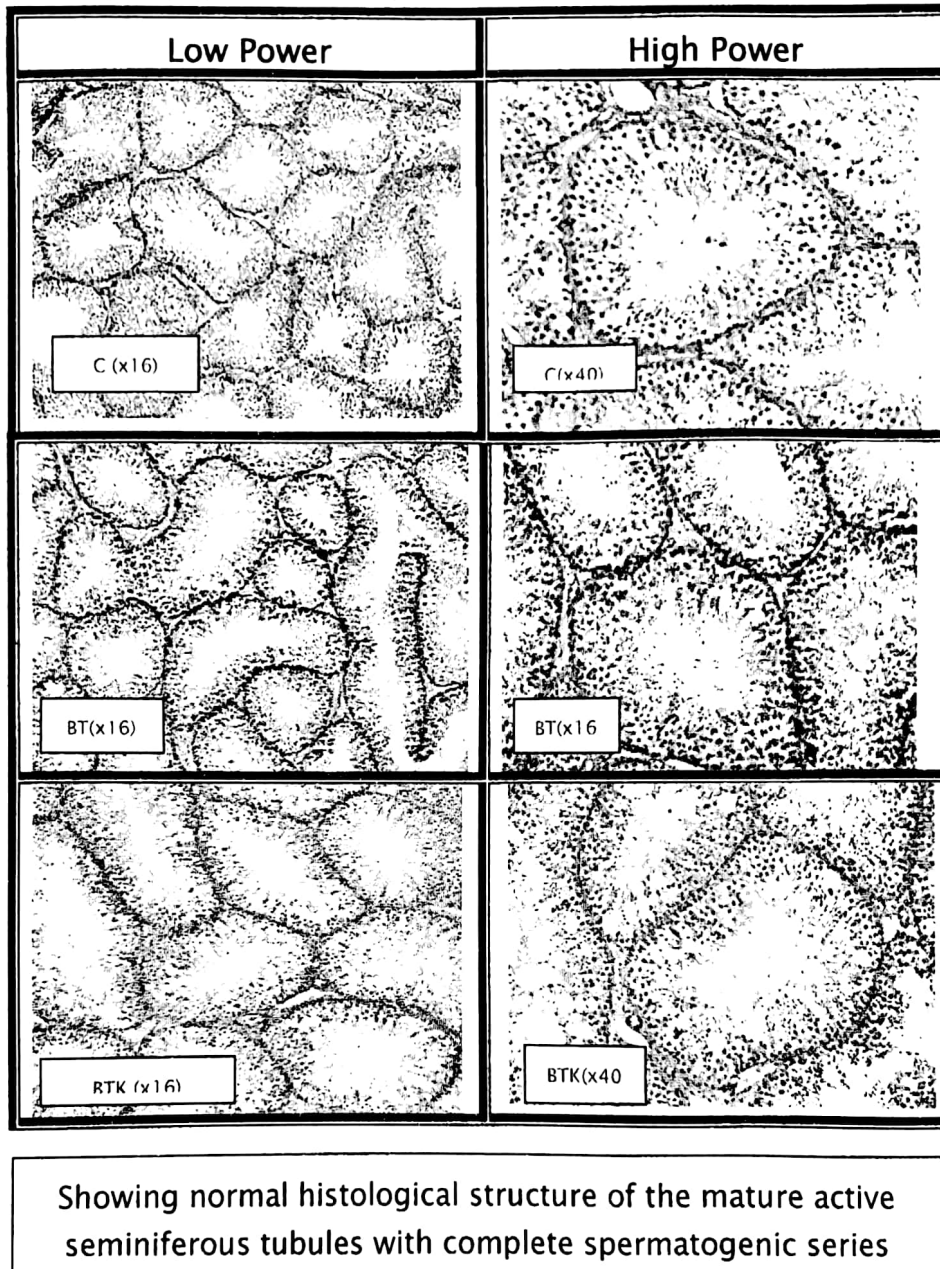
Data indicate mean  $\pm$  standard error at  $p \leq 0.05$ ,  $n=3$ /group. C (control), B (Basil), T (Thyme), BT (Basil+Thyme), K (Kemzyme) and BTK (Basil+Thyme+ kemzyme).

**Fig. (1a).** Photomicrographs of testes from male turkey chicken showing the seminiferous tubules in Control (C), Basil (B), Thyme (T) and Kemzyme (K):



Showing normal histological structure of the mature active seminiferous tubules with complete spermatogenic

**Fig. (1a).** Photomicrographs of testes from male turkey chicken showing the seminiferous tubules in Control (C), Basil+ Thyme (BT) and Basil+ Thyme +Kemzyme (BTK):



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#### الملخص العربي

#### مقارنة ودراسة بعض الإضافات الطبيعية والتجارية لتحسين الكفاءة التناسلية للديوك الرومي

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قسم الفسيولوجيا- كلية الطب البيطري - جامعة القاهرة

أجريت هذه الدراسة بهدف معرفة تأثير إضافة بعض الأعشاب (الريحان والزعتر) والانزيمات الهاضمة الخارجية (الكيمزيم والزيموجين) منفردة أو معا على الكفاءة التناسلية لذكور الرومي فكانت عدد الطيور المستعملة 36 من ذكور الرومي والتي تبلغ من العمر ثلاثة أشهر وامتدت التجربة لمدة عشرة أسابيع قُسمت عشوائيا الي 6 مجاميع ( 6ديوك في كل مجموعة منها)، المجموعة الأولى (C) تغذي علي العليقة الأساسية بدون إضافات، المجموعة الثانية (B) تغذي علي العليقة الأساسية التي أضيف إليها مسحوق الريحان بجرعة 3 جم /كجم عليقة و المجموعة الثالثة (T) التي تغذت علي العليقة الأساسية التي تمت إضافة مسحوق الزعتر لها بجرعة 2 جم / كجم من العليقة القاعدية ، وتمت تغذية المجموعة الرابعة (BT) بحصة غذائية أضيف إليها الزعتر ومسحوق الريحان بنفس الجرعات السابقة، المجموعة الخامسة (K) بجرعة 0.5 جم / كجم من العليقة الأساسية ، والمجموعة السادسة (BTK) ، تم جمع عينات مصل الدم لقياس بعض المؤشرات البيوكيميائية و هرموني الاستراديول والتستسترون وفي الوقت نفسه تم جمع بعض الأنسجة الاثني عشر ( V.L, CD. V/C ratio, No. of G.C. والخصية وإعدادها للدراسات المورفولوجية والجزيئية

أظهرت النتائج ارتفاع معنوي في دهون المصل في المجموعات (BT) (T) (B) وعلي النقيض انخفض مستوي دهون الدم في كل من المجموعة الضابطة والمجموعة (K) كما أكدت الدراسة ارتفاع هرموني الاستراديول والتستسترون في مصل الديوك التي تناولت كيمزيم والتي صاحبها انخفاض في مستوي دهون مصل الدم. لوحظ ارتفاع النشاط الكلي لمضادات الأكسدة (TAC). أثبتت الدراسة المورفولوجية في المجموعة التي تناولت (BTK) ارتفاع نسبة الخلايا المعوية الي عمق الجزء الغدي (Crypt) وعدد الخلايا المخاطية (Goblet). علاوة علي وجود تحسن في الحمض النووي (DNA) ، (RNA) ونسبها مع البروتين في خلايا الاثني عشر الذي يشير إلى تحسن في وظائف الأمعاء الدقيقة (حجم الخلية، انقسام الخلية، والقدرة الريبوسومية).

الكلمات الدالة: ديوك رومي - ريحان - زعتر - كيمزيم - زيموجن.