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## Research Article

# Impact of Some Medicinal Plants Supplement on Pregnant Rabbits Diet During Hot Summer Season

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## Abstract

**Background and Objective:** Rabbits are suffering from the heat stress conditions during summer season of Egypt so, productive and reproductive traits of rabbits are deleteriously affecting by more than 50%. Effect of ginger and curcumin addition in the diet of female rabbits during hot summer season to alleviate the negative effect of heat stress was the objective of this study. **Materials and Methods:** Forty five virgin mature healthy New Zealand White (NZW) female rabbits were used in this experiment. One week before mating, animals were divided into 3 equal groups, the 1st group was fed the basal ration (control group) while the 2nd and 3rd groups were fed the same basal ration supplemented with 250 mg daily from roots crushed of ginger or curcumin per doe, respectively. **Results:** Results showed that conception rate, litter size and litter weight at both birthing and weaning improved significantly due to addition of ginger or curcumin in the diet of female rabbits. Water intake values were lower while body weight and feed intake values were higher in rabbits received ginger or curcumin than rabbits not received any supplement. Cortisol level was lower while thyroid hormonal levels and progesterone level were higher in rabbits received ginger or curcumin in their diet than control. The physiological thermoregulatory parameters were lower in rabbits received ginger or curcumin than control rabbits. **Conclusion:** Supply of ginger or curcumin to rations of rabbits succeeded in improving the reproductive traits of female rabbits under stressful conditions of hot summer season in Egypt.

**Key words:** Ginger, curcumin, heat stress, rabbits, pregnancy, lactation, breeding

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

In tropical and sub-tropical states, climatic specific is the major restriction on animal productivity. Production and reproduction performance are deleteriously affected due to the severe heat stress conditions<sup>1</sup>. Exposure of rabbits to >30 THI (temperature-humidity index) units as severe heat stress during summer season in Egypt obligate unfavorably affects and extreme variations happen in biological purposes<sup>2</sup>. Alleviations of heat stress syndrome can be eliminating the negative effects on farm animals. The process of minimizing these effects is titled "amelioration process"<sup>3</sup>. Oxidative stress generally occur following exposure animals to heat stress in tropical regions which affects negatively on animals performance. The adverse influence of the heat stress conditions is a harmful impact on glutathione, ATP-ase and cholinesterase enzymes actions. In addition, glutathione is an antioxidant in animals to protect cells from oxidative damages and avoiding damage of important cellular components caused by reactive oxygen species such as free radicals, peroxides and lipid peroxides<sup>4</sup>. The ATP-ase and cholinesterase enzymes are also consider the main parts in metabolic pathways inside the living cell of any animal. Activities of ATP-ase, cholinesterase and glutathione enzymes are inhibition in any heat stressed animals<sup>5</sup>. The antioxidant activity is high in medicinal plants and antioxidants play an important role in protects cells from oxidative damages<sup>6</sup>. The gingerols increase the motility of the gastrointestinal tract<sup>7</sup>. Ginger significantly depressed lipid peroxidation by maintaining the activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase and the blood glutathione level increased in ginger fed rats<sup>8</sup>. The antioxidant action of curcumin was due to it turns as a scavenger of oxygen free radicals and keeps haemoglobin from oxidation<sup>9</sup>. curcumin also decreases the production of reactive oxygen species like super oxide anions, H<sub>2</sub>O<sub>2</sub> and nitrite radical generation and prevents oxidative damage during indomethacin-induced gastric lesion<sup>10-12</sup>. Moreover, curcumin contains essentials antioxidant activity and reduces cholesterol levels and increase high-density lipoprotein<sup>13</sup>. Curcumin can too enhance high glucose prompted neural faults by suppressing cellular stress and apoptosis<sup>14</sup>.

The present work was conducted to study the effect of ginger and curcumin addition to the diet of rabbits exposed to severe heat stress conditions during hot summer season in Egypt for improving the productive and reproductive traits of female rabbits during pregnancy and lactation periods.

## MATERIALS AND METHODS

**Experimental location:** This study was carried out in the Egyptian Atomic Energy Authority in Rabbits Farm, Biological Application Department, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, Inshas, Egypt (Latitude 31°12'N to 22°2'N, Longitude 25°53'E to 35°53'E) with cooperation with Animal Production Department, Faculty of Agriculture, Zagazig University.

**Time of duration:** The experimental work was carried out during summer season, July and August of 2017.

**Experimental ethics:** Experimental animals were cared using husbandry guidelines derived from Egyptian Atomic Energy Authority standard operating procedures with Animal Ethics Committee guidelines. These ethics contain relevant information on the endeavour to reduce animal suffering and adherence to best practices in veterinary care according to the International Council for Laboratory Animal Science guidelines.

**Experimental procedure:** Forty five healthy mature New Zealand white (NZW) virgin female rabbits were used in this research. Before 1 week from mating, animals were divided into three similar groups, 15 animals in each group, the 1st group was fed the basal ration (commercial pelleted diet) and kept as control group while the 2nd and 3rd groups were fed the same commercial pelleted diet but supplemented with 250 mg daily from roots crushed of ginger and curcumin per animal, respectively. The supplementation was lasted 2 months during summer season (July and August, 2017) including pregnancy and lactation periods. The powder of ginger or curcumin additives were mixed manually with commercial pelleted diet and offered individually once in the morning at 10:00 am. First 2 days from the beginning of the experiment was consider adjustment period and then female rabbits of the three experimental groups were offered to NZW male rabbits for mating through 2 days. Diagnosis of pregnancy was carried out after one week from mating by the abdominal palpation and female rabbits not pregnant were excluded from the experiment and continuation only on pregnant animal (8, 12 and 11 animals in 1st, 2nd and 3rd groups, respectively).

**Estimation of conception rate:** Conception rate was estimated and after kidding average litter size and litter weight at birth, litter size and litter weight at weaning and

morality rate during lactation period were also estimated. Conception rate (is the ratio of the number of does conceived to the total number of does mated multiplied by 100) was estimated. Litter size at birth (is the total number of kits given birth including still birth) and litter weights at weaning one by one were also estimated according to Habeeb *et al.*<sup>2</sup>.

**Rabbits feeding:** Rabbits of experimental groups were vaccinated with clostridia enterotoxaemia bloat at weaning. Female rabbits in the three groups were fed the same diet during experimental period according to Habeeb *et al.*<sup>2</sup>. The ingredients and chemical analysis of the commercial pelleted diet were as shown in Table 1.

**Rabbits housing:** The rabbitry building was naturally ventilated through wired windows. The animals were individually housed in galvanized wired battery cages (50×55×39 cm) and each cage was provided with a feeder, automatic nipple drinker and a crock. The crock was used to measure the water consumption after separating the automatic nipple drinker. Urine and faeces dropped from cages were cleaned daily.

**Estimation of meteorological data:** Air temperature (°C) and relative humidity (%) were measured inside the rabbitry

Table 1: Ingredients and chemical composition of the commercial pelleted diet<sup>a</sup> used in feeding of experimental rabbits during the experiment

Ingredients and chemical composition of the commercial pelleted diet	Percentage
<b>Ingredients of pelleted diet</b>	
Clover hay	41.50
Wheat bran	25.00
Yellow corn	15.00
Soybean meal (44% CP)	10.00
Molasses	5.00
Bone meal	1.75
Calcium carbonate	0.70
Sodium chloride	0.55
Vitamins and minerals premix*	0.35
DL-Methionine	0.15
<b>Chemical composition as DM (%)</b>	
Crude protein	18.10
Crude fiber	12.35
Ether extract	3.17
Nitrogen free extract	57.20
Ash	9.18
Digestible energy (kcal kg <sup>-1</sup> DM)	2600.00

\*Each kilogram of vitamins and minerals premix contained: 10000 IU vitamin A, 3900 IU vitamin D, 2 mg vitamin K, 50 mg vitamin E, 12 mg vitamin B<sub>12</sub>, 6 mg vitamin B<sub>2</sub>, 2 mg vitamin B<sub>6</sub>, 20.01 mg vitamin B<sub>1</sub>, 20 mg pantothenic acid, 50 mg niacin, 5 mg folic acid, 1.2 mg biotin, 12000 mg choline, 3 mg copper, 0.2 mg iodine, 75 mg iron, 30 mg manganese, 70 mg zinc, 0.1 mg selenium, 0.1 mg cobalt and 0.04 mg magnesium, <sup>a</sup>El-Mostafa Establishment for feed manufacturing, Shubra El-Enab, Minya El-Qamh, Sharkia Province state, Egypt

building using automatic thermo-hygrometer (Table 2). Each value from air temperature and relative humidity was the average of three measurements recorded at 12.00, 13.00 and 14.00 h once a day weekly. The combined effect of the ambient air temperature and relative humidity as temperature humidity index (THI) was calculated using the equation as follows<sup>15</sup>:

$$THI = db^{\circ}C - [(0.31 - 0.31 RH\%)(db^{\circ}C - 14.4)]$$

where, db°C is dry bulb temperature in Celsius and RH is relative humidity (%)/100. The obtained THI values were classified as follow: <27.8 = absence of heat stress, 27.8 to <28.9 = moderate heat stress, 28.9 to <30.0 = severe heat stress and 30.0 and more = very severe heat stress. Data in Table 2 shows that rabbits exposed to very severe heat stress during experimental period due to that average THI during experimental period was more than 30.0.

**Estimation of feed and water intakes:** Feed and water intakes were estimated for each rabbit at day 15 and at day 28 from pregnancy for the three experimental groups. Food intake was measured by subtracting the residuals of food from that offered for each doe. Water intake was estimated by measuring the difference in the water volume in the crocks of each rabbit. The daily difference in water volume within the crock at 10.00 h was calculated according to Habeeb *et al.*<sup>2</sup>. The evaporative water was considered in estimation of water intake. Each doe from the experimental groups were weighted at day 15 and at day 28 of pregnancy period.

**Estimation of blood biochemical components:** Blood sample were collected from ear vein into vacutainer tubes at day 15 and at day 28 of pregnancy for the three experimental groups. Serums were separated by centrifugation at 3,000 rpm for 15 min and were frozen and stored at -20°C until analysis. The concentrations of total protein, albumin, creatinine and urea-N were measured by quantitative enzymatic colorimetric methods using chemical commercial kits (Diamond Diagnostic Company, Egypt). The concentration of globulin calculated as the difference between total protein and albumin<sup>2,15</sup>. The Level of cortisol, T<sub>3</sub>, T<sub>4</sub> and progesterone hormones were estimated in serum by Radioimmunoassay (RIA) using coated tubes kit (DIAsource ImmunoAssays S.A. Rue du Bosquet, 2B-1348 Louvain-la-Neuve, Belgium)<sup>2</sup>. The tracer in the three hormones was labeled with iodine-125. The unknown samples or standards are incubated with 125I-labeled hormone in antibody-coated tubes. After incubation, the liquid contents of the tube are aspirated and the radioactivity is determined

Table 2: Air temperature, relative humidity and temperature-humidity index during experimental period (2017)

Experimental period	Temperature (°C)	Relative humidity (%)	Temperature humidity index (THI)
<b>July 2017</b>			
1st week	35.2	82.0	31.2
2nd week	35.5	83.0	31.2
3rd week	32.4	82.0	31.2
4th week	34.0	80.5	32.6
Mean ± SE	34.5 ± 0.64	81.8 ± 0.75	31.6 ± 0.70
<b>August 2017</b>			
1st week	35.0	70.0	33.1
2nd week	34.1	83.0	33.1
3rd week	34.7	83.0	28.9
4th week	36.5	86.0	30.7
Mean ± SE	35.07 ± 0.51	80.50 ± 3.75	31.45 ± 1.02
Overall mean	34.5	81.2	31.6 very severe heat stress

in using the computerized gamma counter (ISOCOMP1-MGM) in Biological Applications Department at Nuclear Research Centre. The sensitivity of hormonal assay values was 5.0, 4.0, 3.2 and 4.0 pg mL<sup>-1</sup> for P<sub>4</sub>, T<sub>3</sub>, T<sub>4</sub> and cortisol, respectively. The respective intra- and inter-assay coefficients of variation were 5.7 and 7.4% for P<sub>4</sub>, 7.2 and 8.5% for T<sub>3</sub>, 3.5 and 4.6% for T<sub>4</sub> and 3.5 and 4.1% for cortisol, respectively.

**Estimation of thermoregulatory parameters:** Rectal temperature was recorded by inserting a digital clinical thermometer into the rectum for one minute. The skin temperature was measured at one location between the neck and loin on the body surface while the thermometer was fixed on the bare skin. The ear temperature was measured by placing the thermometer into direct contact with the central area of the ear. Respiration rate was measured when the rabbit was at rest by counting the number of breaths for one minute by counting how many times the chest has risen. Thermoregulatory parameters were estimated once at day 15 and at day 28 of pregnancy for the three experimental groups according to Habeeb *et al.*<sup>2</sup>.

**Statistical analysis:** Data were statistically analyzed using procedure of Statistical Package for the Social Sciences (SPSS)<sup>16</sup> according to the following model:

$$Y_{ijc} = \mu + T_i + P_j + TP_{ij} + e_{ijc}$$

where,  $\mu$  is the overall mean,  $T_i$  is the fixed effect of treatments (1 = Control, 2 = Ginger, 3 = Curcumin),  $P_j$  is the fixed effect of pregnancy day (1 = day 15, 2 = day 28),  $TP_{ij}$  is interaction between treatment and pregnancy day and  $e_{ijc}$  is residual error. The significant differences among means were compared using Duncan's new multiple-range test<sup>17</sup>. Statistical differences between treatments in probability of the

conception rate and mortality rate were conducted by Chi-square test and significant results were subsequently evaluated using the multiple Z-tests to compare corresponding proportions.

## RESULTS

**Effect of ginger and curcumin treatments on conception rate in rabbits:** Supplemented pregnant female rabbits diet with ginger improved ( $p \leq 0.01$ ) conception rate (CR) from 53.3% to reach 80% and supplementation with curcumin increased ( $p \leq 0.01$ ) CR to reach 73%. The improvements in CR due to supplementation with ginger and curcumin were 50 and 37%, respectively compared with CR of pregnant rabbits not received any supplement (Table 3).

**Effect of ginger and curcumin treatments on total kits born, litter weight at both birthing and at weaning and mortality rate:** Total kits born at birth increased ( $p \leq 0.001$ ) by 85.7 and 77.1% due to supplement of ginger and curcumin in the diet of pregnant rabbits compared with the control group. Total kits at weaning increased ( $p \leq 0.001$ ) by 96 and 86% due to supplement of ginger and curcumin in the diet of pregnant rabbits compared with the control group. Litter size per animal increased in ginger and curcumin treated groups both at birth and weaning as compared to control group. Same trends were followed by increase in average kit weight and litter weight per animal both at birth and weaning. In addition, mortality rate decreased ( $p \leq 0.02$ ) by 23.0% and 19.5% due to ginger and curcumin treatments, respectively, compared with the control group (Table 4).

**Effect of ginger and curcumin on feed intake and water consumption:** The overall treatment effect showed that live body weight (LBW), feed intake (FI) and feed/water ratio (F/W) ratio increased and decreased water consumption due to

Table 3: Effect of ginger and curcumin treatments on conception rate

Items	Group one control	Group two ginger supplement	Group three curcumin supplement
Number of does at mating	15.0	15.0	15.0
Number of does pregnant	8.0	12.0	11.0
Conception rate, CR (%)*	53.3 <sup>b</sup>	80.0 <sup>a</sup>	73.0 <sup>a</sup>
Increase CR (%)	-	+50.0	+37.0

\*Statistical differences between treatments in probability of the conception rate were conducted by Chi-square test and significant results were subsequently evaluated using the multiple Z-tests to compare corresponding proportions

Table 4: Effect of ginger and curcumin treatments on litter size litter weight at birth and at weaning and mortality rate in kits of female rabbits during lactation period

Items	Group one control (8)	Group two ginger (12)	Group three curcumin (11)
<b>At birthing</b>			
Total kits born at birth	35.0	65	62
Number of kits increase	-	30	27
increase kits number (%)	-	85.7	77.1
Litter size per animal	4.37±0.29 <sup>b</sup>	5.80±0.20 <sup>a</sup>	5.70±0.18 <sup>a</sup>
Increase over control (%)	-	32.7 (p>0.01)	30.43 (p>0.01)
Average kits weight (g)	48.90±2.44 <sup>c</sup>	64.55±3.54 <sup>a</sup>	58.70±2.26 <sup>b</sup>
Increase over control (%)	-	32.0 (p>0.01)	20.04 (p>0.01)
Litter weight (g) per animal	213.69±10.0 <sup>c</sup>	374.4±14.3 <sup>a</sup>	334.6±10.52 <sup>b</sup>
Increase litter weight (%)	-	75.2 (p>0.01)	52.2 (p>0.01)
<b>At weaning</b>			
Total kits at weaning	28.0	55	52
Number of kits increase	-	27	24
Increase kits number (%)	-	96	86
Litter size per animal	3.50±0.20 <sup>c</sup>	5.0±0.10 <sup>a</sup>	4.5±0.20 <sup>b</sup>
Increase over control (%)	-	42.9 (p>0.01)	28.6 (p>0.01)
Average kits weight (g)	493.4±11.1 <sup>b</sup>	531.17±14.80 <sup>a</sup>	522.60±12.5 <sup>a</sup>
Increase over control (%)	-	7.7 (p>0.05)	5.9 (p>0.05)
Litter weight (g) per animal	1726.9±27 <sup>c</sup>	2655.9±30 <sup>a</sup>	2351.7±33 <sup>b</sup>
Increase over control (%)	-	53.8 (p>0.01)	36.2 (p>0.01)
No of dead kits	7.0	10.0	10.0
Mortality rate (%)*	20.0 <sup>a</sup> (7/35)	15.4 <sup>b</sup> (10/65)	16.1 <sup>b</sup> (10/62)
Reduction mortality rate (%)	-	23.0	19.5

Means in the same row within each item having different superscripts are differ significantly at p<0.05, \*Statistical differences between treatments in probability of the mortality rate were conducted by Chi-square test and significant results were subsequently evaluated using the multiple Z-tests to compare corresponding proportions. between brackets are number of pregnant rabbits in each group

supplemented diets of rabbits with ginger or curcumin. In addition, there are significant increases ( $p \leq 0.05$ ) in LBW and FI and F/W ratio values with advanced in pregnancy period. These differences between at 15 and at 28 days of pregnancy may be due to the change in age of animals as well as increase the embryos weight. However, water intake decreased significantly ( $p \leq 0.05$ ) with advanced in animal age. No significant differences were observed due to interaction between treatment and pregnancy day on live body weight, feed intake and water consumption as well as feed/water ratio in pregnant rabbits (Table 5).

### Effect of ginger and curcumin on blood biochemical components

**Plasma protein fractions concentrations:** Supplemented ginger or curcumin in the diet of female rabbits improved significantly total protein, albumin and globulin concentrations at both 15 and 28 days from pregnancy period. In addition, total protein and globulin concentrations in the blood of female rabbits supplemented with curcumin were

higher than total protein and globulin concentrations in blood of female rabbits supplemented with ginger. No significant ( $p > 0.05$ ) differences were observed in each of total protein, albumin and globulin concentrations due to progressive in the pregnancy period (Table 6).

**Plasma urea and creatinine concentrations:** Supplemented diets of rabbits with ginger or curcumin decreased significantly urea and creatinine concentrations with compared to control group (Table 6). No significant ( $p > 0.05$ ) differences was observed in urea and creatinine concentrations due to progressive in the pregnancy period as observed in Table 6. However, no significant differences due to interaction between treatment and pregnancy day on these blood parameters in pregnant rabbits.

### Effect of ginger and curcumin on hormonal levels

**Cortisol hormonal level:** The level of cortisol in female rabbits at day 15 and at day 28 of pregnancy decreased ( $p \leq 0.0001$ ) due to supplement with ginger and curcumin.

Table 5: Effect of ginger and curcumin treatments on live body weight, feed intake and water consumption in pregnant rabbits

Items	Experimental days	Group one control (8)	Group two ginger (12)	Group three curcumin (11)	Pregnancy day effect
Body weight (g)	At day15	2818.50±23.7	2822.0±21.7	2824.0±20.0	2821.5±3.6 <sup>b</sup>
	At day 28	2970.00±20.9 <sup>b</sup>	3189.7±17.1 <sup>a</sup>	3208.1±18.9 <sup>a</sup>	3122.6±7.9 <sup>a</sup>
	Treatment effect	2894.25±25	3005.9±24	3016.1±26	
Increase over control (%)			7.4 (p<0.05)	8.1 (p<0.05)	
Feed intake (g/day)	At day15	120.20±1.43 <sup>b</sup>	138.2±1.26 <sup>a</sup>	136.7±1.38 <sup>a</sup>	131.7±5.9 <sup>b</sup>
	At day 28	135.53±1.23 <sup>b</sup>	168.2±1.66 <sup>a</sup>	168.9±1.28 <sup>a</sup>	157.5±1.2 <sup>a</sup>
	Treatment effect	127.90±6.4	153.2±12.5	152.8±13.4	
Increase over control (%)			19.8 (p<0.01)	19.5 (p<0.01)	
Water consumption (mL/day)	At day15	758.0±6.27 <sup>a</sup>	699.0±6.12 <sup>b</sup>	703.3±6.27 <sup>b</sup>	720.1±6.1 <sup>a</sup>
	At day 28	730.0±6.17 <sup>a</sup>	658.6±5.90 <sup>b</sup>	659.6±6.51 <sup>b</sup>	682.7±3.4 <sup>b</sup>
	Treatment effect	744.0±5.9	678.8±4.3	681.5±5.2	
Decrease over control (%)			8.8 (p<0.05)	8.4 (p<0.05)	
Feed/water ratio	At day15	0.159±0.001 <sup>b</sup>	0.198±0.001 <sup>a</sup>	0.194±0.002 <sup>a</sup>	0.174±0.01 <sup>b</sup>
	At day 28	0.186±0.001 <sup>b</sup>	0.255±0.002 <sup>a</sup>	0.256±0.001 <sup>a</sup>	0.223±0.02 <sup>a</sup>
	Treatment effect	0.172±0.009	0.226±0.027	0.224±0.025	
Increase over control (%)			31.4 (p<0.01)	30.2 (p<0.01)	

Means in the same row within each item having different superscripts are differ significantly at p<0.05, between bracts are number of pregnant rabbits in each group

Table 6: Effect of Ginger and curcumin treatments on blood parameters

Items	Experimental days	Group one control (8)	Group two Ginger (12)	Group three curcumin (11)	Pregnancy day effect
Total protein (g dL <sup>-1</sup> )	At day 15	5.00±0.12 <sup>b</sup>	5.00±0.18 <sup>a</sup>	6.02±0.13 <sup>a</sup>	5.64±0.33
	At day 28	5.10±0.11 <sup>c</sup>	5.70±0.17	6.01±0.58 <sup>a</sup>	5.60±0.27
	Treatment effect	5.05±0.04	5.80±0.08	6.02±0.004	
Increase over control (%)			14.90 (p<0.01)	19.20 (p<0.01)	
Albumin (g dL <sup>-1</sup> )	At day 15	2.80±0.08 <sup>b</sup>	3.10±0.10 <sup>a</sup>	3.30±0.12 <sup>a</sup>	3.07±0.15
	At day 28	2.90±0.06 <sup>b</sup>	3.30±0.09 <sup>a</sup>	3.10±0.08 <sup>a</sup>	3.10±0.12
	Treatment effect	2.85±0.04	3.20±0.08	3.20±0.08	
Increase over control (%)			12.30 (p<0.05)	12.30 (p<0.05)	
Globulin (g dL <sup>-1</sup> )	At day15	2.20±0.16 <sup>b</sup>	2.80±0.17 <sup>a</sup>	2.70±0.20 <sup>a</sup>	2.57±0.19
	At day 28	2.20±0.13 <sup>c</sup>	2.40±0.63 <sup>b</sup>	2.90±0.12 <sup>a</sup>	2.50±0.21
	Treatment effect	2.20±0.004	2.60±0.17	2.80±0.08	
Increase over control (%)			18.20 (p<0.01)	27.30 (p<0.01)	
Urea (mg dL <sup>-1</sup> )	At day15	13.19±0.54 <sup>a</sup>	11.01±0.43 <sup>b</sup>	11.23±0.29 <sup>b</sup>	11.81±0.71
	At day 28	12.65±0.45 <sup>a</sup>	10.46±0.24 <sup>b</sup>	10.16±0.64 <sup>b</sup>	11.09±0.80
	Treatment effect	12.92±0.23	10.74±0.23	10.70±0.45	
Decrease over control			16.90 (p<0.01)	17.20 (p<0.01)	
Creatinine (mg dL <sup>-1</sup> )	At day15	1.17±0.56 <sup>a</sup>	0.94±0.05 <sup>b</sup>	0.83±0.03 <sup>c</sup>	0.98±0.10
	At day 28	1.11±0.51 <sup>a</sup>	0.88±0.07 <sup>b</sup>	0.91±0.04 <sup>b</sup>	0.97±0.07
	Treatment effect	1.14±0.03	0.91±0.03	0.87±0.03	
Decrease over control (%)			20.20 (p<0.01)	23.70 (p<0.01)	

Means in the same row within each item having different superscripts are differ significantly at p<0.05, between bracts are number of pregnant rabbits in each group

**Thyroid hormonal (T<sub>4</sub> and T<sub>3</sub>) levels:** Adding ginger or curcumin to the diet of female rabbits affect positively on thyroid hormonal levels. The levels of thyroid hormones in rabbits treated with ginger were significantly increased for T<sub>3</sub> and for T<sub>4</sub> at both 15 and 28 days of pregnancy. These levels were higher (p≤0.001) than levels of T<sub>3</sub> and T<sub>4</sub> hormones in control rabbits at day 15 and day 28 of pregnancy. No significant differences in the levels of cortisol, T<sub>3</sub> and T<sub>4</sub> hormones due to advance in pregnancy period. However, no significant differences due to interaction between treatment and pregnancy day on hormonal levels in pregnant rabbits (Table 7).

**Progesterone (P<sub>4</sub>) hormonal level:** Adding ginger or curcumin to the diet of female rabbits affect positively on P<sub>4</sub> hormonal level. The levels of P<sub>4</sub> hormone increased (p≤0.001) due to supplemented diets of rabbits with ginger and curcumin. P<sub>4</sub> level decreased significantly (p≤0.05) with advanced in animal age. P<sub>4</sub> level was higher significantly by 22.65% at 15 day of pregnancy than that at 28 day of pregnancy before kidding (Table 7).

**Effect of ginger and curcumin on physiological thermoregulatory parameters:** Rectal, skin, ear temperature and respiration rate values were lower significantly in pregnant rabbits received ginger or curcumin than those

Table 7: Effect of ginger and curcumin treatments on hormonal levels

Hormones (ng mL <sup>-1</sup> )	Experimental days	Group one control (8)	Group two ginger (12)	Group three curcumin (11)	Pregnancy day effect
Cortisol	At day15	26.20±0.69 <sup>a</sup>	18.80±0.49 <sup>b</sup>	19.90±0.41 <sup>b</sup>	21.66±2.3
	At day 28	25.60±0.19 <sup>a</sup>	19.30±0.34 <sup>b</sup>	18.90±0.39 <sup>b</sup>	21.27±2.2
	Treatment effect	25.90±0.25	19.10±0.17	19.40±0.20	
Decrease over control (%)			26.30 (p<0.01)	25.10 (p<0.01)	
T <sub>3</sub>	At day15	1.20±0.04 <sup>b</sup>	1.34±0.03 <sup>a</sup>	1.40±0.04 <sup>a</sup>	1.31±0.06
	At day 28	1.08±0.02 <sup>b</sup>	1.25±0.05 <sup>a</sup>	1.33±0.05 <sup>a</sup>	1.22±0.08
	Treatment effect	1.14±0.05	1.30±0.04	1.37±0.03	
Increase over control (%)			14.00 (p<0.01)	20.20 (p<0.01)	
T <sub>4</sub>	At day15	33.50±1.83 <sup>b</sup>	46.00±0.68 <sup>a</sup>	44.10±1.02 <sup>a</sup>	41.2±3.96
	At day 28	32.70±1.71 <sup>b</sup>	44.90±0.23 <sup>a</sup>	44.70±0.49 <sup>a</sup>	40.8±4.11
	Treatment effect	33.10±0.33	45.45±0.46	44.40±0.25	
Increase over control (%)			37.30 (p<0.01)	34.10 (p<0.01)	
P <sub>4</sub>	At day15	11.85±0.35 <sup>b</sup>	13.30±0.58 <sup>a</sup>	13.80±0.75 <sup>a</sup>	12.98±0.60 <sup>a</sup>
	At day 28	8.26±0.55 <sup>c</sup>	10.80±0.59 <sup>b</sup>	11.07±0.89 <sup>a</sup>	10.04±0.91 <sup>b</sup>
	Treatment effect	10.06±1.49	12.05±1.04	12.44±0.14	
Increase over control (%)			19.80 (p<0.01)	23.70 (p<0.01)	

Means in the same row within each item having different superscripts are differ significantly at p<0.05, between bracts are number of pregnant rabbits in each group

Table 8: Effect of ginger and curcumin treatments on physiological thermoregulatory parameters

Items	Experimental days	Group one control (8)	Group two ginger (12)	Group three curcumin (11)	Pregnancy day effect
Rectal temperature (°C)	At day15	40.02±0.04 <sup>a</sup>	39.40±0.05 <sup>b</sup>	39.50±0.06 <sup>b</sup>	39.64±0.20
	At day 28	40.01±0.03 <sup>a</sup>	39.50±0.10 <sup>b</sup>	39.40±0.98 <sup>b</sup>	39.64±0.19
	Treatment effect	40.02±0.01 <sup>A</sup>	39.45±0.05 <sup>B</sup>	39.45±0.10 <sup>B</sup>	
Skin temperature (°C)	At day15	40.10±0.02 <sup>a</sup>	39.30±0.07 <sup>c</sup>	39.50±0.09 <sup>b</sup>	39.63±0.24
	At day 28	40.03±0.05 <sup>a</sup>	39.30±0.06 <sup>c</sup>	39.40±0.04 <sup>b</sup>	39.58±0.23
	Treatment effect	40.07±0.04 <sup>A</sup>	39.35±0.05 <sup>B</sup>	39.45±0.05 <sup>B</sup>	
Ear temperature (°C)	At day15	39.20±0.03 <sup>a</sup>	38.70±0.05 <sup>b</sup>	38.30±0.08 <sup>c</sup>	38.73±0.27
	At day 28	39.30±0.04 <sup>a</sup>	38.80±0.08 <sup>b</sup>	38.40±0.06 <sup>c</sup>	38.83±0.27
	Treatment effect	39.25±0.05 <sup>A</sup>	38.75±0.10 <sup>B</sup>	38.35±0.10 <sup>B</sup>	
Respiration rate (rpm)	At day15	109.93±0.90 <sup>a</sup>	90.80±1.46 <sup>b</sup>	89.90±1.22 <sup>b</sup>	96.88±6.7
	At day 28	110.30±0.73 <sup>a</sup>	91.10±1.15 <sup>b</sup>	91.70±1.09 <sup>b</sup>	97.70±6.4
	Treatment effect	110.12±0.2 <sup>A</sup>	90.95±0.15 <sup>B</sup>	90.80±0.90 <sup>B</sup>	

Means in the same row within each item having different superscripts (a,b,c, A,B) are differ significantly at p<0.05, between bracts are number of pregnant rabbits in each group

values of non-treated animals at both day 15 or at day 28 of pregnancy. No significant difference between ginger and curcumin effects on these parameters. In addition, no significant ( $p>0.05$ ) differences in all thermoregulatory parameters was found due to pregnancy day. These results indicate that supplemented diet of rabbits with ginger and curcumin succeeded in alleviate the negative effect of heat stress conditions on pregnant rabbits. However, no significant differences due to interaction between treatment and pregnancy day on physiological thermoregulatory parameters in pregnant rabbits (Table 8).

## DISCUSSION

Supplemented female rabbits diet with ginger or curcumin improved CR. The improved in CR in supplemented rabbits may be due to the increase in P<sub>4</sub> level compared to non-treated animals. The increase in P<sub>4</sub> in treated groups may be owing to that curcumin has strong phytoestrogenic

properties and its action on the reproductive system is mediated by ovarian steroid hormone synthesis and/or steroid receptors in the ovaries<sup>17-19</sup>. The ovaries of rabbits fed high doses of turmeric released more P<sub>4</sub> in vitro than the ovaries of control does and suggest that ovarian follicular genesis, oogenesis and fertility are affected by turmeric through changes in the output of ovarian steroid hormones<sup>20</sup>. Supplementation with ginger or curcumin in female diet improved the productive and reproductive performance of female rabbits under heat stress condition. Supplemented female rabbits diet with ginger or curcumin improved litter size and kit weight at birth, litter weight and bunny weight at weaning and increased viability percentage in bunnies from birthing till weaning. The increase in litter size at birth due to treatment by curcumin or ginger may be due to the increasing number of both primary and secondary follicles and consequently increase the ovulations number. The generation and growth of rabbit follicles were caused by the action of curcumin on ovarian cell proliferation<sup>21</sup>. The improved in



viability in bunnies from birthing to weaning may be due to the antioxidant activity and the antimicrobial activity and essential oils in these medicinal plants which were secreted in the milk of their mothers<sup>22</sup>. Dietary curcumin can improve rabbit production, ovarian function, growth, or viability and increased the number of primary follicles, as well as the diameter of primary, secondary and tertiary follicles<sup>21</sup>. The same authors suggested that curcumin stimulates rabbit fecundity in two ways by encouraging the manufacture of primary ovarian follicles and by stimulating follicle growth throughout follicular genesis. In addition, curcumin increased the number of live born and weaned rabbits due to that curcumin promotes rabbit reproductive efficiency by decrease bunny mortality and also improves the viability of adult does<sup>21</sup>.

Supplementing with ginger or curcumin in the feed of heat stressed rabbits alleviate the negative effect of heat stress conditions. The two supplements affect positively on live body weight and feed intake and decreased water intake. The improvement in does supplemented with ginger or curcumin may be due to the increase in feed consumption, especially, increased digestible crude protein intake in the medicinal plants<sup>23</sup>. The essential oils and antioxidant activity in these medicinal plants and increase in the levels of anabolic hormonal levels ( $T_4$  and  $T_3$ ) in supplemented groups may be responsible the positive effect in body weight of does. In addition, curcumin or ginger may be having a stimulating effect on the rumen proper functions and digestion and the higher digestibility for groups supplemented led to increase the absorbed nutrients from small intestine, increased efficiency of nutrient utilization and led to more gain<sup>24,25</sup>.

The increase in doe weight due to day of pregnancy may be due to the change in age of animals as well as increase the embryos weight. The antioxidant activity in medicinal plants as well as increases the feed consumption in supplemented groups may be increased blood proteins fraction. Ginger and curcumin supplementation may be increased protein biosynthesis due to an increase in thyroid activity, as well as higher protein digestibility, which led to higher blood plasma total protein and albumin concentrations and decreased the end product of protein catabolism i.e. urea and creatinine. The enhancement in the metabolism of essential and volatile oils included in the medicinal plants may be also had the positive effect on some blood metabolic parameters and improved animal immunity function<sup>26,27</sup>. In addition, the increase in the concentration of protein fractions in supplemented rabbits may reflect an increase in the hepatic function when rabbits

fed on these medicinal plants and these findings suggest that these medicinal plants may increase the metabolic rate and increased muscle protein synthesis and protein deposition<sup>28,29</sup>. Supplemented with ginger or curcumin in the feed of heat stressed rabbits increased blood anabolic hormonal levels ( $T_4$ ,  $T_3$  and  $P_4$ ) and decreased the level of stress hormone (cortisol). The increases of  $T_3$  and  $T_4$  levels in supplemented rabbits may be due to that the essential and volatile oils included in the medicinal plants had the positive effect on thyroid gland activity to increase its hormones to blood as a result of increasing the metabolic rate. The decrease in cortisol level due to treatments may be due to that supplements affect positively on adrenal cortex and ACTH hormone to decrease secretion of cortisol as a result of alleviation the negative effect of heat stress conditions<sup>27</sup>. The increase in  $P_4$  in treated groups may be owing to that curcumin and ginger have strong phyto-estrogenic properties which improved the reproductive system by ovarian steroid hormone synthesis and/or steroid receptors in the ovaries<sup>19</sup>. The physiological thermoregulatory parameters of supplemented pregnant female rabbits with ginger or curcumin exposed to hot summer season were significantly lower than those of the control ones. Similarly, rectal temperature and respiration rate decreased as a function of feeding medicinal plant as ginger to growing NZW rabbits when compared with control group<sup>25</sup>. The reduced in physiological thermoregulatory parameters of supplemented pregnant female rabbits with ginger or curcumin may be attributed to the that supplements increase the antioxidant activity and antioxidants play an important role in alleviated the negative effects of heat stress on treated rabbits<sup>30</sup>.

Ginger or curcumin addition to the diet of rabbits has a useful implication in alleviating the adverse effects of heat stress conditions during hot summer season on rabbits. These medicinal plants may be applied in rabbit nutrition in the future without any side effect or limitation in application

## **CONCLUSION AND RECOMMENDATIONS**

It can be concluded that supply of ginger or curcumin to rations minimize the impact of the hot summer season of Egypt on female rabbits and succeeded in alleviating the adverse effects of heat stress conditions on productive and reproductive parameters of rabbits.

We recommended supply of ginger or curcumin to ration of pregnant rabbits during stressful conditions of hot summer season in Egypt to increase conception rate in does and decrease mortality rate in their kids.

## SIGNIFICANCE STATEMENT

This study discovers supply of ginger or curcumin to ration of rabbits during pregnancy period could be beneficial for increasing reproductive performance. This study will help the researcher to uncover the critical areas of importance of medicinal plant additive to ration of pregnant rabbits that many researchers were not able to explore. Thus a new theory on supply of ginger or curcumin to ration of rabbits during pregnancy period alleviating the adverse effects of heat stress conditions on heat stressed rabbits during summer season of Egypt may be arrived at.

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