3. MATERIALS AND METHODS

The present experimental work was carried out at the Rabbitry Research Laboratory belonging to Animal and Fish Production Department, Faculty of Agriculture (Saba Basha), Alexandria University. This study was undertaken during the summer season of Alexandria City during the period from July to August 2013.

The study investigated the effects of garlic, ginger and their mixtures and ginseng on the growth performance, carcass, organs, haematological indices and serum biochemistry of weaned V-line rabbits.

3.1. Processing of garlic and ginger and source of ginseng:

The garlic bulbs were divided into cloves which were cut into chips and then drying at 37ºC for 24 h. The plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to sunlight was avoided to prevent the loss of active components. Slices of dried ginger were also pounded, milled and stored. Powdered ginger and garlic were later incorporated in the diets.

Ginseng was purchased from the Fathalla market store in Alexandria.

3.2. Experimental animals and management:

Forty five weaned V-line rabbits of both sexes with initial weights of 812.0 ± 11.3 g were used for the study. The rabbits were randomly allocated to five treatments groups of 9 rabbits each. Each treatment was further sub-divided into 3 replicate of 3 rabbits. The rabbits were weighed at the beginning of the experiment to obtain their initial body weight and subsequently biweekly. Feed and water were supplied *ad libitum* throughout the experimental period of 6 weeks. The rabbits were housed in galvanized wire cages with flat deck (50 x 50 x 40 cm) three rabbits in each cage, in a well-ventilated building (natural through the windows). Before starting the experiment, all cages were cleaned and disinfected by fire. All animals were kept under the same managerial and environmental conditions. A cycle of 16 hours of light and 8 hours of dark were provided throughout the experiment. Animals were fed experimental diets in a pellet form. All cages were equipped with feeding hoppers made of galvanized steel sheets with automatic drinkers with nipples for drinking. Feed and water were provided *ad libitum* throughout the experimental period from 6 to 12 weeks of age by using high standard hygiene and careful management. The incidence of dangerous diseases was largely avoided and rabbits have never been treated with any kind of systematic vaccination or medication. Urine and faces dropped from cages on the floor were removing every day in the morning.

3.3. Experimental diets:

Five experimental diets were formulated such that diet 1 contained neither garlic or ginger nor ginseng. Diets 2 and 3 contained 0.25% garlic and 0.25% ginger, respectively. Diet 4 contained a mix of 0.25% garlic and 0.25% ginger, while diet 5 contained 10 g ginseng / 100 Kg diet. The composition and calculated analysis of the basal experimental diet was presented in Table 1. Pellets of the experimental diets were made as follow, pelleting was initiated by molasses addition as binding material and then all diet ingredients were pressed at 70Cº, after that pellets were cooled. The basal experimental diet was formulated to cover all essential nutrient requirements for growing rabbits according to NRC (1977).

3.4. Performance traits:

3.4.1. Body weight, feed intake, feed conversion ratio and performance index:

Individual body weight and feed intake were taken weekly from 8 weeks until 16 weeks of age.

Table 1: Composition and chemical analyses of basal diets.

Ingredients	Experimental diet %
Barley	20.00
Yellow corn	14.80
Wheat bran	10.00
Berseem hay	30.20
Soybean meal (44 %)	19.60
Molasses	3.00
Limestone	1.00
Di-calcium phosphate	0.30
Salt	0.50
Vit. And Min. Mix. *	0.30
Lysine	0.15
Methionine	0.15
Total	100
Chemical Analysis (as fed**)	
Crude protein, %	17.06
Ether extract, %	2.75
Crude fiber, %	13.80
Nitrogen free extract, %	54.10
Ash, %	8.50
Methionine, %	0.18
Cystine, %	0.24
TSAA, %	0.42
DE Kcal / Kg	2696

*Vitamin/trace mineral premix provides the following (per kg of diet): vitamin A, 1.800 mg retinol; vitamin E, 6.67 mg D-a-tocopherol; menadione, 2.5 mg; vitamin D3, 50mg cholecalciferol; riboflavin, 2.5 mg; Ca pantothenate, 10 mg; nicotinic acid, 12 mg; choline chloride, 300 mg; vitamin B12, 4 mg; vitamin B6, 5 mg; thiamine, 3 mg; folic acid, 0.50 mg; biotin, 0.02 mg; Mn, 80 mg; Fe, 40 mg; Cu, 4 mg; Se, 0.10 mg.
** Calculated according to NRC (1977).

From these data the relative growth rate, feed conversion ratio and performance index were calculated on a group basis as follows:

$$\text{Relative growth rate} = \frac{W2 - W1}{1/2\,(W2+W1)} \times 100$$

Where as:

W1= the initial body weight

W2 = the final body weight

Feed conversion ratio (FCR) was calculated as:

$$= \frac{\text{Feed consumed (g) during a certain period}}{\text{Body weight gain (g) during the same period}}$$

Performance index was calculated according to (North, 1981):

$$\text{Performance index} = \frac{\text{Final live body weight (kg)}}{\text{Feed conversion ratio}} \times 100$$

3.4.2. Mortality rate:

Mortality and the clinical health status of all rabbits were monitored daily and mortality percentage was calculated as follows if occared:

$$\text{Mortality \%} = \frac{\text{Number of dead animals}}{\text{Total number of animals at start}} \times 100$$

3.5. Carcass traits:

At the end of growing period, six rabbits of 12 weeks of age were taken randomly from each treatment, fasted for 12 hrs, weighed, slaughtered and weighed after complete bleeding, skinned and eviscerated. Immediately after evisceration weight of the dressed carcass free from any internal organs was recorded. Small intestine, caecum and large intestine weights (full and empty) were taken. Dressed carcass percentages were evaluated as described by Fernell *et al*. (1990) as follows:

$$\text{Dressed percentage (\%)} = \frac{\text{Fat weight + Giblets + Carcass weight}}{\text{Live body weight}} \times 100$$

3.6. Blood hematological study:

At the end of the feeding trial, 6 rabbits were selected from each treatment group, starved of food but not water for 12 hours. 4 ml of blood sample was taken from the ear vein with a sterile syringe. 2 ml of the blood was put into a bijon bottle containing ethylene diamine tetracetic acid (EDTA) as an anticoagulant for haematological assay. The remaining 2ml of the blood sample was put into a sterile vacutainer tube without an anticoagulant for serum biochemical analysis. The haematological assay was carried out to determine erythrocyte indices such as packed cell volume (PCV), and haemoglobin (Hb) values.

Red blood cell (RBC) counts were counted on an AO Bright line hemocytometer using a light microscope at 400X magnification after diluting blood samples 200 times with a physiological saline (0.9% NaCl solution) before counting (Natt and Herrick, 1952). White blood cell (WBC) were counted on an AO Bright line hemocytometer using a light

microscope at 100X magnification after diluting blood samples 20 times with a diluting fluid (1% acetic acid solution with a little of Leishman's stain) before counting (Hepler, 1966).

Differential leucocytic count was examined according to the method of Lucky (1977). A drop of heparinzed blood was spread on a glass slide, quickly air dried, fixed by methyl alcohol for 3- 5 min. then stained with Giemsa's stain for 20 minutes after that rinsed under slow water current and dried gently between two filter paper. Stained blood sample was examined using oil immersion lens. The percentage of each type of cells was calculated according to Schalm, (1986). Hemoglobin (Hb) concentration as (g/dl) was estimated by cyanomethe-moglobin method according to Eilers (1967). Wintrobe hematocrit tubes were used for determination of packed cell volume (PCV) as (%). Blood was centrifuged for 20 minutes on 4000 rpm, and then PCV volume was obtained by reading the packed cell volume on the graduated hematocrit tubes.

3.7. Biochemical parameters of blood:

Blood samples were taken from four rabbits from each group at the end of the productive experiment (at 12 weeks of age) to study the influence of experimental diets on some blood constituents. All tests; total protein, albumin, total lipids, cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, creatinine, uric acid, urea and glutamine aminoacid transferease (GOT), alkaline phosphatase, glucose, total antioxidant capacity (TAC), lipid peroxide Malondialdehyde (MDA) and glutathione peroxidase, were carried out using the commercial kits produced by Human (Max-Planck-Ring 21-D-65205 Wiesbaden, Germany). Globulin concentration was calculated by the difference between total protein and albumin, since the fibrinogen usually comprises a negligible fraction (Sturkie, 1986).

3.8. Digestibility trial:

At 13 weeks of age, fifteen male rabbits (three rabbits from each treatment) were randomly taken after the termination of fattening trial. Rabbits within each treatment were randomly housed individually in metabolic cages that allowed separation of feces and urine. A preliminary period of 7 days was followed by five days for measurements of actual consumed feed, feces and collection of feaces and urine. The animals were fed twice daily at 8 a.m. and 4 p.m. Water was available all time. Feces of each rabbit was collected quantitatively once a day before offering the morning meal at 8 a.m. Samples(100%) of daily feces of each rabbit were stored at -20^0C. The five days combined collection fecal samples were kept for routine analysis. Fecal samples were oven dried at 60^0C for 48 h (partial drying) then ground through a 1 mm screen on a wiley mill grinder. Samples were composite per treatment per animal for analysis.

Representative samples of feed offered and feces of each rabbit were chemically analyzed for determinations of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash which were carried out according to A.O.A.C. (1995) methods. Nitrogen free extract (NFE) was determined by difference.

3.9. Statistical analysis:

Data of each growing and digestion experiment were analyzed using one-way ANOVA of GLM procedure of SAS® (SAS Institute, 2000). Significant differences between means were detected using mew Duncan multiple range test (Duncan, 1955).