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GROWTH PERFORMANCES AND SEMEN CHARACTERISTICS OFNZW RABBITS FED BIOLOGICALLY TREATED JOJOBA MEAL WITH OR WITH OUT *ALPINIA GALANGA* UNDER NORTH SINAI CONDITION

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SUMMARY

This study included two experiments, the first aimed at investigating the impact of biologically treated Jojoba meal with or without Alpinia galanga on growth performance, some blood parameters and histological indices of some organs in growing rabbits. The second experiment aimed at assessing the effect of these dietary treatments on the semen characteristics and histological characteristics of testicular sections of rabbit bucks. For the first experiment, thirty unsexed weanling NZW rabbits about 35 days were randomly divided into three groups of initial mean weight of 653 ± 45.03 g. The 1st group was fed on the basal diet and served as a control group (CON), The 2nd group was fed basal diet containing 10% Jojoba meal treated with Lactobacillus acidophilus (JML) and the 3rd group was fed basal diet containing 10% Jojoba meal treated with Lactobacillus acidophilus and 0.25% Alpinia galangal rhizomes (JMLA). Results revealed that dietary treatments had no significant effects on growth traits, feed intake, carcass traits and blood parameters of growing rabbits.

In the 2nd experiment, four males from the first experiment were chosen from each group at 13 weeks of age with an average weight of 2.270 ± 0.057 kg and were allowed the same dietary treatments and kept until six months old. Results showed that rabbit bucks fed JML diet showed a decrease (P>0.05) in semen characteristics. While, rabbits fed JMLA diet exhibited improvements in semen characteristics.

It could be concluded that using biologically treated Jojoba meal with Alpinia galanga can enhance the productivity and improve the testicular structure and function of rabbits.

Keywords: Rabbits, Jojoba meal, Alpinia galanga, growth performance, semen characteristics

INTRODUCTION

There is a large gap between demand and available of protein sources used for animal feed, which results in high feeding cost. The main protein source in rabbit diets is soybean meal. So, it is necessary to evaluate untraditional protein sources that could be used in feeding rabbits and decrease the cost of rabbit feeding.

Jojoba (*Simmondsia chinensis*) is desert shrub that grows in arid or semi-arid regions. In Egypt, the cultivated areas were concentrated in Ismailia, New Valley, El-Sharkia and Assiut governorates (El-Rayes, 2010). Jojoba meal (JM) is the residual meal remaining after oil extraction, and it includes 20–33% crude protein that can be added to animal diets as a partial alternative to conventional sources of protein (Elsanhoty *et al.,* 2017). Jojoba meal has some anti-nutritional compounds like simmondsin, bitter compounds, trypsin enzyme inhibitors and phytic acid. These components cause a decrease in feed intake and body weight in animals fed JM (Abbott *et al.,* 2004). Bellirou *et al.* (2005) explained that a variety of methods, including heating, solvent extraction, chemical treatments, and biological fermentation, can be used to remove the anti-nutritional compounds from JM. However, it contains alkaloids, carbohydrates, flavonoids, proteins, phenols, and tannins. These components give JM various physiological properties, such as antibacterial, antioxidant, and antitumor capabilities (Imchen *et al.,* 2022).

Alpinia galanga, also known as "Khulanjan" in Arabic and "Galanga" in English, is a native of Java and Sumatra. *A. galanga* contains volatile oil, consisting of cineol and resin composed of galangol, amino acids, sterols and triterpenoids in the rhizome (Akhtar *et al.,* 2010).

The rhizome of *A. galanga* is a high-quality aphrodisiac medication that also has anti-inflammatory, anti-ulcer, and anthelmintic properties. Its essential oils have demonstrated antibacterial action against Gram-positive bacteria. (Puri, 1971 and Akhtar *et al.,* 2010).

The aim of this study was to evaluate the influences of biologically treated Jojoba meal with *Lactobacillus Acidophilus* with or without *Alpinia galanga* on rabbits' growth performance, blood parameters and reproductive performance under North Sinai conditions, Egypt.

MATERIALS AND METHODS

This study was conducted at the Rabbitry Farm of the Department of Animal and Poultry Production, Collage of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. Jojoba meal (*Simmondsia chinensis*) was purchased from private Oil Mill at Sadat City, Menoufyia Governorate, Egypt.

This study is consisted of two experiments. The first experiment (1st Exp.) aimed to investigate the impact of tested diets on growth performance, hematological parameters, lipid profile and immunity response and histological features of some organs in growing New Zealand White (NZW) rabbits. The farm trial lasted for eight weeks (September-October, 2023). The second experiment (2ndExp.) aimed to assess the effects of the same dietary treatments on semen characteristics, reproductive performance and histological characteristics of testicular sections of the bucks.

Treatment of Jojoba meal by biological treatment:

A bacterial strain (*Lactobacillus acidophilus*) was obtained from the Friendly Human Bacterial Unit at the National Research Center, Dokki, Cairo, Egypt. It was cultivated in MRS broth (De Man–Rogosa–Sharpe) and then placed in skimmed milk (120 mg skimmed milk powder per liter of distilled water) under sterile conditions. The mixture was then incubated at 37°C for 48 hours to curdle. Bulk cultures were prepared one day before the treatment application. The resulting culture was sprayed onto the sterilization Jojoba meal, JM (10% v/w). The JM was then placed in polyethylene bags, tightly sealed and incubated for 21 days at 26°C under anaerobic and dark conditions, as described by Verbiscar *et al.* (1981). The treated JML was sun-dried and stored in room temperature until it used.

Experimental design and diets:

The first experiment (1st Exp.)

Thirty unsexed weanling New Zealand White (NZW) rabbits aged 35 days were randomly assigned to three equal treatment groups (10 rabbits each). Each group contained five replicates with two rabbits each. The 1st group was fed on basal diet and served as a control group (CON), The 2nd group (JML) was fed basal diet containing 10% JM treated with *Lactobacillus acidophilus* and the 3rd group (JMLA) was fed basal diet containing 10% jojoba meal treated with *Lactobacillus acidophilus* and 0.25% *Alpinia galangal* rhizomes. The diets were formulated to meet nutrient requirements of rabbits (NRC, 1977) and to be nearly iso-nitrogenous and iso-caloric. Composition and calculated proximate chemical analyses of the experimental diets are shown in Table 1.

Table 1. Ingredients, proximate chemical analyses and nutritive value of the experimental diets

Ingredients (%)	Experimental diets[1]		
	CON	JML	JMLA
Soybean meal (46%)	15	9.7	9.7
Treated jojoba meal	0	10	10
Wheat bran	22	24.5	24.25
Yellow corn	10	8.5	8.5
Alfalfa hay	31	31	31
Barley grains	16.7	11	11
Alpinia galanga	0	0	0.25
Molasses	3	3	3
Dicalcium Phosphate	0.5	0.5	0.5
Limestone	0.9	0.9	0.9
Sodium chloride	0.3	0.3	0.3
Premix (Vitamins & Mineral)	0.3	0.3	0.3
Antifungal	0.1	0.1	0.1
L- Lysine	0.1	0.1	0.1
Dl-Methionine	0.1	0.1	0.1
Total	100	100	100

Proximate chemical composition of on DM%							Nutritive value		
	OM	CP	EE	CF	NFE	Ash	NDF[2]	DE[3] (kcal/kg)	DCP[4](g/kg.DM)
Diet (1): CON	90.40	18.04	4.56	13.04	54.76	9.60	37.49	2523	123.360
Diet (2): JML	90.97	18.45	5.35	16.32	50.85	9.03	39.65	2417	126.952
Diet (3): JMLA	90.96	18.42	5.37	16.31	50.86	9.04	39.64	2413	126.689

[1]CON= Control (basal diet), JML =10% Treated Jojoba meal, JMLA =10% Treated Jojoba meal and 0.25% *Alpinia galanga*
[2]NDF%= 28.924+ 0.657 (CF%) and [3]DE (Kcal/kg) = 4.36 – 0.0491 (%NDF)
[4]DCP= 0.876 CP – 34.67 according to (Villamide and Fraga, 1998)

Housing and management:

Weanling rabbits were housed collectively as two per metal cage (30 x 40 x 40 cm), supplied by automatic stainless nipple for drinking and a feeder in good natural ventilated house. All rabbits had free access to feed and water which were available all the time. Rabbits were subjected to identical managerial, hygienic and environmental conditions. The temperature humidity index (THI) was calculated according to LPHSI (1990) using the modified formula. THI = db°C – [(0.31 – 0.31 RH) (db°C – 14.4)]. The THI values were classified as following: (<27.8) = absence of heat stress, (27.8 to 28.9) = moderate heat stress, (29.0 to 30.0) = severe heat stress and (> 30.0) = very severe heat stress (Marai *et al.,* 2001). Animals of each treatment group were weighed at the start of the study period and then every week. Growth performance in terms of body

weight (BW), and body weight gain (BWG) were determined. Feed intake (FI)was recorded and feed conversion ratio (FCR) was computed as the ratio between feed consumption and BWG per period. Viability rate was calculated by recording the number of dead bunnies during the trial. Relative growth rate (RGR) was calculated according to the equation of Hassan *et al.* (2009) as the following: RGR (%) = [(W2-W1)/ 0.5* (W2+W1)] x 100. Whereas: W1 = initial Live Body Weight and W2 = final LBW.

Carcasses traits:

At the end of the study period (56 days), four rabbits (two males and two females) from each treatment group were slaughtered to study carcass traits. Rabbits were weighed after fasting for 12 hours and slaughtered, skinned, and eviscerated. Carcass traits were estimated according to Cheeke (1987). Edible giblets (heart, liver, and kidney) were separated and weighed. Empty carcass with head and hot carcass were weighed. Carcass traits were estimated as relative to pre-slaughter live body weight.

Blood biochemical assay:

During slaughter, two blood samples for each rabbit were collected (four rabbits/group). One sample was withdrawn in plastic vials containing heparin for determination of blood picture, which was analyzed on the same day of collection. The other sample was withdrawn in a dry plastic tube and left for half an hour at room temperature to clot, then centrifuged at 3500 rpm for 15 min. to obtain serum. Hematological parameters: blood hemoglobin (Hb) was measured colorimetrically using kits manufactured by Randox, England. The fraction of packed cell volume (PCV, %) was determined following the method of using Wintrobe (2009). Red blood cell (RBCs) count, platelets (PLTs) and red cell distribution width (RDW)were determined according to Dacie and Lewis (2006). Absolute erythrocyte indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)were calculated as following:

MCV (fl) = (PCV/ RBC) ×10
MCH (pg) = (Hb/ RBC) ×10
MCHC (%) = (Hb/ PCV) × 100
RDW (CV) %= 1SD of RBC volume/MCVx100%.

Total and differential leukocyte count were analyzed as described by Dacie and Lewis (2006).

Serum lipid profile in terms of total cholesterol (Cho) and triglycerides (TG) were determined according to Zollner and Kirsch (1962). High-density lipoprotein (HDL)-cholesterol was measured according to the method outlined by Lopez-Virella*et al.,* (1977). The serum low-density lipoprotein (LDL)- cholesterol and very low-density lipoprotein (VLDL)- cholesterol were estimated using Friedewald equation: LDL = Total cholesterol – HDL– (Triglycerides/5). VLDL= Triglycerides/5 (Friedewald *et al.,* 1972). The analyzed triiodothyronine (T3), thyroxine (T4), insulin (Ins), IgE, IgM and IgG were determined using an Enzyme Immunoassay test kit (PerkinElmer Health Sciences, Inc., Hayward, CA, USA).

Physiological parameters:

Physiological parameters have been recorded on all growing rabbits weekly until finish experimental period. Rectal temperature (RT) was determined using a clinical thermometer inserted at two cm depth into the rectum for one min. Respiration rate (RR) was recorded by counting the flank movements per minute using a hand counter. Pulse rate (PR) was recorded by placing a fingertip on the femoral arteries of the hind limb for one minute (Abdel-Monem, 1995 and Ayyat *et al.,* 2018).

Economic efficiency:

The economic efficiency (EE) was determined according to price of feed and live weight of rabbits at local market as following equation: profit= income from BW gain – feed cost, assuming that the costs of other inputs were constant.

The second experiment (2nd Exp.):

At finishing the first experiment, four males of each treatment group, all about 13 weeks old were housed individually in galvanized cages (40 x 55 x 60 cm) and fed the same experimental diets used during the first experiment up to six months old. The semen characteristics were evaluated from each buck for a month.

Semen collection and evaluation:

Semen was collected using an artificial vagina (IMV, France) and a teaser doe. The ejaculates were collected from each buck twice weekly and two times daily at 15 min intervals (two ejaculates per buck) and then promptly kept in a water bath at 37°C until evaluation. Within 15 to 30 min. following the removal of the gel mass, the volume of each ejaculate was measured directly using a graded collection tube (ml). Sperm evaluation was carried out by the Computer Assisted Semen Analysis (CASA) system (MiraLab's Spermolyzer).

Sperm concentration, sperm motility percentage, live/dead sperm percentage, total sperm output per ejaculate and live normal sperm output were measured with the CASA system.

Testosterone hormone:

After the experiment ended and the bucks were slaughtered. Blood were collected and blood serum separated as above-mentioned procedures. Blood serum testosterone (T) concentration was determined by using an Enzyme Immunoassay test kit (PerkinElmer Health Sciences, Inc., Hayward, CA, USA).

Histological examination for two experiments:

At the end of each experiment, after slaughter, specimens of liver, spleen (from four growing rabbits

at 56 days of age, from each treatment group) and testes (from four bucks at seven months of age, from each treatment group) were taken and prepared for histological examination. The samples were fixed with 10% neutral buffered formalin (40% conc.), then trimmed, washed in distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Blocks were sectioned at 3-6 µm thickness, mounted to glass slides, stained by Hematoxylin and Eosin (H&E) (Bancroft, 2008), and examined for histological study.

Histopathological grading of spermatogenic cells:

Evaluation was carried out to identify spermatogenic cells in various stages and forms in seminiferous tubules, using a modified Johnson score (Teixeira*et al.,* 2019). Twenty seminiferous tubule cross sections were chosen, and each one was given a score from 1 to 5 according to the following criteria: (1) no spermatogenic cells within the seminiferous tubules; (2) Seminiferous tubule hyalinization with vacuolization; (3) incomplete spermatogenesis, not going past the spermatocyte stage (spermatogenic arrest); (4) all stages of germ cells, including spermatozoa, are present, but there is a noticeable decrease in the quantity of germ cells (hypospermatogenesis) and (5) normal spermatogenesis. The number of tubules with a given score was multiplied by the score. The final Johnsen score was calculated by adding up all of the scores and dividing that total by the number of tubules that were evaluated. The average score for each tubule is then calculated by dividing the final score by the total number of tubules (20) that were examined.

Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA). Using the general linear model (GLM) procedure of SAS (2004) according to Steel and Torrie (1980). Whenever F value was significant ($P<0.05$) means were compared using the least significant difference (LSD) test. The following model was used:

$Y_{ij} = \mu + T_i + e_{ij}$ Where:

Y_{ij} = Observation made on j^{th} rabbit of treatment i.

μ = Overall mean.

T_i = Fixed effect of treatment i.

e_{ij} = The experimental random error.

Ethical approval:

The Scientific Research Ethics Committee of Arish University approved all procedures of the study (No. ARU/Agri.37).

RESULTS AND DISCUSSION

The first experiment:

Temperature humidity index:

Results in Table 2 show that overall mean of ambient temperature (db) was 27.82°C and overall mean of relative humidity (RH%) was 69.71%. The overall mean of temperature humidity index value (THI) was 26.56indicated the absence of heat stress (Marai *et al.,* 2001).

Table 2. Ambient temperature (AT), relative humidity (RH) and temperature humidity index (THI) of the rabbitry farm

Weeks	AT °C (db.)[1]			RH%[2]			THI
	Max.	Min.	Aver.	Max.	Min.	Aver.	
1-4 week	31.36	26.10	28.73	62.77	76.71	69.74	27.38
4-8 week	29.25	24.59	26.92	64.69	74.65	69.67	25.74
Overall mean	30.31	25.34	27.82	63.73	75.68	69.71	26.56

[1]**db°C**: dry bulb temperature in Celsius**.** [2]**RH**: relative humidity percentage/100

Productive performance o f growing rabbits:

Non-significant differences in growth performance traits in terms of live body weight (LBW), total weight gain (TWG) and daily weight gain (DWG) among experimental groups. LBW, TWG and DWG of rabbits fed JML diet decreased ($P>0.05$) compared to that fed CON diet. However, LBW, TWG and DWG of rabbits fed diet containing treated JM (JML diet) were reduced with (2.95, 4.27 and 4.24%), respectively compared with that fed CON diet. Also, rabbits fed JMLA diet were increased in LBW, TWG and DWG with (3.44, 4.70 and 4.70%), respectively in comparison to that fed CON diet, while rabbits fed JMLA diet were enhanced in LBW, TWG and DWG with (6.59, 9.37 and 9.34%), respectively compared to JML group. Our findings agree with Abd El-Maksoud (2011) who found that inclusion of JM treated with fungus (*Aspergillus funigotus*) in rabbit diet had no significant effects on growth performance (LBW and TWG) in comparison to control diet. Contrarily, Khayyal *et al.* (2009) showed that feeding JM treated rabbit-diet with lactic acid bacteria significantly improved growth performance compared with control diet. This may be due to reducing simmondsin in treated JM which consider major toxicants in JM (Decuypere *et al.,* 1995). El-Speiy *et al.* (2022) found that supplementation of *Alpinia galangal* into rabbit diet at level of 100 mg/Kg diet improved significantly weight gain. Improvement of growth performance in JMLA group may be attributed to slightly improved feed intake, as *Alpinia galangal* is considered rich source of minerals and trace elements, which play an effective function in amelioration physiological and nutritional efficiency (Imchen *et al.,* 2022 and Khalifah *et al.,* 2022). Kattab *et al.* (2017) showed that adding *Alpinia galanga* to lactating Barki goat diet at level of 4 g/kg DM increased significantly propionate concentrations and stimulating the digestive effect compared with control group. Ibrahim *et al.* (2011) demonstrated that adding lesser galanga at 1% to

rabbit diet significantly improved the final BW (2.31%). Daily feed intake (DFI), total feed intake (TFI), feed conversion ratio (FCR) and relative growth rate (RGR)of growing rabbits were not significantly affected by dietary treatments (Table 3). DFI of rabbit fed diet (JMLA) increased by2.75 and 2.95 g/day compared to the control, respectively. Similarly, El-Speiy *et al.* (2017) observed that adding *Alpinia galanga* at level
of 100 mg/kg diet significantly increased feed intake of rabbits compared with control diet. Also, non-significant differences between treatment groups in feed intake impales the tested diets were balanced in energy and protein (Khadr and Abdel-Fattah, 2008).

The FCR and RGR, rabbits fed on JMLA diet had the best values followed by that fed CON diet, while that fed JML diet had the worst values. The improvement in FCR of JMLA diet may be due to the components found in *Alpinia galangal* which stimulate the digestive enzymes and so increase digestibility coefficients of nutrients, which reflected on increasing body weight (Viveros *et al.,* 2011and El-Speiy *et al.,* 2017).

Table 3. Effect of dietary treatments on growth performance of growing NZW rabbits (Exp.1)

Items	Treatment[1]			SE[2]	Sig.test[3]
	CON	JML	JMLA		
No. weaning rabbits	10	10	10		
Viability %	100	100	100		
Initial live body weight (LBW, g)	652.00	654	654	45.03	NS
Final live body weight (LBW, kg)	2.267	2.200	2.345	0.057	NS
Total weight gain (TWG, kg)	1.615	1.546	1.691	0.061	NS
Daily weight gain (DWG, g)	28.840	27.615	30.196	1.097	NS
Total feed intake (TFI, g)	5340	5330	5495	199	NS
Daily feed intake (DFI, g)	95.4	95.2	98.1	3.57	NS
Feed conversion ratio (FCR)	3.27	3.41	3.20	0.12	NS
Relative growth rate (RGR%)	111.20	108.93	113.15	4.53	NS

[1]Treatments; CON, Control-basal diet, JML, 10% Treated jojoba meal, JMLA, 10% Treated jojoba meal and 0.25% *Alpinia galangal,* [2]SE = standard error, [3]**NS**= Not significant

Carcass traits:

Results of carcass characteristics show that the replacement of 40% soyabean protein by protein of biologically treated JM without or with *Alpinia galanga* did not significantly affect all carcass traits as percentage of pre- slaughter weight (Table,4). Our findings agree with those reported by Abd El-Maksoud (2011) who found that pre-slaughter weight as percentage of rabbit fed on diet containing treated JM with fungus at level (10%) did not differ significantly than that fed control diet. Also, El-Adawy *et al.* (2013) reported that carcass traits were not affected as result of replacement of 30% soyabean protein by protein of treated JM with *L. acidophilus*, except for dressing and liver weights and carcass %. In this regard, Ibrahim *et al.* (2011) showed that addition of *Alpinia galangal* at levels of 0.5 or 1 % did not significantly affect carcass cuts and digestive tract. El-Speiy*et al.* (2022) found that hot carcass, heart, kidneys and dressing percentage of rabbits improved (P<.001) by addition *Alpinia galanga* at level of 100 mg/kg diet compared to control diet.

Table 4. Effect of dietary treatments on carcass traits of growing NZW rabbits (Exp.1)

Item	Treatment[1]			SE[2]	Sig.test[3]
	CON	JML	JMLA		
No. of animals	4	4	4		
Pre-slaughter wt (kg).	2.157	2.166	2.240	0.028	
Empty carcass with head wt. (%)	59.50	59.25	59.75	1.45	NS
Liver (%)	2.79	2.74	2.72	0.09	NS
Kidney (%)	0.59	0.63	0.64	0.04	NS
Heart (%)	0.30	0.31	0.31	0.02	NS
Edible giblets (%)	3.69	3.70	3.68	0.11	NS
Head (%)	6.45	6.21	6.30	0.23	NS
Spleen (%)	0.07	0.07	0.09	0.02	NS
Abdominal Fat (%)	1.04	0.96	1.08	0.08	NS
Dressing (%)	63.25	63.16	63.49	1.55	NS

[1]Treatment, CON, Control, basal diet, JML, 10% Treated jojoba meal, JMLA, 10% Treated jojoba meal and 0.25% *Alpiniagalanga,* [2]SE = standard error, [3]NS= Not significant

Blood measurements:

Hematological parameters of growing rabbits:

Feeding rabbit on biologically treated JM without or with *Alpinia galangal* did not significantly affect all hematological parameters (erythrocyte indices and total and differential leukocyte count). The figures obtained are within the normal range (Shoushab *et al.,*2017 and Popoiu *et al.,* 2021). There were slight

declines in RBCs count, HB and PCV for rabbit fed JML diet when compared to the CON or JMLA groups (Table 5).

It was evident from the normal range of MCV, MCH, and MCHC obtained in this study for the rabbits fed experimental diets that there was no anemia in any of the experimental groups. In this context Johnson-Delaney (1996) clarified that the MCV and MCH assessments might be indicative of anemia as well as the bone marrow's ability to generate RBCs with normal size and metabolic capability. Njidda *et al.* (2006) reported that MCV, MCH and MCHC are used in diagnosing anaemic conditions.

Platelets (PLTs), white blood cell count (WBCs) and differential leukocyte count (neutrophils, lymphocyte, monocytes and eosinophils%), all of these parameters were within normal range (Shousha *et al.,* 2017 and Popoiu*et al.,* 2021). Value of eosinophils of rabbits fed JMLA diet was higher than the range reported by (Popoiu *et al.,* 2021). This impliesthat the rabbits fed JM had a healthy immune system. Reda *et al.* (2009) showed that values of hematological parameters (RBCs, HB, WBCs and Platelets) of rats supplied with Jojoba extract did not show significant variables differences compared to the control.

Table 5. Effect of dietary treatments on erythrocyte indices and total and differential leukocyte count of growing NZW rabbits (Exp.1)

Item		Treatment[1]			SE[2]	Sig. test[3]
		CON	JML	JMLA		
Erythrocyte indices	Number of samples	4	4	4		
	Hemoglobin (Hb) (g/dl)	12.70	11.67	12.02	1.14	NS
	PCV (%)	35.00	32.75	34.75	3.38	NS
	RBCs (10^6/µl)	6.17	5.63	5.72	0.60	NS
	MCV (fl)	57.25	58.25	61.25	1.63	NS
	MCH (pg.)	20.75	20.55	21.50	0.47	NS
	MCHC (%)	35.75	35.25	34.75	0.62	NS
	RDW (%)	16.12	15.62	16.75	1.13	NS
	PLT (10^3/µl)	279.00	255.75	296.25	66.70	NS
Total and differential leukocyte count	WBCs (10^3/µl)	16.27	13.62	15.09	3.51	NS
	Neutrophils (%)	51.50	53.50	51.50	5.44	NS
	Lymphocyte (%)	41.00	39.25	39.25	5.39	NS
	Monocytes (%)	5.50	5.00	6.00	1.36	NS
	Eosinophils (%)	2.00^{b}	2.25^{ab}	3.25^{a}	0.46	0.0001

[1]Treatment, CON, Control, basal diet, JML, 10% Treated jojoba meal, JMLA, 10% Treated jojoba meal and 0.25% *Alpinia galanga.,* *a*[b]Means in the same row with different superscripts differ (P<0.05), [2]SE = standard error, [3]**NS**= Not significant

Blood biochemical changes:

Lipide profile:

Values reported in Table (6) reveal no significant differences (P>0.05) among experimental groups concerning lipide profile in terms of Cho, TG, HDL, LDL and VLDL.

These findings are in agreement with those of El-Kady *et al.* (2008), who showed an increase in total Cho, TG, and TL as the JM increased in diets. On the other hand, El-Speiy *et al.* (2022) indicated that theCho, TG, and LDL, insignificantly decreased in JMLA group compared to control group. This could be because bioactive phytochemicals like tannins and saponins have a hypolipidemic impact (Dong et al., 2007). Matsura (2001) mentioned that saponins from different sources can cause low serum cholesterol levels in various animals. Several dietary saponins were found to have a hypo-cholesterolemic action (Francis*et al.,*2002). Additionally, saponins were found to delay the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (Han *et al.,* 2000). Also, tannins were found to play a significant role in lipid digestibility by forming complexes with fatty acids (Romero *et al.,* 2000), leading to decrease in cholesterol absorption and an increase in fat excretion (Bravo *et al.,* 1993).

Immune response:

It is evident that partial replacement of soybean meal protein by biologically treated JM protein without or with *Alpinia galanga* numerically improved in IgM and IgG immune response of rabbit (Table 6). These findings reveal the beneficial impact of treated Jojoba meal and *Alpinia galanga* on the immune response of rabbits. In this respect, Wisniak (1994) showed that JM contains high level of cysteine, which as a reducing agent, the liver and lymphocytes use it to get rid of harmful immunosuppressive toxins (Doubt and Rosell,2013). Richard *et al.* (2008) reported that JM has high levels of unsaturated fatty acids, which can enhance antioxidant mechanisms and minimize mitochondrial depolarization *Alpinia galangal* includes bioactive compounds like vitamins, flavonoids, phenolic acids and saponin that have immuno stimulatory properties by stimulating mononuclear cells to release cytokine IL-1β, IFN-γ and TNF-α (Liao *et al.,* 2015). In addition, *Alpinia galanga* contains lectin, which has an important role for the immuno modulatory response as it helps in the development of cells, supporting communication between them (Yuandani *et al.,* 2023). Similarly, El-Speiy *et al.* (2022) showed

that rabbits fed diets supplemented *Alpinia galanga* at level of100 or 200 mg/kg diet significantly enhanced IgG and IgM production.

Hormonal parameters:

Thyroid hormonal activities ofT3 and T4 results are presented in Table (6). Serum T3 level was significantly higher (P<0.05) in rabbits fed JML diet compared with rabbits fed CON diet. While serum T4 level was insignificantly decreased in rabbits fed JMLA diet compared with rabbits fed the other diets (CON and JML). There are also no significant differences in the T3/T4 ratio among experimental treatments. These findings are consistent with those of Abdou and El-Essawy, (2018). The increase in T3 and T4 levels could be due to a shortage of relative protein induced by simmondsin (Rothwell *et al.,* 1982) and it indicates high heat production (Buyse *et al.,* 1992).

Elghalid *et al.,* (2021) found that levels of T3 and T4 increased significantly in broiler chicks fed a diet that contains *Alpinia galanga*.

The lowest (P>0.05) plasma insulin (Ins) values were observed in JML group compared with CON group, while it was higher (P<0.05) in JMLA group compared with JML group. The decrease in insulin values of JML is due to simmondsin (Flo *et al.,* 1999 and 1998). These findings are in accordance with data reported by Arnouts *et al.* (1993), who found that insulin values were decreased (P<0.05) in the JM supplementation (4 to 12%) groups compared with control group.

Table 6. Effect of dietary treatments on lipid profile, immune response and hormonal parameters (Exp.1)

Item	Treatment[1]			SE[2]	Sig.test[3]
	CON	JML	JMLA		
No. of animal	4	4	4		
Lipidprofile					
Cholesterol (mg/dl)	81.33	117	101	26.21	NS
Triglycerides (mg/dl)	41	52.67	44.67	12.50	NS
HDL-Cholesterol (mg/dl)	23	24.67	23.33	4.00	NS
LDL-Cholesterol(mg/dl)	50.13	81.80	69.40	22.75	NS
VLDL (mg/dl)	8.20	10.53	8.93	2.50	NS
Immune response					
IgE (IU/ml)	0.20	0.20	0.20	0.00	NS
IgM (mg/dl)	20.15	21.80	36.87	11.23	NS
IgG (mg/dl)	367.50	468.50	537.75	143.19	NS
Hormonal parameters					
Total T3 (ng/dl)	19.92^{b}	40.35^{a}	28.87^{ab}	5.21	0.0001
Total T4 (ug/dl)	4.05	4.22	3.87	0.18	NS
T3/T4 ratio	0.21	0.13	0.13	0.03	NS
Insulin (uIU/ml)	15.10^{ab}	13.27^{b}	20.70^{a}	2.44	0.0001

[1]Treatment, CON, Control, basal diet, JML, 10% Treated jojoba meal, JMLA, 10% Treated jojoba meal and0.25% *Alpinia galanga.,* [2]S.E. = standard error, [3]**NS**= Not significant

Physiological parameters of growing rabbits:

Results in Table (7) show the effect of dietary treatment on some physiological parameters of growing rabbits. Rectal temperature (RT)was decreased (P<0.05) in JML and JMLA groups compared to the control group.Also, Respiration rate (RR) was lower (P<0.05) in JMLA and JML groups (93.83and 94.54, respectively) than control group (99.31). Pulse rate (PR)was decreased (P<0.05) in JML and JMLA groups compared to control group. The decrease in RT, RR and PR can be attributed to the fact that *Alpinia galanga* contains many important components such as (borneol, 1,8-cineole, Eugenol, Myrcene and lectin) which have different therapeutic functions that include antipyretic, anti-inflammatory, anti-allergic, sedative and immune response (Raina *et al.,* 2002, Duke, 2003, Valenzuela-Grijalva *et al.,* 2017and Yuandani *et al.,* 2023). As for the improvement of JML, it is also attributed to the biological and pharmacological activity for Jojoba such as anti-inflammatory, antipyretic and analgesic activities (Gad *et al.,*2021). Astrup *et al.* (2011) mentioned that Jojoba typically has a high iodine value, indicating a high content of unsaturated fatty acids. As a result, jojoba can ease inflammation, stabilize heart rhythms and improve blood cholesterol levels. Histopathological findings: Histological examination illustrated in Fig.1, Plates A1 and A2 show that liver sections of control group (CON) especially histological structure of portal area and hepatocytes, hepatic blood sinusoid was dilated and engorged with blood. Liver of JML group indicated portal fibrosis with congestion of portal blood vessels, newly formed bile ductulus, some hepatocytes showing necrobiosis changes including nuclear pyknosis and acidophilic cytoplasm and hepatic sinusoid engorged with blood (Fig.1, Plate B1 and B2). While, Liver of JMLA group indicated mild portal fibrosis with infiltration by mononuclear inflammatory cells and newly formed bile ductulus, mild dilatation of hepatic sinusoid and engorged with blood (Fig.1, Plate C1and C2).

These results are consistent with those of Cokelaere et al. (1993) and Sobhy et al. (2003), who

found that rats given 3% defatted JM showed vasodilatation of the majority of the vasculature along with a number of degenerative liver alterations.

Examined sections of spleen showing normal histological structure of white pulp in the JML group (Fig.2, Plate B). While, spleen of group CON showed a marked depletion of white pulp (Fig.2, Plate C). Moreover, JMLA group (Fig.2, Plate C) showed depletion of white pulp with appearance of mega karyocytes.

Table 7. Effect of dietary treatments on physiologic parameters for weaning rabbits (Exp. 1).

Item	Treatment[1,2]			S.E.[3]	Sig.test[4]
	CON	JML	JMLA		
Rectal temperature, (°C)	39.16	39.12	39.12	0.034	NS
Respiration rate, (rpm)	99.31[a]	94.54[b]	93.83[b]	1.04	0.0001
Pulse rate, (bpm)	97.16[a]	95.29[ab]	94.50[b]	0.69	0.0001

[1]Treatment, CON, Control, basal diet, JML, 10% Treated jojoba meal, JMLA, 10% Treated jojoba meal and0.25% *Alpinia galanga,* [2]No significant differences were found between treatment means. (P>0.05), [a,b]Means in the same row with different superscripts differ(P<0.05). [3]S.E. = standard error, [4]NS= Not significant

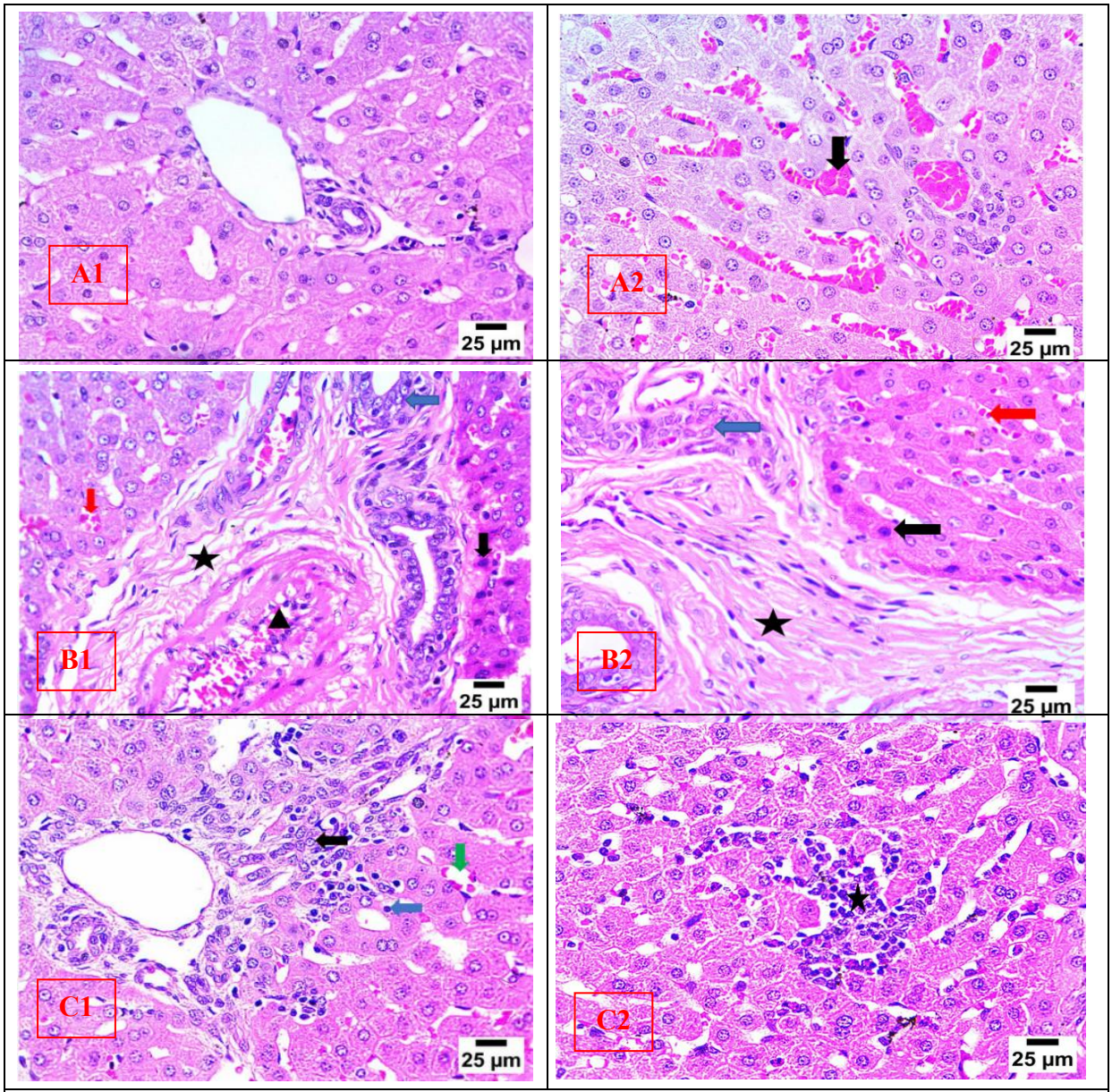

Fig. 1. Photomicrograph of liver sections of different experimental groups
A) CON group: (Plate A1) showing normal histological structure of portal area and hepatocytes;(Plate A2) showing dilated hepatic sinusoid and engorged with blood (arrow), B) JML group: (Plate B1&B2) showing portal fibrosis (star) with congestion of portal blood vessels (arrowhead), newly formed bile ductulus (blue arrow), some hepatocytes showing necrobiosis changes including nuclear pyknosis and acidophilic cytoplasm (black arrow) and hepatic sinusoid engorged with blood (red arrow), C) JMLA group: (Plate C1) showing mild portal fibrosis with infiltration by mononuclear inflammatory cells (blue arrow) and newly formed bile ductulus (black arrow), mild dilatation of hepatic sinusoid and engorged with blood (green arrow); (Plate C2) showing aggregates of inflammatory cells mainly lymphocytes and eosinophils between hepatocytes (star), (H&E stain, X 400, Scale bar 25µm).

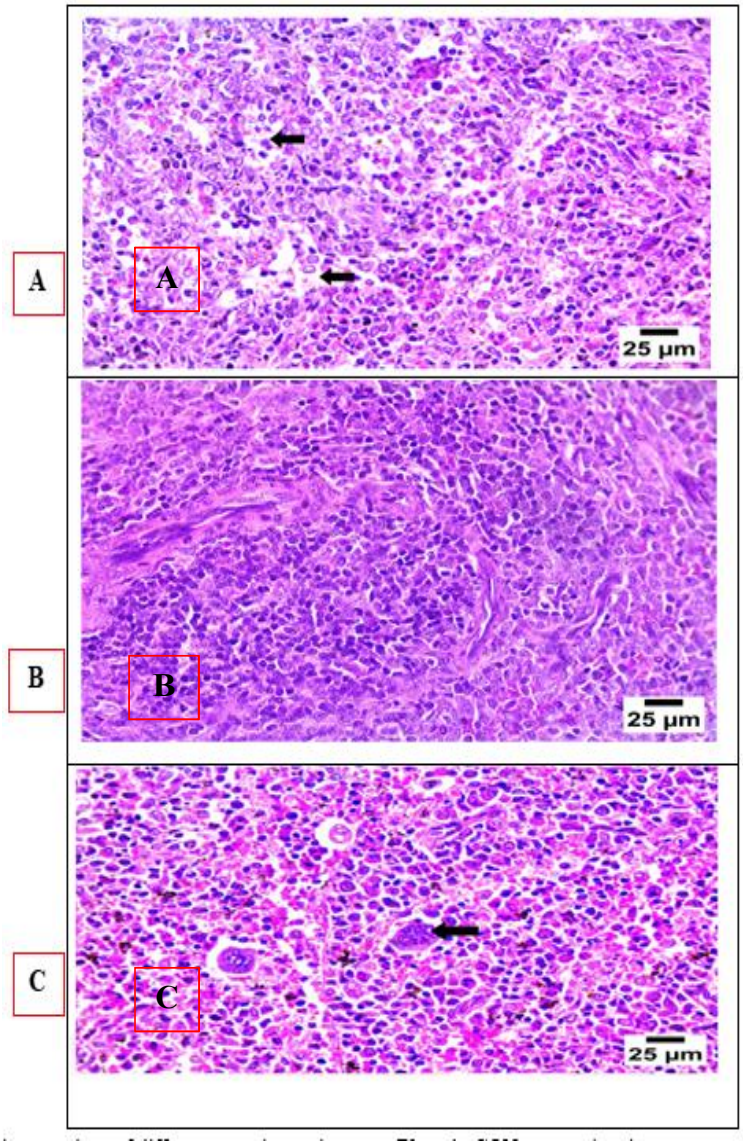

Fig. 2. Photomicrograph of spleen sections of different experimental groups. **Plate A:** CON group: showing marked depletion of white pulp (arrows)**. Plate B:** JML group: showing normal histological structure of white pulp. **Plate C:** JMLA group: showing depletion of white pulp with appearance of mega karyocytes (arrow)(H&E stain, X 400, Scale bar 25µm).

Economic efficiency:

The decline of the price of treated Jojoba meal compared with the price of soyabean meal was reflected on the cost of the experimental diets. The resultsas seen in Table 8 show that JML treated with *L. Acidophilus* with *Alpinia galanga* gave the best net revenue (LE). While JML treated with *L. Acidophilus* without or with *Alpinia galangal* had the best economic efficiency and relative revenue when compared to the control group. These findings are consistent with those of Khayyal *et al.* (2009), Abd El-Maksoud (2011) and El-Speiy *et al.* (2022).

The second experiment:

Reproductive Performance of Rabbit Bucks:

Table (9), illustrates the effect of dietary treatments on some semen traits including advanced sperm motility, live sperm, live normal sperm output, dead sperm and abnormal sperm. Results did not bear significant treatments' differences for all studied variables. While, ejaculate volume, sperm cell concentration and total sperm output were significantly increased as result of feeding JMLA diet. According to El-Speiy *et al.* (2023), *Alpinia galanga* has antioxidant activity and bioactive substances such flavonoids, phenolic acids, and alkaloids. It also includes a variety of flavones, including galangin, kaempferol, and alpinin (Rachkeeree *et al.* 2018).

These findings are in agreement with those of Akbar (2020) and Kolangi *et al.* (2019) They observed Alpinia officinarum (*A. officinarum*) increased both the quantity and quality of sperm. These benefits were attributed to plant components including galangin antioxidant and scavenging properties against reactive oxygen species.

Non-significant differences among dietary treatment groups in free testosterone (Table 9).

Table 8. Economic efficiency of the dietary treatments in growing rabbits (Exp.1)

Item	Experimental diets of does		
	CON	**JML**	**JMLA**
Total feed intake / rabbit (kg)	5.340	5.330	5.495
Price/kg diet (L.E.)	14.56	13.42	14.32
Total feed cost / rabbit (L.E.) *	77.75	71.53	78.69
Total weight gain / rabbit (kg)	1.615	1.546	1.691
price/ kg gain (L.E)	100	100	100
Selling price (L.E.) **	161.5	154.6	169.1
Net revenue (L.E.) ***	83.75	83.07	90.41
Economic efficiency (E. EF) × 100******	107.72	116.14	114.90
Relative revenue (%) *******	100	107.818	106.667

* Based on prices of the Egyptian market during the experimental period (2023)
* Total Feed cost = Total feed intake (kg) ×Price/kg of diet (L.E)
**Selling price (L.E) = Total weight gain / rabbit (kg) × Price/kg gain (L.E)
*** Net revenue = different between selling price (L.E) and Total feed cost (L.E)
**** Economic efficiency (E. EF) = (Net revenue / Total feed cost) × 100
***** Relative Economic efficiency (R. E. E.), assuming control treatment = 100%

Table 9. Effect of dietary treatments on semen characteristics and serum testosterone levels of NZW rabbit bucks (Exp.2)

Item	Treatment[1]			SE[2]	Sig. test[3]
	CON	**JML**	**JMLA**		
Ejaculate volume (EJ) (ml)	1.02[a]	0.72[b]	0.90[ab]	0.06	0.0001
Advanced sperm motility (AM) (%)	71.25	65.00	73.75	3.28	NS
Live sperm (LS) (%)	83.25	82.75	82.00	3.71	NS
Sperm cell concentration (N*ml. - count per ml.)	365.00[ab]	328.00[b]	397.25[a]	21.97	0.0001
Total sperm output (count per Ej)	372.17[a]	237.82[b]	356.00[a]	27.79	0.0001
Live normal sperm output (Ln*N/Ej - count per Ej)	74.50	73.75	71.50	4.69	NS
Dead Sperm (DS) (%)	16.75	17.25	18.00	3.71	NS
Abnormal Sperm (Abs) (%)	8.75	9.00	10.50	1.35	NS
Testosterone free (Pg/ml)	4.51	3.93	4.44	0.22	NS

[1]Treatment, **CON**, Control, basal diet, **JML**, 10% Treated jojoba meal, **JMLA**, 10% Treated jojoba meal and 0.25% *Alpinia galanga*, [2]S.E. = standard error of the mean, [3]**NS**= Not significant

Histopathological findings:

The histological examination of testicular sections showed uniform morphology of the seminiferous tubules with full spermatogenesis in CON and JMLA groups (Fig.2, Plate A and C), and JML group (Fig.3, Plate B). Several seminiferous tubules showed incomplete spermatogenesis not reaching full spermatids (spermatogenic arrest) with the presence of few tubules showing full spermatogenesis but with reduced sperm formation.

These findings are in agreement with those of Bebars *et al.* (2021). They observed *A. officinarum* extract greatly improved testicular structure, function and sexual behavior of adult male albino rats. Sobhy *et al.* (2003) found that rats given 3% defatted JM showed vasodilatation of the majority of the vasculature along with a number of degenerative testes changes. Furthermore, Abd El-Hakim *et al.* (2012) demonstrated that Japanese quail fed jojoba meal supplemented with 8% and 12% significantly reduced Leydig cells, exhibiting a greater number of primordial spermatocytes and a lack of mature sperm.

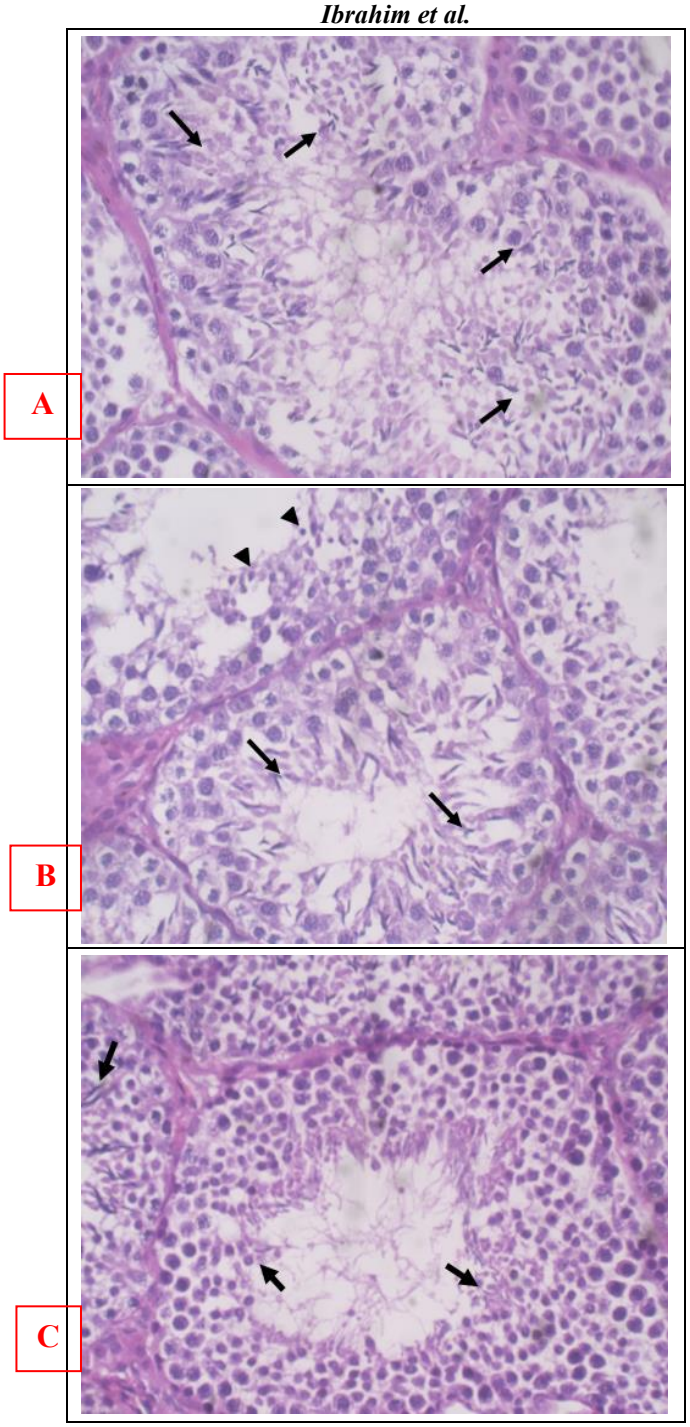

Fig. 3: Photomicrographs of Seminiferous tubules of different experimental groups. **Plate A:** CONgroup: Seminiferous tubules show uniform morphology with full spermatogenesis (Black arrows). **Plate B:** JML group: Several seminiferous tubules showing incomplete spermatogenesis, not reaching full spermatids (spermatogenic arrest) (Black arrowheads). Few tubules show full spermatogenesis with reduced sperm formation (Black arrows)**. Plate C:** JMLA group: Most of the seminiferous tubules show uniform morphology with full spermatogenesis (Black arrows). Few tubules show decreased spermatogenesis. (H&E stain, X400, Scale bar 20µm).

Histological grading findings:

Data presented in Table (10) reveal that average score of histopathological grading of seminiferous tubules was given higher score in treatment groups CON and JMLA (5 and 4.1 = normal spermatogenesis), respectively compared with JML group (3.1 = spermatogenicarrest). These findings are in align with Abd El-Hakim *et al.* (2012) who found that Japanese quail fed jojoba meal treated with 8% and 12% showed hypocellularity of spermatogenic layer cells, with a rise in primary spermatocytes and absence of mature sperm.

Table 10. Effect of dietary treatments on histopathological grading score of seminiferous tubules of rabbit bucks *(Exp. 2)

TRT	CON	JML	JMLA
Score	5x20=100 "20 tubules, each with score 5 = normal spermatogenesis"	3x18=54 "18 tubules, each with score 3 = spermatogenic arrest" 4x2=8 "2 tubules, each with score 4 = hypospermatogenesis"	5x19=95 "19 tubules, each with score 5 = normal spermatogenesis" 4x1=4 "1 tubule, with score 4 = hypospermatogenesis"
Final score	100	54+8=62	95+4=99
Average score for each tubule	5 normal spermatogenesis	3.1 spermatogenic arrest	4.9 normal spermatogenesis

CONCLUSION

It is concluded that using biologically treated jojoba meal, along with the use of *Alpinia galanga* at a level of 0.25%, can enhance the productivity and improve the testicular structure and function of rabbits.

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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أداء النمو وخصائص السائل المنوي للأرانب النيوزيلاندي البيضاء المغذاه على كسب الجوجوبا المعالج بيولوجيا مع أو بدون الخولنجان

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تضمنت هذه الدراسة تجربتين، هدفت التجربة الأولى إلى دراسة تأثير كسب الجوجوبا المعالج بيولوجيا مع أو بدون جذور نبات الخولنجان *Alpinia galanga* على أداء النمو وخصائص الدم والفحص النسيجي لبعض الأعضاء في الأرانب النامية. بينما هدفت التجربة الثانية إلى تقييم تأثير هذه العلائق التجريبية على بعض الصفات التناسلية والخصائص النسيجية للخصية في ذكور الأرانب.

التجربة الأولى: تم تقسيم عدد ثلاثون أرنب من كلا الجنسين حديثة الفطام عمر ٣٥ يوم بشكل عشوائي إلى ثلاث مجموعات متماثلة (١٠ لكل معاملة) بمتوسط وزن ٦٥٣ ± ٤٥,٠٣ جم. غذيت المجموعة الأولي علي العليقة الأساسية كمجوعة مقارنة كنترول (بدون كسب جوجوبا)، بينما المجموعتان الثانية (الجوجوبا المعالجة بالبكتريا) والثالثة (الجوجوبا المعالجة بالبكتريا مضاف اليها جذور نبات الخولنجان) غذيتا علي العليقة الأساسية بعد إستبدال ١٠٪ منها بكسب الجوجوبا المعالج بالبكتريا (*Lactobacillusacidophilus*) بدون او مع جذورالخولنجان *Alpinia galanga* (٠,٢٥٪)، على التوالي.

وكانت اهم النتائج المتحصل عليها: أن العلائق التجريبية لم تؤثر معنويا على وزن الجسم الحي النهائي، والزيادة الكلية والزيادة اليومية في وزن الجسم، ومعدل المأكول اليومي، ومعدل التحويل الغذائي، ومعدل النمو النسبي كما أنه لا توجد فروق معنوية بين المجموعات التجريبية في صفات الذبيحة، ومكونات الدم، كما أظهر الفحص النسيجي لمقاطع الكبد في مجموعة الكنترول بنية نسيجية طبيعية بينما ظهر بالمجموعة الثانية والثالثة تليفًا مع إحتقان الأوعية الدموية. أما بالنسبة لنسيج الطحال فقد أظهر الفحص الميكروسكوبي بنية نسيجية طبيعية اللب الأبيض في المجموعة الثانية بينما ظهر إستنزافًا ملحوظًا للب الأبيض في مجموعة الكنترول والمجموعة الثالثة.

التجربة الثانية: عند الانتهاء من التجربة الاولي، تم إختيار أربعة ذكور من التجربة الأولى من كل مجموعة عمر ١٣ أسبوعا ومتوسط وزن ٢,٢٧٠ ± ٠,٠٥٧ كجم/ المجموعة. استمرت الذكور في التغذية على نفس العلائق التجريبية المستخدمة في التجربة الأولى. وعند عمر ٦أشهر تم قياس خصائص السائل المنوي ثم الذبح وقياس تركيز هرمون التستوستيرون في مصل الدم واجراء الفحص النسيجي للخصية.

وأظهرت النتائج أن: الأرانب بالمجموعة الثانية المغذاه علي العليقة التجريبية المحتوية على كسب الجوجوبا المعالج بيولوجيا بدون إضافة الخولنجان أظهرت إنخفاضا معنويا في خصائص السائل المنوي مقارنة بالأرانب المغذاه علي عليقة الكنترول، في حين أن الأرانب بالمجموعة الثالثة المغذاه علي عليقة كسب الجوجوبا المعالج بيولوجيا مع إضافة الخولنجان أظهرت تحسناً غير معنوياً في خصائص السائل المنوي مقارنة بمجموعة الكنترول، وأظهر الفحص النسيجي للأنابيب المنوية شكلا مورفولوجيا موحداً مع تكوين الحيوانات المنوية الكاملة في مجموعة الكنترول والمجموعة الثالثة، ولكن في المجموعة الثانية لوحظ بالعديد من الأنابيب المنوية تكوين حيوانات المنوية غير مكتمل والتي لا تصل إلى الحيوانات المنوية الكاملة (توقف تكوين الحيوانات المنوية).

الخلاصة: خلصت هذه الدراسة إلى أن استخدام كسب الجوجوبا المعالج بيولوجياً بالبكتريا، إلى جانب إستخدام جذور نبات الخولنجان بمستوى ٠,٢٥٪عزز الإنتاجية وحسن بنية الخصية وخصائص السائل المنوي لدى الأرانب.