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IMPACTS OF ENZYMES AND PROBIOTIC IN IMPROVING THE UTILIZATION OF SIEVED OLIVE PULP MEAL ON REPRODUCTIVE PERFORMANCE AND SOME BLOOD TRAITS OF RABBIT BUCKS

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SUMMARY

Olive pulp is considered a waste of agricultural processing with low nutritional value. The current study aimed to study the effect of enzymes and dry yeast in enhancing the benefit of sieved olive pulp (SOP) on reproductive performance and some blood parameters of rabbit bucks. The question arises as to what is the maximum amount of sieved olive pulp that can be incorporated into a buck's diet to achieve maximum benefits using some additives? A total of 49 adult V-Line buck rabbits were divided into seven dietary treatments and housed in individual batteries. Treatments included a basal diet with no supplement as a control and the other six treatments involved substituting the basal diet with two levels of 20 and 25% of SOP supplemented with 0.1 g/kg econase enzymes (E), 0.5 g/kg dry yeast (DY), or a combination of E+DY for 8 weeks. The results showed that rabbit bucks fed diets containing SOP_{20}-E and SOP_{25}-DY exhibited significant improvement ($P \leq 0.05$) in viability compared with other treatments, as well as those feeding rabbit bucks diets containing SOP_{25} with any supplements resulted in significant improvements ($P \leq 0.05$) in sperm concentration as compared to other groups. While bucks received SOP diets with any supplements, there was a significant ($P \leq 0.01$) increase in total sperm output compared with the control group. Additionally, the inclusion diet SOP_{25}-DY produced the fewest abnormal sperm and dead spermatozoa in comparison to other treatments. Supplementations or SOP levels did not significantly impact the majority of hematological parameters, but platelets were significantly ($P \leq 0.05$) lower in all dietary treatments than the control group. Blood cholesterol levels were significantly ($P \leq 0.01$) lower in rabbit bucks that received SOP, regardless of supplementation compared to the control group. Conversely, diets containing SOP_{20} or SOP_{25} with E+DY resulted in a statistically significant increase in glucose levels ($P \leq 0.01$). In conclusion sieved olive pulp can be integrated into buck's diet up to 25% supplemented with enzymes or dry yeast without any negative effects on some semen quality, blood constituents or hematological parameters.

Keywords: *olive pulp, rabbit bucks, enzymes, dry yeast and blood traits*

INTRODUCTION

Rabbit production is considered one of the ways to solve the problem of persistent animal protein deficiency, especially in developing countries, including Egypt, due to their numerous advantages, which include their high growth rate, high prolificacy, high efficiency in converting feed to meat and comparatively inexpensive production costs. However, the high costs of feed ingredients are the most limiting factor for the intensive production of rabbits. In Egypt, the problem of animal feed shortages remains a major concern for nutritionists. Therefore, there is an urgent need to search for alternative sources of feed. Olive cake is one of the agricultural by-products that can contribute to solving the problem. It is a good source of calcium, copper, and cobalt and a reasonable source of manganese and zinc, although it is low in phosphorus, magnesium, and sodium. The cell walls of olive cake contain anti-nutritive factors like xyloglucan (non-starch polysaccharides), as reported by Gil-Serrano *and* Tejero-Mateo (1988) and Coimbra *et al*. (1995) and high levels of crude fiber (27–41%), tannin, and phenols, which may have a negative impact on the productivity and profitability of animals. (Abo Omar, 2005). The presence of non-starch polysaccharides, which reduce digestion of carbohydrates, proteins, and fats by the NSPs, can bind a lot of water, the fluid's viscosity increases. These problems can be resolved by adding of enzymes or yeast to animals' diets. By minimizing the effects of anti-nutritional factors, enzymes could be used to increase the efficiency of feed utilization. The response is often enhanced by using enzyme mixtures (such as econase), which contain many enzymes. Diets supplemented with 0.05% Allzyme SSF to the 8 and 16% olive cake diets may enhance the cock's performance (Hassan *et al*., 2016). In recent years, the use of yeast and enzymes as feed additives has received considerable attention. Chandra *et al*. (2014) found that adding yeast and exogenous enzymes to the rabbit diet improved growth and health status. Additionally, Falcao-e-Cunha *et al*. (2007) showed that supplementing rabbit diets with dietary enzymes improved nutrient utilization.

Saccharomyces (S) cerevisiae is considered a valuable and high-quality growth promoter due to its availability, safety, and price. They are beneficial in the treatment and prevention of diseases that result from either their direct nutritional impact as biologically valuable proteins, vitamin B-complex, essential traces of minerals, and various other special factors, or their health-promoting effects such as acting as a bioregulator of the intestinal microflora and improving the host's natural defenses (Hassanein and Soliman, 2010).

There is literature on the effects of olive pulp on the production performance of farm animals. However, there is no literature on the inclusion of olive pulp in the rabbit buck's diets. Besides, there is little research on how yeast and enzyme blends affect rabbit bucks. Therefore, the purpose of the current study was to determine how much sieved olive pulp can be incorporated into the diet of rabbit bucks to maximize the benefits of using some additives.

MATERIAL AND METHODS

The present study was carried out at Gimmizah, El-Gharbia Governorate, Experimental Station of Animal Production, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. (APRI/132429/191214).

Collecting and preparing sieved olive pulp*:*

During the olive pressing season, a sample of olive pulp was collected and brought to the testing location. Olive pulp was laid out on a plastic sheet and exposed to the sun for ten days to dry. To ensure that the material dried efficiently, olive pulp was periodically stirred, and it was covered overnight to keep out moisture. After the sample was air-dried, the endocarp started to separate. The majority of the endocarps were removed during this process using a 2-mm sieve, and the chemical composition of the sieved olive pulp sample shown and feed ingredients was done according to the standard method of the AOAC (2005) in Tables 1 and 2.

Enzyme preparation:

The econase enzyme used in this study was a readily available product from the Indian company Tex Biosciences (P) Ltd. It is a multi-enzyme contains amylase 125.000 U/kg, Phytase 200.000 U/kg, cellulose 100.000.000 U/kg, lipase 10.000 U/kg, xylanase 1.500.000 U/kg, protease 15.000 U/kg, pectinase 30.000 U/kg, arabinase 7.000 U/kg, α-galactosidase 10.000 U/kg, and β-glucosidase 10.000 U/kg. 100 g/ton of feed is the dose that the producer recommends.

Experimental animals, design and diet:

A total of adult 49 V-Line rabbit bucks ranged from 3000-3200 g were randomly divided into 7 dietary treatments and housed in individual wire cages (40W x 60L x 50H cm) in completely randomize design.

Table (1): The chemical composition of sieved olive pulp (% as dry matter basis).

Items	Sieved olive pulp
Dry matter	91.42
Organic matter	88.70
Crude protein	9.77
Crude fiber	31.50
Ether extract	12.50
Nitrogen free extract	34.93
Ash	11.30
Neutral detergent fiber	49.62
Acid detergent fiber	38.16
DE* (Kcal/g diet)	1930

DE; digestible energy was calculated according to Cheeke (1987).*

The first treatment group was fed a basal diet without supplements as a control and the other six treatments included substituting the basal diet with two levels of 20 and 25% of sieved olive pulp (SOP) supplemented with 0.1 g econase enzymes (E), 0.5 g/kg dry yeast (DY), or a combination of E+DY. for 8 weeks. Rabbits were provided with manual feeders and a nipple system for watering. The rabbits were kept under similar hygienic and environmental circumstances, including galvanized batteries, a well-ventilated building, an ambient temperature of 23±2°C, 55-65% humidity, and a photoperiod of 16 h light: 8 h dark. All experimental diets were formulated as pellets to meet their nutritional requirements, according to the NRC (1977) and De Blas & Weiseman (2010). The ingredients of the experiment's diet are shown in Table 2. All of the rabbits had free access to feed and water for the whole length of the experimental period.

Table (2): The ingredients and chemical composition of experimental diets.

Indices	**Control**	**Dietary levels of sieved olive pulp**	
		20%	**25%**
Ingredients (%)			
Sieved olive pulp	0.00	20.00	25.00
Yellow corn	26.00	20.00	17.00
Soybean meal (44 % CP)	19.00	18.00	20.00
Barley	10.00	08.00	9.00
Wheat bran	14.00	13.65	13.50
Clover straw	5.20	0.00	0.00
Sunflower meal	10.00	8.00	6.50
Alfalfa hay	10.00	8.00	4.60
Limestone ground	1.50	0.00	0.0
Di-calcium phosphate	1.50	1.50	1.55
Molasses	2.00	2.00	2.00
Vit. and min. premix*	0.30	0.30	0.30
Common Salt	0.50	0.50	0.50
Total	100	100	100
**Calculated analysis			
***DE kcal/kg	2621	2617	2601
Crude protein ,%	18.14	18.91	19.18
Crude fiber,%	10.4	13.16	13.78
Calcium,%	1.19	2.23	2.62
Available phosphorus,%	0.46	0.46	0.46

**Each 3 Kg contains: Vitamins A (12,000,000 IU), D3 (2,200,000 IU), E (10,000 mg), K3 (2,000 mg), B1 (1,000 mg), B2 (5,000 mg), B6 (1,500 mg), Pantothenic acid (10,000 mg), B12 (10 mg) Manganese (60,000 mg), Zinc (50,000 mg). Niacin (30,000 mg), Biotin (50 mg), Iron (30,000 mg), Folic acid (1,000 mg), Iodine (1,000 mg), Copper (4,000 mg) Selenium (100 mg), Cobalt (100 mg), and Calcium Carbonate.*

***According to Feed Composition Tables for rabbits (NRC of rabbits, 1977 and De Blas & Weiseman, 2010). [NDF% = 28.924 + 0.657 (%CF), ADF= 9.432+0.912 (CF %). Hemicellulose = NDF% - ADF%, according to Cheeke (1987) ***DE (M Cal /Kg) = (4.36-0.0491*NDF)].*

Collecting and evaluating semen:

Thirty days after the bucks started out feeding the experimental diets (the adaptation period), semen samples were collected from rabbit bucks using an artificial vagina (Morrell, 1995). The artificial vagina was held in the hand, palm downward at an angle of 45^{o}, and put under the female between hind limbs. When the bucks mount the female, orgasm takes place and ejaculation usually occurs within seconds, as in natural mating. Most of the ejaculate passes into the collecting tube. Two ejaculates were collected per buck weekly, with an interval of 30 minutes between collections for 6 consecutive weeks (García-Tomás *et al.*, 2006). Gel plugs were taken out right away after semen collection using pasture pipettes and a gift. Using a graded semen conical tube, the fresh ejaculate volume (in ml) without gel fractions for each buck was measured. Semen samples were then immediately taken to the lab, put in a 37°C water bath, and examined. Physical semen characteristics include ejaculate volume, viability (g/dl), sperm concentration (N10^6/mL), total sperm output (N10^6/ejaculate), live sperm (%), dead sperm (%) and abnormal sperm (%).

Blood collection and serum preparation:

Blood samples were taken from the ear veins for 5 bucks per treatment at the end of the trial. Each sample was divided into two tubes, the first of which was heparinized and the second not. Heparinized blood samples were used to measure the contents of the haematological parameters red blood cells (RBCs), white blood cells (WBCs), and hemoglobin (HGB), according to Emad El-Eslam's (1997) description. Also, platelets (PLT), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes (LCTs) and neutrophils (%) were determined.

Blood samples that had not been heparinized were immediately centrifuged at 3000 rpm for 15 minutes to separate the serum, which was then stored at -20°C for biochemical and hormonal analysis, which determined total protein, albumin (A), total cholesterol, triglycerides, and glucose using commercial diagnostic kits provided by (Bio-diagnostic Co. Giza, Egypt) accordance with the manufacturer's instructions. The difference between total protein and A was used for calculating the plasma globulin (G) and the albumin/globulin ratio (A/G ratio). In accordance with Demetriou (1987) approach, radioimmunoassay kits were used to measure the serum level of testosterone.

Statistically analysis:

The data were statistically examined by analysis of variance (ANOVA) and the following model was used: $Y_{ij} = \mu + T_i + E_{ij}$, where y_{ij} is the measured value, μ is the overall mean effect, T_i is the i_{th} treatment impact, and E_{ij} is the random experimental error. Duncan's multiple ring test was used to distinguish differences among treatment means (Duncan, 1955) by using SPSS Software 20 for Windows from 2011.

RESULTS AND DISCUSSION

Semen quality:

The effects of dietary treatments on the semen characteristics of rabbit's bucks are illustrated in Table 3. Sieved olive pulp supplemented with E, DY, or E+DY displayed insignificant improvement on testosterone level, ejaculate volume (ml), live spermatozoa, dead spermatozoa, and sperm abnormalities. Otherwise, the results showed a significant increase in viability, sperm concentrations ($P \leq 0.05$) and total sperm output ($P \leq 0.01$). Rabbit bucks fed diets containing SOP_{20}-E and SOP_{25}-DY showed a significant improvement in viability ($P \leq 0.05$) compared to other treatments. In contrast, rabbit bucks fed a control diet exhibited a significant ($P \leq 0.01$) decrease in total sperm output compared to other treatment groups. The lowest sperm abnormalities and dead spermatozoa were recorded in the buck's diet containing SOP_{25}-DY compared with other treatments. Generally, diets including SOP supplemented with E, DY, or a combination of them resulted in improvements in all semen traits evaluated.

These results support findings by El-Azzazi *et al*. (2015), who observed that bucks fed olive cake up to 12.5% showed significantly improved desire, sperm motility, sperm cell concentration, and semen ejaculate volume when compared to controls. Younan *et al*. (2017) stated that total semen volume was significantly increased in rabbits fed diets supplemented with olive leaf extract (OLE) up to 1.5 ml/kg as compared to the control group. Additionally, they stated that OLE had a beneficial impact on testosterone secretion by increasing male fertility by inhibiting 6 β-hydroxylation of testosterone and raising the level of the hormone. On the other hand, Hassan *et al*. (2016) showed that cocks' diets inclusion of 8% of olive cake resulted in a reduction in the volume of ejaculate, total number of motile sperm, semen quality, and increased hydrogen ion. The addition of olive cake to the diets of cocks did not significantly change sperm concentration, living spermatozoa, dead spermatozoa, sperm abnormalities, or the concentration of testosterone hormone. Additionally, most of the semen quality parameters significantly decreased when the percentage of OC was increased to 16% in the diet of cocks.

It's obviously true that the best improvement in the most previous parameters was obtained by including rabbits in the SOP_{25}-DY diet. These improvements may be attributable to increasing nutrient availability made possible by more effective nutrient absorption throughout the gastrointestinal tract as a result of the addition of probiotics (McDaniel and Sefton, 1991), which stimulate the digestion process (El-Badawi *et al*., 2017) and/or contribute to the gut's microbial equilibrium in order to prevent digestive disorders. This result is consistent with recent studies that found probiotics significantly increased total sperm cells and semen volume in the ejaculate of rabbit bucks (Ewuola, 2013 and Mymrin *et al.*, 2017). In the same trend, Besseboua and Ayad (2021) noted increases in semen volume, mass motility, and individual motility in the rabbits fed diets supplemented with *S. cerevisiae* up to 0.6 g/day. Similarly, Emmanuel *et al.* (2019) and Helal *et al*. (2018) found a notable improvement in epididymal sperm characteristics, testicular, sperm concentration, motility and testosterone of rabbit bucks that received SC supplements. In contrast, over the two-month experiment, the sperm concentration was significantly lower in the rabbits given SC supplementation compared to the control group. When SC supplementation was added to the rabbit's diet, the mortality rate of spermatozoa was reduced compared to that in the control group.

In connect of enzyme effects, our results agree with the findings by Saleh *et al*. (2020), who found that kemzyme supplementation in bucks diets was shown to increase sexual activity indices, semen parameters, and steroidogensis. Similarly, Gado *et al.* (2015) indicated that multienzyme supplementation in the diets of rabbit bucks improved sperm motility, testosterone and decreased abnormal sperm and dead spermatozoa, which may have promoted the proliferation of high-quality sperm cells. This response may be caused by dietary factors that affect reproductive processes, such as the cellular activities in the testis' seminiferous tubules and the neuroendocrine pathways via which nutritional inputs influence the brain centers that regulate reproduction. They added that adding multienzymes to rabbit diets increased the availability of macronutrients and micronutrients as a result of the activity of enzymes required for the production of various spermatozoa components. This might be due to the increasing endocrine activity of rabbit buck gonads, which created a favorable environment for spermatogenesis.

A few reports were available in the literature on the effect of exogenous enzyme supplementation in rabbit buck diets on semen quality.

These results exhibited the beneficial impacts of adding enzymes and dry yeast to diets that contained 20 or 25% SOP in improving the semen quality parameters of rabbit bucks.

Blood hematological parameters:

The results of hematological parameters as affected by dietary treatments are presented in Table 4. According to the results, most hematological parameters, such as WBCs, RBCs, HBG, MCV, MCH, MCHC, HCT, LCTs, and neutrophils, were not significantly affected by SOP levels, enzyme or dry yeast supplementations, with the exception of PLT, which was improved significantly ($P \leq 0.05$) by most dietary treatments in comparison to the control group. Rabbit bucks fed a diet containing SOP20-E attained the greatest PLT value, followed by rabbits provided a diet containing SOP20 with a combination of E+DY supplements compared to the control group. The present results are in line with Afsari *et al*. (2013), who found that adding non-phytate phosphorus and phytase to date stones and olive pulp up to 20% had no significant influence on the white blood cell count (heterophils, eosinophils, basophils, lymphocytes, and monocytes), cholesterol and triglycerides of laying hens, but a level of 5% olive pulp reduced plasma cholesterol concentration. However, Hassan *et al*. (2016) demonstrated that cocks fed 16% OC had significantly lower levels of hemoglobin, globulin, and RBCs compared to those fed the control diet. In comparison to the control group, the cocks fed Allzyme had higher levels of RBCs, HGB, total protein, A, and G. In addition, they showed that OC and enzyme supplementation had a significant interaction in RBCs.

Regarding enzymes, our results are in line with Oloruntola and David (2018), who demonstrated that the addition of commercial enzymes to rabbit diets had no significant effect on the blood constituents WBCs, LYM, MON, GRA, RBCs, HGB, PCV, MCV, MCH, MCHC, and PLA. Also, El-Kelawy and El-Kelawy (2016) revealed that adding enzymes to rabbits' diets had no significant impact on their haemoglobin levels, PCV, MCV, MCH, MCHC, WBCs, or differential leukocyte counts. In the same trend, Kurtong (2014) found that there were no significant differences among all treatments for HGB, WBCs, or PCV in rabbits fed diets supplemented with enzyme. Similarly, Amaravadhi *et al*. (2012) reported that adding kemzyme enzymes to rabbits' diets had no impact on the rabbits' total erythrocyte count, total leucocyte count, lymphocytes, neutrophils, eosinophils, monocytes, haemoglobin, and PCV.

On the other hand, Mohammed (2023) showed that rabbits fed diets containing kemzyme had higher RBCs, PCV, and HGB and a lower MCV than those on the control diet. White blood cells and mean corpuscular hemoglobin concentration were unaffected by the rabbits' diets' kemzyme additions. El-Kelawy and El-Shafey (2017) found that when enzyme supplementation (kemzyme) was added to rabbit diets containing 10% date stone meal, RBC levels increased significantly compared to the control and other groups. However, they have significant effects on the other blood plasma constituents, different leucocyte counts and hematological indices.

Exogenous enzymes may be responsible for this improvement in hematological parameters since they are utilized to increase the digestibility of a variety of feed components, including fiber, phytate, protein, etc. Non-starch polysaccharides (NSP), which are big polymers, are specifically broken down by fiber-degrading enzymes into smaller polymers to reduce their anti-nutritive effects (Choct and Annison 1992). The results were better flock performance and better bird health (Saleh *et al*., 2005 and Cowieson and Ravindran, 2008).

Our findings concur with Abdulhakim *et al*. (2018), who noticed that the effects of supplementing with dry yeast on the rabbit's RBCs, HGB, PCV, MCV, MCH, MCHC, TLC, Lymph., Neutoph., and Monocyt are insignificant changes in hematological parameters. Similar studies revealed that adding SC to rabbits' diets had no effect on hematological characteristics (Seyidoglu and Galip, 2014 and Belhassen *et al*., 2016). Similarly, Seyidoglu *et al*. (2013) reported no significant differences in hematological parameters within treatment groups despite observing a slight increase in hematocrit and hemoglobin concentrations in rabbits supplemented with yeast. In contrast, Besseboua and Ayad (2021) found statistically significant differences between the RBCs, HGB, HCT, GCT, and MCV in rabbits receiving graded amounts of SC and the control group. According to Seyidoglu *et al*. (2013), this variance may be due to the various *S. cerevisiae* concentrations. In a different study, Elghandour *et al*. (2019) showed that yeast-fed rabbits had higher levels of WBCs and LCTs than those fed on the control diet. Based on reports, the LCT content in rabbits fed diets supplemented with SC may be an indication of greater humoral immune responses. Also, research conducted by Al-Mansour *et al*. (2011) and Adebowale *et al*. (2014) demonstrated that including dietary yeast in the diets of poultry and turkey significantly lowered the number of leukocytes and lymphocytes in those animals.

Blood serum parameters:

The nutritional and physiological status of the animal may be determined by changes in blood constituents and metabolites. All serum constituents were within the normal range (Manning *et al.*, 1994). The results in Table (5) showed that total protein, A, G, aspartate transaminase (AST), triglycerides, and creatinine all showed insignificant differences. In contrast, blood cholesterol levels in rabbit bucks fed the dietary treatments were significantly lowered ($P \leq 0.01$) compared to the control treatment. As well as significant differences ($P \leq 0.01$) in glucose levels, rabbit bucks receiving diets containing SOP_{20} or SOP_{25} with a combination of enzyme and dry yeast supplementations achieved the highest values of glucose levels compared to those fed on the control diet. This might be a result of the enzyme and yeast working together synergistically. In agreement with our results, Bakr *et al*. (2019) decided that inclusion of olive cake pulp in rabbit diets up to 25% did not significantly affect total protein, A, G, AST, alanine amino transferees (ALT), creatinine, or cholesterol, but it did significantly lower triglycerides compared to the control treatment. Moreover, the presence of saponins, which exert inhibitory effects on cholesterol uptake in the gut through intra-lumenal physiochemical interaction, may be responsible for the observed decrease in cholesterol levels (Yilkal, 2015). Azazi *et al*. (2020) indicated that total lipids, total protein, globulin, A/G, AST and ALT were not significant, but differences between the control group and growing rabbits fed diets containing 20% olive cake in terms of blood cholesterol and triglyceride were significant. Azazi *et al*. (2018) illustrated that growing rabbits fed a diet containing 10% olive cake without or with 0.1% citric acid had lower cholesterol and triglyceride levels than the other treatment. The dietary treatments had no effect on the levels of total lipids, total protein, A, G, A/G ratio, or the liver enzymes ALT and AST. According to Mousa and Abd El- Samee (2002), the inclusion of olive pulp in growing rabbit 'diets had no significant influence on the experimental groups' serum globulin, total lipid, glucose, creatinine, AST, or ALT concentrations. The same trend was noticed by Mehrez and Mousa (2011), who found that concentrations of total protein, A, G, cholesterol, urea-N, AST and ALT did not differ significantly with olive pulp inclusion in rabbit diets at levels up to 30%. Similar to Hassan *et al*. (2016), when olive cake was used up to 16% in the diet of Mamora cocks, there was no significant difference among treatments in ALT, AST, or urea concentrations.

An elevated triglyceride level may increase the risk of liver disease, pancreatitis, and atherosclerosis. Reduced levels of triglycerides in the study's rabbits fed a diet containing 25% SOP compared to the other test diets indicate the potential presence of bioactive chemicals in SOP that may have impeded fat absorption and led to fat depletion. The fact that the cholesterol levels were lower in the rabbits fed inclusion SOP regardless of any supplementation in the diets than those fed the control diet in this study is further evidence that this is health-beneficial for customers generally, especially for those who are at risk for heart disease. The effect of SOP on diets is increased loss or catabolism of cholesterol or reduced cholesterol uptake.

Our results coincide with those of El-Kelawy and El-Shafey (2017), who revealed that the effects of kemzyme supplementation on blood plasma A, total lipids, cholesterol, creatinine, AST, ALT, and alkaline phosphates were not statistically significant. According to Al-Harthi *et al*. (2020), plasma cholesterol and triglycerides were significantly reduced by adding phytase to 10% and 15% olive cake in broiler chicken diets.

Also, Attia *et al*. (2012) found that rabbits treated with kemzyme had significantly higher blood glucose levels than controls. It could be due to the inclusion of exogenous enzymes improving the digestibility and metabolism of different nutrients in the diet, which then directly impacts the blood levels of essential nutrients (Mathlouthi *et al*., 2003).

As regards dry yeast effects, our results agree with those of Abdalhakim *et al*. (2018), who reported that adding *S. cerevisiae* to rabbit diets significantly reduced blood cholesterol and low-density lipoprotein levels while having no effect on creatinine or urea levels. As well as Galip and Seyidoglu (2012) noticed that rabbits fed *S. cerevisiae* had a significantly decreased serum cholesterol. In addition, Seyidoglu and Galip (2014) showed that rabbits given dietary *S. cerevisiae* supplementation had significantly higher serum glucose levels compared to the control treatment. Edrees *et al*. (2017) found blood total lipids, triglycerides, and cholesterol significantly decreased when used yeast up to 4g/kg diet of laying hens. This demonstrated that adding dietary yeast does not harm kidney tissue and may even help to prevent kidney disease. Shareef and Al-Dabbagh (2009) added that broiler chickens fed *S. cerevisiae* up to 2% showed significantly decreased serum total cholesterol levels. The hypocholesterolemic effects of *S. cerevisiae* may be attributed to its probiotic ability to modulate serum cholesterol levels by deconjunction of bile acid. They further stated that the effects of *S. cerevisiae* on blood triglycerides and ALT and AST activity were insignificant. In another study Matur *et al.* (2010) observed that the serum triglyceride levels of hens fed *S. cerevisiae* extract for 30 days were not significantly affected.

Generally, the absence of negative effects of SOP on semen quality traits or most serum constituents may be linked to the ability of enzymes and dry yeast to improve the utilization of nutrients, especially crude protein in dietary treatments.

Table (3): Semen characteristics of male rabbits as affected by dietary treatments.

Parameters	Control	Dietary treatments						SEM	Sig.
		20% sieved olive pulp			25% sieved olive pulp				
		0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY)	E+DY	0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY)	E+DY		
Ejaculate volume (ml)	1.00	1.27	1.33	1.08	1.05	1.13	1.05	0.12	NS
Viability (%)	66.65^{c}	86.00^{a}	72.50^{bc}	68.15^{bc}	69.00^{bc}	80.00^{ab}	70.50^{bc}	3.33	*
Sperms concentration (N×106/mL)	426.25^{d}	432.50^{cd}	438.75^{bcd}	446.25^{bcd}	515.00^{a}	488.75^{abc}	497.50^{ab}	17.94	*
Total sperm output (N×106/ejaculate)	428.50^{d}	542.75^{ab}	581.25^{a}	484.50^{c}	537.50^{ab}	551.00^{ab}	518.50^{bc}	13.93	**
Live (%) Spermatozoa	86.00	89.25	87.00	87.00	87.75	91.75	91.00	1.60	NS
Dead spermatozoa (%)	7.75	5.50	7.25	7.00	6.50	4.00	4.50	1.24	NS
Sperm abnormalities (%)	6.25	5.25	5.75	6.00	5.75	4.25	4.50	0.85	NS
Blood testosterone concentration	1.34	1.67	1.63	1.57	1.40	1.54	2.12	0.14	NS

$^{a-d}$: Means in the same row without similar superscripts are significantly (P ≤0.05) and (P ≤0.01) different. NS: Not significant.

Table (4): Hematological parameters of rabbits as affected by dietary treatments

Parameters	Control	Dietary treatments						SEM	Sig.
		20% sieved olive pulp			25% sieved olive pulp				
		0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY)	E+DY	0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY	E+DY		
RBCs ($10^6/mm^3$)	5.29	5.48	5.80	5.41	5.74	5.79	5.41	0.21	NS
WBCs ($10^3/mm^3$)	8.33	8.60	7.70	7.03	9.93	9.53	7.63	1.10	NS
HGB (g/dl)	11.90	12.73	13.67	12.60	12.90	13.10	12.65	0.37	NS
MCV (%)	63.70	68.40	73.47	67.20	65.37	67.20	68.07	1.14	NS
MCH (pg)	22.50	23.20	23.50	23.17	22.47	22.60	24.13	0.49	NS
MCHC (g/dl)	35.40	33.93	34.90	35.40	34.37	33.70	35.63	0.56	NS
HCT (%)	33.53	37.50	39.10	35.40	37.43	39.87	35.87	1.40	NS
PLT ($10^3/mm^3$)	121.00^{c}	233.67^{a}	143.33^{bc}	216.67^{a}	200.67^{ab}	187.67^{ab}	207.00^{ab}	18.82	*
LCTs ($10^3/mm^3$)	53.33	53.20	49.90	42.20	51.80	54.57	51.67	2.97	NS
Neutrophil.	27.20	34.13	36.40	43.80	33.83	31.67	34.47	3.68	NS

Red blood cells (RBCs), haemoglobin (HGB), platelets (PLT), haematocrit (HCT), white blood cells (WBCs), lymphocytes (LCTs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

$^{a-b}$: *Means in the same row without similar superscripts are significantly (P≤0.05) and (P≤0.01) different.*

NS: Not significant.

Table (5): Blood serum parameters of rabbits as affected by dietary treatments.

Parameters	Control	Dietary treatments						SEM	sig
		20% sieved olive pulp			25% sieved olive pulp				
		0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY)	E+DY	0.1 g/kg econase enzyme (E)	0.5 g/kg dry yeast (DY)	E+DY		
Total protein(g/dl)	6.83	6.92	7.02	6.91	7.23	6.64	6.53	0.16	NS
Albumin (g/dl)	4.33	4.52	4.61	4.50	4.83	4.31	4.50	0.14	NS
Globulin (g/dl)	2.50	2.40	2.41	2.42	2.40	2.33	2.04	0.12	NS
Aspartate transaminase (AST)	19.00	22.00	22.67	19.00	22.67	22.67	19.00	1.37	NS
Alanine amino transferees (ALT)	33.00	32.67	33.67	31.00	32.67	36.00	31.00	1.23	NS
Total cholesterol (mg/dl)	59.00^{a}	49.00^{b}	52.67^{b}	49.33^{b}	47.00^{b}	50.33^{b}	52.67^{b}	1.49	**
Triglycerides (mg/dl)	63.33	57.67	57.00	61.00	62.00	57.33	60.67	2.93	NS
Creatinine	0.93	0.83	0.87	0.90	0.91	0.92	0.92	0.04	NS
Glucose (mg/dl)	121.33^{c}	131.00^{bc}	127.00^{c}	141.67^{ab}	132.67^{abc}	133.33^{abc}	143.33^{ab}	3.34	**

$^{a-b}$: Means in the same row without similar superscripts are significantly ($P \leq 0.01$) different   NS: Not significant.

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تأثير الانزيمات والبروبيوتيك في تحسين الاستفادة من تفل الزيتون المنخول على الأداء التناسلي وبعض صفات الدم لذكور الأرانب

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يعتبر تفل الزيتون من مخلفات التصنيع الزراعي ذات القيمة الغذائية المنخفضة. هدفت الدراسة الحالية إلى دراسة تأثير الإنزيمات والخميرة الجافة في زيادة الاستفادة من تفل الزيتون المنخول على الأداء التناسلي وبعض مؤشرات الدم لذكور الارانب، السؤال الذي يطرح نفسه هو ما هو الحد الأقصى لكمية تفل الزيتون المنخول التي يمكن دمجها في النظام الغذائي لذكور الارانب لتحقيق أقصى قدر من الاستفادة باستخدام بعض الإضافات؟ تم تقسيم 49 أرنبًا بالغًا من نوع V-Line إلى 7 معاملات غذائية وتم وضعها في بطاريات فردية. تضمنت المعاملات العليقة الاساسية بدون إضافات كمجموعة ضابطة و6 معاملات أخرى تتضمن استبدال العليقة الاساسية بمستويين 20% و25% من تفل الزيتون مضاف اليها انزيم econase 0,1 جم/كجم عليقة، و0,5 جم خميرة جافة /كجم عليقة أو مزيج من الانزيم والخميرة الجافة معا لمدة 8 أسابيع.

أظهرت النتائج أن تغذية ذكور الأرانب على علائق تحتوي على 20% تفل زيتون منخول مع الانزيم و25% تفل زيتون منخول مع الخميرة الجافة أظهرت تحسناً معنوياً (P≤0.05) في الحيوية مقارنة بالمعاملات الأخرى.

ـ أن تغذية ذكور الأرانب على علائق تحتوي على 25% تفل زيتون منخول مع أي إضافات أدت إلى تحسن معنوي. (P≤0.05) في تركيز الحيوانات المنوية مقارنة بالمجموعات الأخرى. بينما أدت التغذية على علائق تفل زيتون المنخول المحتوية على أي إضافات غذائية إلى زيادة معنوية (P≤0.01) في إجمالي إنتاج الحيوانات المنوية مقارنة بالمجموعة الضابطة.

ـ أدت تغذية ذكور الارانب على علائق محتوية على 25% تفل زيتون منخول مع الخميرة الجافة الى قلة عدد الحيوانات المنوية الشاذة والميتة مقارنة بالمعاملات الأخرى. لم تؤثر الاضافات الغذائية أو مستويات تفل الزيتون معنويا على معظم قياسات الدم، ولكن PLT كان انخفض معنويا (P≤0.05) في جميع المعاملات الغذائية مقارنة بالمجموعة الضابطة.

ـ كانت مستويات الكوليسترول في الدم أقل معنويا (P≤0.01) في ذكور الأرانب التي تغذت على تفل الزيتون بغض النظر عن الاضافات الغذائية مقارنة. بالمجموعة الضابطة على العكس من ذلك، أدت التغذية على علائق تحتوي على 20% و25% تفل زيتون المنخول مع خليط الانزيم والخميرة إلى زيادة معنوية في مستويات الجلوكوز .(P≤0.01)

الخلاصة: يمكن دمج تفل الزيتون المنخول في غذاء ذكور الارنب بنسبة تصل إلى 25% مع اضافة الأنزيمات أو الخميرة الجافة دون أي آثار سلبية على نوعية السائل المنوي أو مكونات الدم أو الصفات البيوكيمائية للدم.