

## **IMPACT OF DIETARY SUPPLEMENTATION WITH MULTI ENZYME AND/OR PROBIOTIC ON GROWTH PERFORMANCE, NUTRIENTS DIGESTIBILITY AND BLOOD CONSTITUENTS OF GROWING RABBITS**

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### **SUMMARY**

A total of 60 weaned New Zealand White rabbits at five weeks of age with an average body weight of  $536 \pm 29.3$ g were used in the present study. The study aimed to investigate the effect of feeding a basal commercial diet supplemented with either an enzyme complex (Kemzyme) or yeast preparation (Actisaf) or mixture of them on growth performance, nutrient utilization and health status of growing rabbits. The animals were divided into four equal experimental groups (each experimental group contains 15 rabbits distributed among 5 replicates, each containing 3 rabbits) fed on four diets, basal commercial diet control (diet 1), control + 1g/kg diet Kemzyme (diet 2), control + 10g/kg diet Actisaf (diet 3) and control + 1g/kg diet Kemzyme + 10g/kg diet Actisaf/kg (diet 4). At the 13 week of age, 5 rabbits from each experimental group were taken for the digestibility trial. The results of the study revealed that final live body weight, daily body gain and economical efficiency were significantly ( $P < 0.01$ ) higher with rabbits fed supplemented diets than those fed the control one. However, the previous parameters were the highest ( $P < 0.01$ ) with rabbits fed diet 4. The daily weight gain values (at 5-13 weeks old) recorded 25.3, 27.5, 27.0 and 28.7 g for those fed the four diets, respectively. Feed conversion ratio (FCR) was significantly ( $P < 0.01$ ) improved with supplemented diets than control (3.97, 3.83, 3.88 and 3.70 for diets 1, 2, 3 and 4, respectively). Apparent digestibility (%) of DM, CP and DCP were improved by dietary supplementation. However, the improvement was significantly the highest ( $P < 0.01$ ) by rabbits fed Kemzyme + Actisaf supplemented diet (diet 4). In spite of the elevation of levels of blood plasma total protein, globulin, albumin and phagocytic activity by dietary supplementation, all blood biochemical and hematological parameters were within the normal physiological range. The results indicate that Kemzyme and Actisaf are good growth stimulators to the growing rabbits and can be added together to the commercial diet to improve feed utilization and growth performance without any adverse effects on health status of rabbits.

**Keywords:** Rabbits, multienzyme, probiotic, growth performance, digestibility and blood parameters.

### **INTRODUCTION**

Monogastric animals such as rabbits are suffering from lack enzymes necessary to hydrolyze non-starch polysaccharide components of plant cells in their foregut and this can lead to less utilization on nutrients and may even be a factor in clinical conditions such as nutritional diarrhea.

Several attempts were devoted to improve feed utilization and eliminate the indigestible feeds in the animal waste including use of probiotics, prebiotics, organic acids, enzymes and plant extracts (Onifade *et al.*, 1998; Attia *et al.*, 2012a; Attia *et al.*, 2014a,b and Goli and Aghdam Shahryar, 2015).

Enzyme mixture could support the endogenous enzymes of the poultry and rabbits, break down some components of cell wall, which cannot be broken down into absorbable nutrients by endogenous enzymes (Abd El-Latif, *et al.* 2008; Attia *et al.*, 2012a and Attia *et al.*, 2014a and b). Exogenous enzymes supplemented was nested to lowering the gastrointestinal viscosity in digestive tract (Simon, 2000), reducing nutrient entrapment and releasing other nutrients like minerals (Ibrahim *et al.*, 2010), decreasing the anti-nutritional substances and improving animal performance (Abd El-Latif, *et al.* 2008; El-Sagheer and Hassanein, 2014 and Goli and Aghdam Shahryar, 2015). Moreover, Latorre *et al.* (2014) suggested that higher amount of untraditional feedstuffs could be used in the presences of enzymes. The enhancement in body weight gain due to enzymes mixture supplementation was reported by Ibrahim *et al.*

(2010) on growing rabbits and Attia *et al.* (2014a) on broilers. Ghazalah *et al.* (2005) reported that enzyme supplementation improved monogastric animal performance and reduce the energy formulation of the diets.

Yeast has been known as a probiotic added in the animal feed (Saegusa *et al.*, 2004). Probiotics are live microorganisms that have many beneficial effects including promoting growth rate and enhancing protection against pathogenic gram positive and negative bacteria inside the intestine, through competitive exclusion, and lower the intestinal pH (Attia *et al.*, 2011). Yeast also provides some essential nutrients such as vitamin B complex and many amino acids and aids in digestion by producing many enzymes. The inclusion of *Saccharomyces cerevisiae* in the diet of rabbits increased feed intake and growth of rabbits (Maertens and Ducatelle, 1996). Also, *Saccharomyces cerevisiae* was noted to improve protein digestion in domestic rabbits (Kamra *et al.*, 1996). Onifade *et al.* (1998) observed the positive effect of *Saccharomyces cerevisiae* on weight gain, feed intake and feed conversion ratio in rabbits. Ezema and Eze(2012) suggested that *Saccharomyces cerevisiae* had a beneficial effect on growth and health status of rabbits. The growth rate of rabbits fed yeast supplemented diets was significantly ( $P<0.05$ ) higher as compared to non-supplemented group irrespective of the housing systems (Khanna *et al.*, 2014).

Therefore, the objective of this study was to assess the influence of dietary addition of *Saccharomyces cerevisiae* and enzymes on productive performance, digestibility of nutrients, blood biochemical and hematological traits of NZW rabbits.

## MATERIALS AND METHODS

The present experiment was carried out in El-Behaira Governorate, Egypt. The present work lasted two month (from mid-December 2014 to mid-February 2015) in order to evaluate the effects of supplementation of multienzyme and probiotic in the basal diet on growth performance, blood biochemical parameters, and nutrient digestibility of weaning New Zealand White (NZW) rabbits. For this purpose, sixty male NZW rabbits, 5 weeks of age with an average initial body weight  $536 \pm 29.33$ g were randomly allotted into four experimental groups, each experimental group contains 15 rabbits distributed among 5 replicates, each containing 3 rabbits (cage). The 1<sup>st</sup> group was used as control and fed only a basal pelleted ration without any supplementation of multienzyme or probiotic. Rabbits in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were fed respectively the basal pelleted ration supplemented with multienzyme (Kemzyme at 1g/kg diet), probiotic (10g Actisaf® yeast, i.e 107CFU/g/kg DM) and multienzyme plus probiotic (1g Kemzyme /kg diet + 10g/kg Actisaf®), respectively. The enzyme complex preparation kemzyme was a product of Kemin Agrifoods Industry, European United and composed analysis of 300 µg beta-glucanase , 5000 µg cellulase, 450 µg alfa amylase and 450 µg protease and lipase.

The Actisaf preparation was product of Lesaffree Feed additive, Marquette-Lez-Lille, France and composed of live yeast ( *Saccharomyces cerevisiae* ) , NCYC Sc 47,  $10^7$  CFU/g of dry matter, coated with saccharides. The rabbits were housed in wire cages (60 × 55 × 40 cm) provided with galvanized feeders and automatic nipple drinkers. The experimental diets were offered to rabbits *ad libitum* and fresh tap water was available to rabbits all the time. Rabbits were kept under the same hygienic and environmental conditions during the experimental period. The experimental basal diet was formulated to meet the nutrient requirements of rabbits according to NRC (1977). Ingredients and chemical composition of the experimental diets are shown in Table (1).

During the growing period from 5 to 13 weeks, rabbit live body weight, daily weight gain (DWG), feed consumption and feed conversion ratio were weekly recorded. European Production Efficiency index was calculated as cited by Attia *et al.* (2012a). Economical evaluation for all experimental rations was calculated as described by Zeweil (1996).

At the 13 week of age, 5 rabbits from each experimental group were taken for the digestibility trial. A total number of 20 male rabbits were housed individually in metabolic cages that allow collected feces throughout the digestibility trial. Quantitative collection of feces was started 24 hours after offering the daily feed. Feces of each male rabbit were collected quantitatively once a day before the morning at 9.00 am and feed intake was recorded every day in the morning for 6 days as a collection period. Feces samples were stored at  $-20^{\circ}$  C. The six days combined collections were sampled and the samples were kept for routine analysis. Feed samples was collected and prepared for proximate analysis. Fecal samples were dried at  $65^{\circ}$ C for 24 hours and ground through a 1 mm screen on a Wiley grinder. They were composited 50 gm per sample per treatment per animal for analysis. The composite samples of the offered

diet and dried feces of each rabbit were chemically analyzed for crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen free extract (NFE) according to the method of A.O.A.C. (1995).

**Table (1): Ingredients and chemical analysis of the experimental basal diet.**

Item	%
Yellow Corn	6.22
Soybean meal, 44%	22.33
Wheat bran	23.33
Barley	15.00
Alfalfa hay	30.12
Ground limestone	1.00
Dicalcium Phosphate	1.20
Common salt	0.50
Vit. + Min. Premix *	0.30
Total	100.00
Chemical analysis:	
Dry matter	90.55
Organic matter	80.97
Crude protein	17.28
Crude fiber	13.26
Ether extract	2.69
Nitrogen-free extract	47.74
Ash	9.58
Calculated digestible energy (kcal/kg diet)**	2680
Cell Wall Constituents (%):	
Neutral detergent fiber (NDF)	4.99
Acid detergent fiber (ADF)	2.85
Hemicellulose	2.14

\* Each 3kg of premix contains: Vit. A:12,000,000 IU; Vit. D3: 3,000,000 IU; Vit. E:10.0 mg; Vit. K3: 3.0 mg; Vit. B1: 200 mg; Vit. B2: 5.0 mg Vit. B6: 3.0 mg; Vit. B12: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg; Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 70 g; Fe:35 g; I: 1 g; Co: 0.15g and Se: 0.3g.

\*\* was calculated according to Carpenter and Clegg (1956).

NDF and ADF were calculated according to Cheeke (1987) where:

$$\text{NDF} = 28.924 + 0.657 (\%CF) \text{ and } \text{ADF} = 9.432 + 0.912 (\%CF).$$

ME calculated according to Carpenter and Clegg (1956) by applying the equation:- ME (Kcal/kg)=(35.3\*CP%)+(79.5\*EE%)+(40.6\*NFE%)+199.

At the end of the experiment (at week 13 of age), six blood samples were collected from each treatment. Blood samples were collected from ear vein using sterilized syring needle and placed into a heparinized test tube. Blood plasma was obtained by centrifugation at 2500 r.p.m. for 15 minutes, and then stored in deep freezer at -20 °C until biochemical tests.

Blood hemoglobin concentration (g/dl) was determined using hemoglobinometers as the method described by Tietz (1982). Red blood cell counts were counted on bright line hemocytometer using light microscope at 400X magnification. RBC's were counted according to the method of Hawkeye and Dennett (1989). White blood cell counts were counted according to the method of Hawkeye and Dennett (1989) using a light microscope at 100X magnification.

PCV (%) was recorded directly according to Wintrobe (1965). Blood MCH and MCHC were determined according to the following equations of Jain (1986):

$$\text{Mean corpuscular hemoglobin (MCH) (Pg)} = \text{HbX10/ RBC's}$$

$$\text{Mean corpuscular hemoglobin concentration (MCHC) (g/dl)} = \text{HbX100/PCV}$$

Blood film was prepared according to the method described by Lucky (1977). Ten drops of any Gunwale stain stock solution with a dry, unfixed smear were added to equal amount of distilled water, then mixed and left for 1 minute for staining. The dye was decanted without rinsing. Diluted Giemsa's solution (10 drops of the dye were added to 10 ml distilled water) was poured over the film as counter stain and left for 20 minutes, then rinsed in water current and absolute value for each type of cells were counted and calculated relative to total WBC's. Phagocytic activity was determined according to Kawahara *et al.* (1991). *Candida albicans* culture (50 µg) was added to 1 ml of citrated blood and shaken in water bath at 23-25°C for 3-5 hr, smears of the blood were then stained with Giemsa solution. Phagocytic was estimated by determining the proportion of macrophages which contained intracellular yeast cell in a random count of 300 macrophages and expressed as percentage of phagocytic cells containing yeast cells (PA). The number of phagocytized organisms was counted in the phagocytic cells and expressed as number of yeast cells phagocytes/number of phagocytic cells (PI).

All blood biochemical parameters were determined using commercial kits (Diamond Diagnostics, Egypt) according to the following methods. Plasma total protein (Doumas *et al.*, 1981), albumin (Doumas *et al.*, 1971), Plasma globulin was obtained by subtracting the concentration of albumin from that of plasma total protein. Plasma creatinine (Fabiny and Ertingshausen, 1971), urea (Sampson *et al.*, 1980), total lipids (Chabrol and Charonnat, 1973), total cholesterol (Allain *et al.*, 1974), triglycerides (Bogen and Kaller, 1987), glucose (Hyvarinen and Nikkila, 1962) and ALP (Belfield and Goldberg, 1971) as well as activities of plasma transaminase ALT and AST were measured (Reitman and Frankel, 1957).

#### **Statistical analysis**

Data were statistically examined by analysis of variance (ANOVA) according to Snedecor and Cochran (1982) using SPSS system (2006). The differences between means were tested by using Duncan's New Multiple Range test (Duncan, 1955).

## **RESULTS AND DISCUSSION**

### **Effect of dietary supplementations on:**

#### **1- Growth performance parameters:**

Data in Table (2) show that average live body weight at 9 and 13 weeks of age and daily weight gain, daily feed intake and feed conversion ratio from 5-9, 9-13 and 5-13 weeks of age were significantly improved ( $P < 0.01$ ) in rabbits fed the diet supplemented with multienzymes or probiotic than the control. Live body weight at 13 weeks of age and daily weight gain from 9-13 and 5-13 weeks of age were significantly higher ( $P < 0.01$ ) with rabbits fed multienzyme + probiotic diets than those fed multienzyme or probiotic diets. Supplementation with multienzymes or probiotic improved ( $P < 0.01$ ) the economic efficiency and production index compared to control group. Rabbit fed diet supplemented with multienzymes plus probiotic had the best values of growth performance parameters, followed by those fed diet supplemented with multienzymes and probiotic, respectively. These findings are in agreement with those of Onifade *et al.* (1998), El-Kelawy *et al.* (2002), Onu and Oboke (2010) and Khanna *et al.* (2014) who found that the growth performance of the rabbits fed diet supplemented with yeast was significantly ( $P < 0.05$ ) higher as compared to the control one. In addition, live yeast, such as *Saccharomyces cerevisia*, contains numerous enzymes that could be released into the intestine and aid existing enzymes in the digestive tract in the digestion of feed (Kornegay *et al.*, 1995). Moreover, yeast supplementation can inhibit pathogenic bacteria and increase the number of anaerobic and cellulolytic bacteria as reported by (Abd El-Azeem, 2002). Also, Yamani *et al.* (1992) found that, a pelleted diet supplemented with probiotic improved weight gain in NZW rabbits during the growing period. Sarhan (2001) found that under Egyptian conditions, feeding rabbits diet supplemented with 0.5 g/kg of either Kemzyme or Optizyme (multi enzyme mixtures) significantly improved daily weight gain from 5 to 9 and from 5 to 13 weeks of age. Also, Makled *et al.*, (2005) found that average daily feed intake of rabbits during the growth period from 6 to 12 weeks of experimental period was increased due to adding optizyme at a levels of 500 or 750 mg / kg feed. The enhancement in feed conversion efficiency as a result of adding enzymes may be due to the effect of enzymes in improving the digestibility of nutrients (El-Mandy *et al.*, 2002). A significant increase in the average body weight was observed in growing rabbits supplemented with Kemzyme for 8 weeks (Attia *et al.*, 2012b). Eiben *et al.* (2002) found that feeding rabbit's diet supplemented with cellulase enzymes significantly improved weight gain from 23 to 63 and from 63 to 77 days of age, by 17 and 3%, respectively. Onu and Oboke (2010) reported that feed conversion ratio of rabbits fed diets supplemented with enzyme and probiotics were significantly superior

over the other groups. Gutiérrez *et al.* (2002) showed that addition of enzymes has improved body weight gain of young rabbits, from 25 to 39 days of age by 3.1%. El-Sagheer and Hassanein (2014) found that the addition of commercial enzyme mixture (Veta-zyme) at the level of 1g/ kg commercial diet for growing female rabbit of different strains has a beneficial effect on growth performance due to improvement in body weight and body weight gain. Improvement in live body weight and body weight gain of the rabbits fed enzymes diet may be due to the enhancing effect of enzymes on microflora growth in the gut and caecum as well as increase in the volatile fatty acids production and organic matter digestibility (Abd El-Latif *et al.*, 2008). In addition, adding a multienzymes product (Optizyme) which contain protease amyloglucosidase, xylanase,  $\beta$  - glucanase, cellulase and hemicellulase monogastric animal diets suffer from under utilization of nutrients due to absence of enzymes necessary for hydrolyzing non-starch polysaccharides in the foregut that reduces the viscosity of intestinal content and improves nutrients absorption (Sullivan, 1987).

**Table (2): Effect of supplementation with multienzymes or probiotic on growth performance of growing NZW rabbits.**

Item	Control (Diet 1)	Multienzyme(MZ) (Diet 2)	Probiotic(Pro) (Diet 3)	MZ plus Pro (Diet 4)	Sig.
Live body weight (g):					
Initial BW (5 weeks)	529±4.22	527±4.85	546±9.98	541±9.07	NS
At 9 weeks	1336 <sup>b</sup> ±10.86	1394 <sup>a</sup> ±14.50	1385 <sup>a</sup> ±16.43	1427 <sup>a</sup> ±15.16	**
Final BW (13 weeks)	2049 <sup>c</sup> ±18.07	2176 <sup>b</sup> ±19.92	2167 <sup>b</sup> ±23.42	2263 <sup>a</sup> ±22.77	**
Daily weight gain (g) from:					
5-9 weeks	26.93 <sup>c</sup> ±0.23	28.90 <sup>a</sup> ±0.33	27.98 <sup>b</sup> ±0.25	29.52 <sup>a</sup> ±0.26	**
9-13 weeks	23.74 <sup>c</sup> ±0.25	26.05 <sup>b</sup> ±0.26	26.06 <sup>b</sup> ±0.24	27.86 <sup>a</sup> ±0.30	**
5-13 weeks	25.33 <sup>c</sup> ±0.23	27.47 <sup>b</sup> ±0.25	27.02 <sup>b</sup> ±0.24	28.69 <sup>a</sup> ±0.26	**
Daily feed intake (g/day) from:					
5-9 weeks	87.46 <sup>c</sup> ±0.22	90.33 <sup>a</sup> ±0.25	88.94 <sup>b</sup> ±0.43	90.52 <sup>a</sup> ±0.40	**
9-13 weeks	113.55 <sup>b</sup> ±0.82	119.76 <sup>a</sup> ±0.97	120.72 <sup>a</sup> ±1.02	121.62 <sup>a</sup> ±1.00	**
5-13 weeks	100.51 <sup>b</sup> ±0.52	105.05 <sup>a</sup> ±0.58	104.83 <sup>b</sup> ±0.71	106.07 <sup>a</sup> ±0.68	**
Feed conversion ratio(g feed/g gain) from age:					
5-9 weeks	3.25 <sup>a</sup> ±0.02	3.13 <sup>bc</sup> ±0.03	3.18 <sup>b</sup> ±0.02	3.07 <sup>c</sup> ±0.02	**
9-13 weeks	4.78 <sup>a</sup> ±0.02	4.60 <sup>b</sup> ±0.03	4.63 <sup>b</sup> ±0.01	4.37 <sup>c</sup> ±0.02	**
5-13 weeks	3.97 <sup>a</sup> ±0.02	3.83 <sup>c</sup> ±0.01	3.88 <sup>b</sup> ±0.01	3.70 <sup>d</sup> ±0.01	**
Mortality (%) from age :					
5-13 weeks	0.00	0.00	0.00	0.00	
Economic efficiency:					
Feed cost	16.89 <sup>c</sup> ±0.364	17.78 <sup>b</sup> ±0.403	17.64 <sup>b</sup> ±0.494	18.14 <sup>a</sup> ±0.473	**
Total cost	26.89 <sup>c</sup> ±0.364	27.78 <sup>b</sup> ±0.403	27.64 <sup>b</sup> ±0.494	28.14 <sup>a</sup> ±0.473	**
Total revenue	40.97 <sup>c</sup> ±1.40	43.51 <sup>b</sup> ±1.54	43.35 <sup>b</sup> ±1.81	45.25 <sup>a</sup> ±1.76	**
Net revenue	14.08 <sup>c</sup> ±1.04	15.74 <sup>b</sup> ±1.14	15.71 <sup>b</sup> ±1.32	17.12 <sup>a</sup> ±1.29	**
Economic efficiency	52.32 <sup>c</sup> ±3.11	56.61 <sup>b</sup> ±3.24	56.79 <sup>b</sup> ±3.73	60.77 <sup>a</sup> ±3.55	**
Production index	61.46 <sup>c</sup> ±2.98	67.52 <sup>b</sup> ±3.26	66.52 <sup>b</sup> ±3.21	71.96 <sup>a</sup> ±3.49	**

Means in the same row within the same classification having different letters are significantly differ ( $P < 0.05$ ).

NS = not significant, \*\* =  $P < 0.01$ .

## 2- Digestibility and nutritive values:

Data in Table (3) show that the average of digestibility coefficient of DM was significantly improved ( $P < 0.05$ ) with rabbits fed basal diet supplemented with multienzymes plus probiotic than those fed the control diet. Moreover, digestibility coefficient of CP and DCP were significantly ( $P < 0.01$ ) higher with rabbits fed multienzymes plus probiotic diet or multienzymes alone compared with those fed the control diet. Moreover, rabbits fed diets multienzymes or probiotic had higher ( $P < 0.05$ ) NFE than those fed the control diet. However, the differences in OM, EE, CF, NDF, ADF and TDN were insignificantly. The improvement in digestibility coefficients DM, CP and NFE of rabbits fed enzymes or probiotic diets, may be due to the enhancing effect of enzymes on microflora growth in the gut and caecum as well as increase in volatile fatty acids production and organic matter digestibility (Abd El-Latif *et al.*, 2008). Also, the enzymes may improve the release of cell bound nutrients; improve the activity of gut ecology and

nutritive values expressed as DCP, TPN and DE (Makled *et al.*, 2005). Onu and Oboke (2010) reported that the higher crude protein digestibility observed in rabbits fed probiotic and enzyme supplemented diets compared to those fed the control and unsupplemented diets may have been responsible for the higher weight gain of the rabbits fed these diets.

**Table (3): Effect of dietary supplementation with multienzymes or probiotic on the apparent nutrients digestibility and feeding value of the experimental diets by NZW rabbits.**

Item	Control (Diet 1)	Multienzyme(MZ) (Diet 2)	Probiotic(Pro) (Diet 3)	MZ plus Pro (Diet 4)	Sig.
Apparent nutrients digestibility (%):					
DM	62.1 <sup>b</sup> ±1.43	69.0 <sup>a</sup> ±2.45	65.0 <sup>ab</sup> ±5.06	67.0 <sup>ab</sup> ±4.20	*
OM	64.7±1.63	69.9±5.69	66.7±4.80	68.6±4.15	NS
CP	72.9 <sup>b</sup> ±1.22	75.8 <sup>a</sup> ±0.84	74.7 <sup>ab</sup> ±2.54	76.6 <sup>a</sup> ±0.83	**
EE	47.1±1.98	48.9±1.60	47.2±2.07	46.6±3.13	NS
CF	42.8±3.68	46.0±5.27	44.4±6.61	47.6±7.05	NS
NDF	57.1±2.42	59.2±3.46	58.1±4.34	60.2±4.63	NS
ADF	48.5±3.35	51.4±4.80	50.0±6.03	52.8±6.43	NS
NFE	68.6 <sup>b</sup> ±1.46	73.5 <sup>a</sup> ±0.73	72.0 <sup>a</sup> ±4.22	74.0 <sup>a</sup> ±1.70	*
Nutritive value as					
TDN	57.1±2.03	60.4±0.80	58.9±3.48	60.8±1.66	NS
DCP	11.8 <sup>b</sup> ±0.20	12.4 <sup>a</sup> ±0.23	12.1 <sup>ab</sup> ±0.42	12.4 <sup>a</sup> ±0.13	**

Means in the same row within the same classification having different letters are significantly differ ( $P < 0.05$ ).  
NS = not significant, \*\* =  $P < 0.01$ .

**3- Blood biochemical components and hematological parameters:**

Data presented in Table (4) show that level of blood plasma total protein was significantly ( $P < 0.01$ ) higher in the rabbits fed the multienzymes plus probiotic diet than those fed the control or probiotic diets. Moreover, globulin and globulin/albumin ratio were significantly ( $P < 0.01$  or  $P < 0.05$ ) higher with rabbits fed multienzymes or probiotic diets than those fed basal diet (control). Also, rabbits fed diet supplemented with multienzymes plus probiotic or multienzymes alone had significantly ( $P < 0.01$ ) higher plasma glucose than those fed basal diet (control) or basal diet supplemented with probiotic alone. Multienzymes plus probiotic and multienzymes alone had significantly ( $P < 0.01$ ) lower plasma urea than the probiotic alone. However, the differences in blood plasma albumin, total lipids, cholesterol, creatinine, urea/creatinine ratio, AST, ALT, AST/ALT ratio and alkaline phosphates were insignificant. Data presented in Table (4) show that all hematological parameters were not affected by either multienzymes or probiotic, except the RBC's, which was higher ( $P < 0.05$ ) in rabbits fed basal diet (control) than those fed probiotic diet. Rabbits fed diet supplemented with multienzymes plus probiotic or multienzymes alone had significantly higher PA% than those fed basal diet (control). On the other hand, there was no significant effect of all supplementations on white blood cells and differential leukocytes counts. Makled *et al.*, (2005) indicated that increasing optizyme level to 750 mg/kg feed in rabbits diet insignificantly increased the concentration of total protein and significantly increased only when rabbit does were fed on diet containing 500 mg Optizyme /kg feed, this enhancement in total protein was also associated with the noticed improvement in CP digestibility. On the other hand, Veslin *et al.* (2003) found no significant change in the level of the total protein, albumin and globulin in the blood of rabbits supplemented with Protozin enzyme (19 mg/kg diet) in the concentrate mixture. Also, El-Tantawy *et al.* (2001) found that rabbits total lipids were insignificantly affected with Kemzyme supplementation in rabbits fed diets supplemented with Kemzyme. A significant increase in blood glucose levels, total lipids and total protein were observed in growing rabbits supplemented with Kemzyme for 8 weeks (Attia *et al.*, 2012b).

**Table (4): Effect of dietary supplementation with multienzymes or probiotic on some blood biochemical components and hematological parameters of growing NZW rabbits.**

Item	Control (Diet 1)	Multienzymes (MZ) (Diet 2)	Probiotic (Pro) (Diet 3)	MZ plus Pro (Diet 4)	Sig.
Blood plasma components:					
Total protein(g/dl)	4.70 <sup>b</sup> ±0.126	5.08 <sup>ab</sup> ±0.475	5.03 <sup>b</sup> ±0.266	5.52 <sup>a</sup> ±0.488	**
Albumin (mg/dl)	3.00±0.369	2.72±0.117	2.65±0.302	2.83±0.082	NS
Globulin (mg/dl)	1.70 <sup>b</sup> ±0.369	2.37 <sup>a</sup> ±0.554	2.38 <sup>a</sup> ±0.360	2.68 <sup>a</sup> ±0.426	**
Albumin /Globulin ratio	0.590 <sup>b</sup> ±0.226	0.878 <sup>a</sup> ±0.235	0.918 <sup>a</sup> ±0.217	0.945 <sup>a</sup> ±0.137	*
Glucose (mg/dl)	78.83 <sup>b</sup> ±2.64	87.17 <sup>a</sup> ±7.60	80.83 <sup>b</sup> ±4.75	89.17 <sup>a</sup> ±3.19	**
Total lipid (mg/dl)	106.4±6.96	104.9±7.71	98.80±4.99	100.7±3.76	NS
Cholesterol (mg/dl)	203.3±3.88	206.3±3.20	201.5±5.21	202.0±6.75	NS
Urea (g/dl)	17.22 <sup>ab</sup> ±0.779	16.42 <sup>b</sup> ±0.917	18.00 <sup>a</sup> ±0.894	16.67 <sup>b</sup> ±1.03	*
Creatinine (g/dl)	0.89±0.128	0.97±0.197	0.86±0.143	0.90±0.089	NS
Urea/creatinine ratio	19.71±3.14	17.61±3.76	21.35±2.52	18.72±2.66	NS
AST (IU/L)	59.11±4.08	61.08±3.61	57.15±1.92	58.50±1.97	NS
ALT (IU/L)	69.50±1.22	69.92±4.01	67.50±1.76	69.42±1.56	NS
AST/ALT ratio	0.85±0.069	0.87±0.041	0.85±0.38	0.84±0.029	NS
Alkaline phosphates (IU/L)	13.17±1.17	12.67±1.37	12.33±1.97	13.00±2.19	NS
Hematological parameters:					
RBC's (10 <sup>6</sup> )	1.63 <sup>a</sup> ±0.121	1.47 <sup>ab</sup> ±0.120	1.38 <sup>b</sup> ±0.194	1.52 <sup>ab</sup> ±0.098	*
Hemoglobin (g/dl)	10.00±1.41	9.67±1.03	9.83±0.75	9.83±0.98	NS
Paced cells volume (%)	29.17±2.71	29.50±2.81	29.83±2.23	29.50±2.07	NS
MCV(micron <sup>3</sup> /RBC)	179.4±21.3	202.6±29.0	217.5±17.6	195.3±20.3	NS
MCH (Ug)	61.45±9.20	66.34±9.62	71.62±4.97	65.20±9.09	NS
MCHC (%)	34.32±4.13	32.75±0.98	32.97±0.87	33.29±1.39	NS
WBC's (10 <sup>3</sup> /mm <sup>3</sup> )	25.00±1.41	23.50±1.05	23.83±1.33	24.33±1.03	NS
Differential leucocytes counts:					
Monocytes (10 <sup>3</sup> )	4.83±0.983	4.33±0.516	4.33±0.520	3.83±0.408	NS
Basophils (10 <sup>3</sup> )	0.83±0.408	0.67±0.516	0.67±0.515	0.83±0.753	NS
Eosinophils (10 <sup>3</sup> )	8.83±0.753	9.67±1.033	9.00±1.41	9.50±0.548	NS
Neutrophils (10 <sup>3</sup> )	48.67±1.51	48.67±2.34	50.67±1.97	50.50±1.64	NS
Lymphocytes (10 <sup>3</sup> )	36.83±1.47	36.67±1.21	35.33±1.22	35.33±1.21	NS
Heterophils /Lym.	1.32±0.081	1.33±0.095	1.44±0.099	1.43±0.088	NS
Phagocytic activity (%)	16.33 <sup>b</sup> ±0.816	16.50 <sup>a</sup> ±1.64	17.83 <sup>ab</sup> ±1.17	18.67 <sup>a</sup> ±1.75	*
Phagocytic index (%)	1.85±0.187	1.83±0.207	1.75±0.187	1.80±0.126	NS

Means in the same row within the same classification having different letters are significantly differ ( $P < 0.05$ ).

NS = not significant \* =  $P < 0.05$  \*\* =  $P < 0.01$ .

## CONCLUSION

1) Kemzyme and Actisaf are good growth stimulators to the growing rabbits and can be added to the commercial diet to improve feed utilization and growth performance traits without any adverse effects on health status of rabbits.

2) Dietary supplementation with a mixture of 1 g/kg of Kemzyme (commercial multienzymes preparation) and 10 g/ kg of Actisaf (commercial yeast preparation) is recommended to obtain the best results of growth performance rather than adding each preparation individually.

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## تأثير إضافة الإنزيمات أو البروبيوتيك على معدل أداء النمو، هضم المركبات الغذائية، ومكونات الدم في الأرانب النامية

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استخدم في هذه الدراسة 60 أرنب نيوزيلندي أبيض مقطوم عمر خمسة أسابيع بمتوسط وزن جسم  $29.33 \pm 536$  جم. وتهدف هذه الدراسة لتقييم تأثير إضافة المخلوط الإنزيمي (Kemzyme) أو البروبيوتيك (Actisaf) أو مخلوط منهما للعليقة التجارية علي معدل أداء النمو وهضم المركبات الغذائية، ومكونات الدم للأرانب النامية. وزعت الحيوانات في أربع مجموعات تجريبية، و تم تغذية المجموعة الأولى على عليقة تجارية أساسية (مقارنة، عليقة 1)، وغذيت المجموعة الثانية من الأرانب على العليقة الأساسية بإضافة مخلوط الإنزيمات (Kemzyme بمعدل 1 جم / كجم عليقة، عليقة 2)، وغذيت المجموعة الثالثة من الأرانب على العليقة الأساسية بإضافة البروبيوتيك (الخميرة Actisaf® 10 جم / كجم عليقة، عليقة 3)، وغذيت المجموعة الرابعة من الأرانب على العليقة الأساسية بإضافة مخلوط الإنزيمات + البروبيوتيك (Kemzyme 1 جم / كجم عليقة + Actisaf® 10 جم / كجم عليقة، عليقة 4).

أظهرت نتائج هذه الدراسة أن الأرانب التي غذيت على العليقة الأساسية مع إضافة مخلوط الإنزيمات + البروبيوتيك تحسنت معنويا (علي مستوي احتمال 1%) في وزن الجسم النهائي والزيادة اليومية لوزن الجسم والكفاءة الاقتصادية عن مجموعة المقارنة. سجلت قيم معدل الزيادة اليومية لوزن الجسم (من 5- 13 اسبوع من العمر) 25.3 ، 27.5 ، 27.0 ، 28.7 جرام للعلائق الأربعة علي التوالي. سجلت قيم الكفاءة التحويلية للغذاء (من 5- 13 اسبوع من العمر) 3.97 ، 3.83 ، 3.88 ، 3.70 للعلائق الأربعة علي التوالي. تحسنت نسبة معامل الهضم الظاهري للمادة الجافة، البروتين الخام، مستخلص الإيثير و القيمة الغذائية معبرا عنها بالبروتين الخام المهضوم أعلى معنويا (علي مستوي احتمال 1%) بإضافة مخلوط الإنزيمات و البروبيوتيك بينما تحسنت معنويا(علي مستوي احتمال 1%) الأرانب التي غذيت علي علائق مضاف لها مخلوط الإنزيمات + البروبيوتيك (عليقة 4). أظهرت النتائج ارتفاع معنوي في مستويات بلازما الدم من البروتين الكلي و الجلوبيولين و كرات الدم البيضاء في الأرانب التي غذيت علي علائق مضاف لها مخلوط الإنزيمات + البروبيوتيك (عليقة 4) عن الكونترول وكانت كل قياسات الدم في المعدل الفسيولوجي الطبيعي لها.

تشير النتائج إلى استخدام Actisaf و Kemzyme كمحفزات نمو جيدة للأرانب النامية ويمكن أن تضاف معا للعلائق التجارية لتحسين الكفاءة الغذائية و معدل أداء النمو دون أي آثار سلبية على الحالة الصحية للأرانب.