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Productive performance response of growing rabbits to dietary protein reduction and supplementation of pyridoxine, protease, and zinc

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Abstract: An 8-week experiment was carried out to assess the impact of supplemental dietary pyridoxine (PY), protease (PR), zinc (Zn) and their mixture (MIX) with low protein diet (LP; 14.76% CP) or high protein diet (HP; 18.53% CP) on rabbit growth, feed utilization, and nutrients digestibility. Rabbits were divided into ten similar groups in a 2 (protein level) ×5 (treatments) factorial design. Treatments included a control group (without any additives), 5 mg PY/kg of diet, 100 mg Zn/kg of diet, 500 mg PR/kg of diet or a mixture of all tested feed additive with the same doses. Results indicated that growth performance, feed utilization, and nutrients digestibility indicators were retarded significantly with reduction of dietary crude protein. Growth performance and feed conversion were significantly enhanced as a result of PY, PR, Zn, and MIX supplementation. All feed supplements had significantly improved the digestibility of crude protein and digestible crude protein. No change in carcass traits was recorded in response to protein level and tested feed supplements. It is concluded that the growing rabbit responded positively to PY, Zn, PR, and MIX (particularly PY) supplemental of LP or HP diets, in terms of growth performance, feed conversion, and nutrient digestibility.

Key words: growing rabbits, growth performance, protease, pyridoxine, nutrient digestibility, zinc.

INTRODUCTION

In developing countries, rabbits are excellent and economical producer animals for protein to cover the ever-increasing human needs (Nehad et al. 2009). Feed is the main component of cost in animal production. In particular, protein is considered the most vital component in the diet as the high-protein diet being viewed as higher than a lower protein one, but it represents a substantial cost (Cunha & Cheeke 2012). A protein deficiency resulted from either one or more limiting amino acids or overall insufficient protein consumption, will result in decreases in parameters such as growth rate, feed intake and utilization (Lei et al. 2004). Also, JI & FUC (2010) confirmed that growth performance, carcass yield and organ weights of rabbits are influenced by dietary crude protein level in the diet of rabbits. There is a growing interest in using feed additives of natural origin in the rabbit industry for consumers safety (Alagawany et al. 2018, Ayyat et al. 2018). Numerous nutritional solutions have been adopted with low-protein diets to improve nutrients utilization with economic efficiency such as supplementation of commercial products of amino acids and enzymes (Rehman et al. 2017). Zinc is an essential element required for many physiological

functions including nutrient metabolism, acid-base balance, the polymeric organization of macromolecules like DNA, cell division, protein synthesis besides immune and antioxidant function (García-Contreras et al. 2011). Improved feed consumption and weight gain were noted in rabbits receiving a supplemented diet with 90 mg Zn /kg (Hossain & Bertechini 1993). Also, Sultan et al. (2018) reported a significant improvement in body weight, feed intake, feed conversion ratio, dressing percentage, blood biochemical, and nutrient digestibility in broiler chicks fed diets supplemented with ZnO at 60 mg/kg.

Pyridoxine (PY) is a water-soluble vitamin and metabolically active in the form of pyridoxal phosphate, an essential cofactor for more than 140 enzymes many of them are incorporated in amino acids metabolism, with efficient roles in growth, and other aspects of metabolism (Combs Jr & McClung 2017). It was reported that growth retardation could result from pyridoxine deficiency in ducks (Xie et al. 2014). In recent years, public attention to the use of enzymes in livestock production has increased (Nijdam et al. 2012). The use of exogenous protease (PR) enzymes is considered as a way to decrease the feed's protein level without inducing adverse effects on animal performance (Giannenas et al. 2017). Several studies have tried to incorporate exogenous enzymes into rabbit diets to improve nutrients availability, nevertheless, in most experiments, rabbits were less responsive and variable effects were observed on their performances (Falcão-e-Cunha et al. 2004, García et al. 2005, Falcão et al. 2007). However, studies conducted in rabbits to study the response to low protein (LP) diets supplemented with PY or PR are limited. Thus, in light of the above backdrop, an attempt has been made in the current experiment to assess the impact of PY, zinc (Zn), PR and their mixture (MIX) with low or high protein (HP) diet levels in growing rabbits. Emphasis was placed on growth performance, feed utilization, nutrients digestibility, carcass traits, and blood biochemistry.

MATERIALS AND METHODS

The current experiment was conducted at the Rabbit Research Farm and laboratories of the Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. All experimental procedures were carried out following the guidelines of the animal ethics of institutional committee of Zagazig University (Zagazig, Egypt).

Experimental animals

In total, seventy growing New Zealand White male rabbits with average body weight 729.8±10.52 g were randomly assigned to ten groups in a 2 × 5 factorial design. Rabbits were divided into two main groups. The first main group was given a basal diet with LP level (14.76% CP), and the second main group was given basal diets containing HP level (18.53% CP). Within each of the last two main groups, the rabbits were divided into five subgroups. The first subgroup was fed on the basal diets (without supplementation), the 2nd, 3rd, 4th and 5th subgroups were given the basal diets containing 5 mg PY/kg, 100 mg Zn/kg or 500 mg PR/kg, a mixture of all tested feed additive (PY, Zn, and PR) with the same concentration. PY and Zn were provided by Multivita Company for Animal Nutrition, Sixth October City, Egypt. PR is a commercial product Cibenza® (Cibenza DP100) obtained from (Navus Company, Hong Kong, China).

Management

All animals were individually house-caged (stainless steel cages) in an artificially illuminated room. The dimension of the cage was 40×30×25

cm. All rabbits were continually provided with freshwater and were maintained under the same managerial, hygienic and environmental conditions all over the experimental period (8 weeks). The rabbits were acclimatized for one week before the commencement of the trials. Rabbits were fed *ad-libitum* during all the experimental period. The experimental diets were completely pelleted and were formulated to cover the recommended nutrient requirements of growing rabbits, according to NRC (1977). The formulation and analysed chemical composition of the basal diets fed to rabbits are presented in Table I.

Measurements

Live body weight (LBW) of rabbits was recorded weekly in grams; the average daily weight gain (DWG) was individually calculated as described by Al-Sagheer et al. (2017). Average DFI and mortality rate were recorded weekly, and the feed conversion ratio was calculated as g feed /g gain. At the last week of each trial, four animals from each group were chosen for digestibility trials. Rabbits were individually housed in cages that permit the partition of feces and urine. Feces of each rabbit were collected quantitatively once a day before offering the morning meal at 9 a.m. The seven days combined collection fecal samples were stored for routine analysis. Feeds and feces samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) and ether extract (EE) according to AOAC (2000). Nitrogen-free extract (NFE) values were calculated by the difference method. The digestible energy (DE) (kcal/ kg diet) of the

Table I. Formulation and analyzed composition of the basal-diets fed to rabbits.

	Low protein diet	High protein diet
Ingredients (%)		
Alfalfa hay	20	29
Yellow corn	24	23
Wheat straw	5	4
Wheat bran	44	29
Soybean meal	5	13
Sodium chloride	0.5	0.5
limestone	1.2	1.2
Minerals ,vitamins mixture*	0.3	0.3
Total	100	100
Chemical analysis (% on DM basis)		
Organic matter	89.42	90.56
Crude protein	14.76	18.53
Crude fiber	12.51	12.39
Ether extract	3.92	4.87
Nitrogen free extract	58.23	54.78
Ash	10.58	90.56

*** Each 1.5 kg of minerals, vitamins mixture contains manganese 80 g, zinc 60 g, iron 30 g, copper 4 g, iodine 0.5 g, selenium 0.1 g and cobalt 0.1 g, vitamin A 12000000 IU, vitamin D3 3000000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, Biotin 75 mg, folic acid 1000 mg, nicotinic 30000 mg and pantothenic acid 10000 mg.**

tested diets was calculated as follows: DE=5.28 DCP (g) + 9.51 DEE + 4.2 (DCF+DNFE).

By the finish of the feeding trial, four rabbits from each group were slaughtered after fasting for 12 hours. After full bleeding, the carcass and some non-carcass components were weighed. The carcasses were prepared by removing the skin, paws, feet, urinary bladder, genital organs, and digestive tract. According to Blasco et al. (2010), hot carcass weight (the main body, head, heart, liver, kidneys, lungs, and other total edible parts) were determined. The weights of the carcasses, liver, kidneys, spleen, heart, and lungs were recorded.

At the end of the trial period, blood samples were collected from 4 rabbits to estimate blood parameters. Red blood cells (RBCs), hematocrit, hemoglobin (Hb), white blood cells (WBCs) and lymphocytes were conducted following the method of Grindem (2011) using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy). Also, the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, glucose, and urea in blood serum were determined by colorimetric enzymatic methods using commercial kits purchased from (Bio-diagnostic, Cairo, Egypt) based on the procedure outlined by the manufacturer.

Statistical analysis

Collected data were subjected to the analysis of variance by using the General Linear Models Procedure according to the following model:

$$Y_{ijk} = \mu + P_i + S_j + PS_{ij} + e_{ijk}$$

Where, μ is the overall mean, P is the fixed effect of protein level (i = 1 ...2), S is the fixed effect of feed supplement; PY, Zn, PR or MIX (j = 1 ...5), PS is the fixed effect of the interaction between protein level and feed supplement and e_{ijk} is random error. Significant differences between treatments were tested using Duncan's multiple range test. Carcass and internal organs data were statistically analyzed by analysis of covariance (factorial experiment) according to the following model: $Y_{ijk} = \mu + P_i + S_j + PS_{ij} + b(X-x) + e_{ijk}$ Where: Y_{ijk}, μ, P_i, S_j, PS_{ij} and e_{ijk} were as defined in the previous model, b = partial linear regression coefficients of Y_{ij} on slaughter weight, X =slaughter weight value and x = overall average of slaughter weight.

RESULTS

Effects of protein level

The results indicated that reduction of protein percentage in growing rabbit diets led to a significant (P≤0.003) increase in DFI, but a significant (P<0.001) retardation in LBW, DWG, and FCR throughout all experiment intervals (Tables II and III). Compared with rabbit fed HP diets, DFI of rabbits fed LP diets was increased by 11.46%, Whereas, LBW, DWG, and FCR were impaired by 11.23, 15.88, and 29.73%, respectively in LP diets during the whole experimental period comparing to HP diets. In the same line, the digestibility of DM, CP, OM, and NFE decreased (P<0.01) due to feeding LP diets. The values of DCP, TDN and digestible energy (DE) diminished (P<0.001) as a result of feeding LP diets (Table IV).

The urea, total protein, and albumin concentrations and ALT activity reduced significantly (P<0.01) in rabbit fed LP diets comparing to HP diets (Table V). While, the AST activity, albumin/globulin ratio, glucose, globulin, hemoglobin, hematocrit concentrations, red blood cells, white blood cells, lymphocyte counts were insignificantly affected by any of protein levels (Tables V and VI).

As presented in Table VII, pre-slaughter and carcass weights were significantly (P<0.001) declined due to dietary crude protein reduction. Dressing (%) and organs weights were

Table II. Live body weight and daily body weight gain (Mean± SE) of New Zealand White rabbits as affected by protein level and tested feed additives.Means in the same column bearing different letters differ significantly (P<0.05).

	Live body weight (g) at			Daily body weight gain (g) at		
	6 weeks	10 weeks	14 weeks	6-10 weeks	10-14 weeks	6-14 weeks
Protein level effect						
Low	724±14.09	1358±18.95 [b]	2000±19.95 [b]	22.65 ±0.57 [b]	22.94±0.35 [b]	22.80±0.36 [b]
High	736±15.60	1517±22.50 [a]	2253±24.39 [a]	27.92 ±0.69 [a]	26.27±0.54 [a]	27.10±0.41 [a]
Supplement effect						
Control	726±18.10	1369±41.63	2017±49.37[b]	22.94±1.53	23.13±0.73 [b]	23.04±0.94[b]
Pyridoxine	726±23.74	1443±35.82	2186±45.13[a]	25.61±0.99	26.52±0.92 [a]	26.07±0.68[a]
ZnO	741±22.50	1460±37.51	2156±49.90[a]	25.68±0.91	24.84±0.84 [ab]	25.26±0.77[a]
Protease	739±26.52	1451±29.93	2137±48.74[a]	25.41±1.22	24.50±0.81 [b]	24.95±0.91[a]
Mixture	717±27.60	1473±50.08	2145±52.29[a]	27.01±1.55	24.00±0.76 [b]	25.50±0.93[a]
Statistical significance						
Protein level	NS	<0.001	<0.001	<0.001	<0.001	<0.001
Supplement	NS	NS	0.006	NS	0.007	0.004
Interaction	NS	NS	NS	NS	NS	NS

NS = Not significant.

insignificantly altered with any of protein levels or tested feed supplements, except liver weight which was increased significantly (P=0.048) with rabbit fed LP diets. However, covariance analysis showed that adjusted carcass and non-carcass components weights did not have any significant effects related to the protein level.

Effects of feed supplements

Overall the experimental period results in Tables II and III showed that all supplemented groups had significantly improved LBW (P=0.006), DWG (P<0.001), and FCR (P=0.005). Rabbit fed diets fortified with PY, Zn, PR or MIX recorded the best values of LBW by 8.38, 6.89, 5.95, and 6.35%; DWG by 13.15, 9.64, 8.29, and 10.68%; and FCR by 9.13, 6.09 and 7.71%, respectively compared with the control group. Conversely, at 6-14 weeks of age, there were no marked impacts on DFI due to the supplementation of the tested feed supplements.

Rabbits fed diet supplemented with PY, Zn and MIX had significantly (P<0.05) increased the digestibility of DM and OM besides nutritive values as TDN and DE. Also, all dietary supplements significantly (P<0.05) improved the digestibility of CP and nutritive value as DCP. Moreover, digestibility of CF was significantly (P=0.019) improved due to PY and MIX supplementation, while digestibility of EE and NFE were not significantly influenced by any of supplementation (Table IV). Generally, the values of blood and hematological parameters were not significantly affected by PY, Zn, PR and MIX supplementation except serum urea concentration which significantly (P=0.002)

Table III. Daily feed intake and feed conversion ratio (Mean± SE) of New Zealand White rabbits as affected by protein level and tested feed additives.

	Daily feed intake (g/day) at			Feed conversion ratio (g food/g gain) at		
	6-10 weeks	10-14 weeks	6-14 weeks	6-10 weeks	10-14 weeks	6-14 weeks
Protein level effect						
Low	89.71±1.88 [a]	146.11±1.81[a]	117.91±1.35[a]	4.05±0.07[a]	6.51±0.11[a]	5.28±0.06[a]
High	72.78±1.54 [b]	138.80±1.65[b]	105.79±1.18[b]	2.70±0.07[b]	5.44±0.12[b]	4.07±0.07[b]
Supplement effect						
Control	75.60±2.16	142.19±3.58	108.90±2.63	3.55±0.28	6.31±0.21 [a]	4.93±0.22[a]
Pyridoxine	83.25±5.08	143.30±1.89	113.28±3.05	3.35±0.23	5.61±0.23 [b]	4.48±0.21[b]
ZnO	83.80±2.79	141.50±1.45	112.65±1.69	3.39±0.17	5.87±0.19 [ab]	4.63±0.16[b]
Protease	80.81±3.81	145.18±2.61	112.99±2.13	3.28±0.20	6.12±0.25 [ab]	4.70±0.20[ab]
Mixture	79.52±2.93	139.66±4.50	109.59±3.27	3.16±0.23	5.94±0.29 [ab]	4.55±0.21[b]
Statistical significance						
Protein level	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
Supplement	NS	NS	NS	NS	0.048	0.005
Interaction	NS	0.018	NS	NS	NS	NS

increased with PY and MIX supplemented groups (Table V).

Dressing (%) and organs weights were insignificantly different with any of tested feed additives. Dietary supplementation of PY, Zn, PR or MIX significantly (P<0.05) improved pre-slaughter and carcass weights compared to non-supplemented rabbits. However, covariance analysis showed that adjusted carcass did not have any significant effects related to dietary feed supplementation (Table VII).

Effects of interaction between protein level and feed supplements

Overall the experimental period, the results of the interaction between protein levels and tested feed additives indicated no significant effects on LBW, DWG, FI, FCR, nutrient digestibility, blood parameters, carcass weight, dressing (%) and organs weights (Tables II-VII). However, within HP groups, PR supplementation significantly (P=0.018) increased the DFI at 10-14 weeks of age (Table III). Generally, the mortality rate was low (only one dead rabbit in the LP control group), and there was no difference among the groups.

DISCUSSION

For animals, protein is one of the most essential dietary macronutrients, and the chief constituent of cells, which plays a vital role in life (Liu et al. 2015). Unfortunately, protein constitutes the most expensive component of rabbit feed. Hence, the goal of the current study is to evaluate the effect of reduction of dietary protein concentration in rabbit diet (from 18.53 to 14.76% CP) with PY, Zn, PR or MIX in the rabbit diets on performance, digestibility, carcasses and blood biochemistry.

Table IV. Digestibility and nutritive value (Mean± SE) of the experimental diets as affected by protein level and tested feed additives.

	Digestibility coefficient (%)						Nutritive values (%)		
	DM	**EE**	**CP**	**CF**	**NFE**	**OM**	**DCP**	**TDN**	**DE**
Protein level effect									
Low	67.27±0.53 [b]	76.24±0.96	70.96±0.93 [b]	52.38±0.85	73.35±0.55[b]	68.95±0.52 [b]	10.47±0.14 [b]	69.39±0.46 [b]	3029±20.50 [b]
High	70.16±0.45 [a]	78.86±0.86	74.08±0.70 [a]	54.48±1.06	75.47±0.50[a]	72.33±0.40 [a]	13.73±0.13 [a]	71.26±0.37 [a]	3143±16.44 [a]
Supplement effect									
Control	66.87±1.17[b]	76.67±1.01	68.78±1.16[b]	50.64±0.45[b]	73.51±1.55	68.86±1.32[b]	11.48±0.71[b]	68.68±0.94[b]	3011±46.77[b]
Pyridoxine	69.59±1.03[a]	77.26±2.24	74.04±1.18[a]	55.51±1.19[a]	74.44±0.93	71.23±1.10[a]	12.36±0.78[a]	70.85±0.79[a]	3111±40.41[a]
ZnO	69.45±1.70[a]	80.07±1.64	73.17±1.14[a]	53.69±1.66[ab]	75.32±0.85	71.53±0.81[a]	12.21±0.75[a]	71.27±0.49[a]	3127±27.69[a]
Protease	67.92±0.63[ab]	77.74±1.28	73.48±1.37[a]	51.14±1.64[b]	73.79±0.50	70.09±0.64[ab]	12.22±0.62[a]	69.84±0.48[ab]	3067±23.78[ab]
Mixture	69.72±0.93[a]	76.03±1.10	73.14±1.56[a]	56.17±1.39[a]	74.97±0.57	71.49±0.87[a]	12.23±0.85[a]	71.00±0.65[a]	3116±32.81[a]
Statistical significance									
Protein level	<0.001	NS	0.004	NS	0.005	<0.001	<0.001	0.001	<0.001
Supplement	0.030	NS	0.016	0.019	NS	0.028	0.010	0.021	0.018
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means in the same column bearing different letters differ significantly (P < 0.05). NS = Not significant.

Herein, throughout the experimental period, a significant reduction in LBW and DWG of growing rabbits fed LP diet. But an increase in both DFI and FCR was evident. Similarly, various studies confirmed the retardation of weight gain following the reduction of dietary protein level (Bassuny et al. 1997, Abdel-Malak 2000, Ayyat & Marai 2000). This could be related to low amino-acid concentrations available for renewing, repairing, and growth of tissues (Lei et al. 2004). In contrary, Deshmukh et al. (1997) reported no change in gain with different dietary protein levels**.**

The increase in FI and FCR subsequent to the reduction of protein level has been previously documented (Bassuny et al. 1997). In contrast, no significant change in the FCR was recorded with different dietary CP levels (Carregal 1993). Less DM intake in the HP diet group was probably because of the higher energetic costs of using amino acids as glucose precursors, as demonstrated by (Fink et al. 2004).

On the contrary, a significant enhancement in both LBW, DWG, FI and FCR in rabbit groups fed diet supplemented with PY, Zn, PR or their MIX compared with those fed the diets without any supplementation. Correspondingly, a dose-dependent improvement in growth was recorded in ducks fed diets supplemented with seven PY levels 0, 0.66, 1.32, 1.98, 2.64, 3.30, and 3.96 mg/kg for 28 days (Xie et al. 2014). Also, rabbits fed diet fortified with 170 mg Zn/kg of diet as $ZnSO_4$ had a significant enhancement in the DWG (El-Rahim et al. 1995). Furthermore, (Ayyat & Marai 2000) reported that supplementing rabbit diets with 100 to 300 Zn mg kg^{-1} significantly ($P<0.05$) increased live weight gains, but had no effect on FI compared with the control group. Besides, in broilers, enhanced growth indices following PR supplementation was recorded in previous reports (Cowieson & Ravindran 2008, Angel

Table V. Blood parameters (Mean± SE) of New Zealand White rabbits as affected by protein level and tested feed additives.

	AST (u/l)	ALT (u/l)	Glucose mg/dl	Urea mg/dl	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin/ globulin ratio
Protein level effect								
Low	16.93±1.27	31.57±2.48^{b}	120.07±5.08	18.57±1.24^{b}	4.79±0.18^{b}	2.71±0.06^{b}	2.09±0.18	1.45±0.16
High	18.71±1.42	40.00±1.24^{a}	116.71±3.95	29.50±2.54^{a}	5.67±0.11^{a}	3.23±0.08^{a}	2.45±0.12	1.36±0.08
Supplement effect								
Control	16.00±1.93	32.33±5.66	119.33±8.75	19.00±2.46^{c}	4.85±0.24	2.73±0.14	2.12±0.13	1.31±0.07
Pyridoxine	19.40±2.16	33.60±1.81	124.00±10.75	31.20±6.30^{a}	5.30±0.38	3.14±0.17	2.16±.29	1.56±0.20
ZnO	18.83±2.82	34.17±2.63	117.17±5.65	24.00±4.03^{bc}	5.12±0.29	3.00±0.15	2.12±0.20	1.46±0.14
Protease	20.80±1.69	42.60±2.64	119.60±3.44	19.80±2.40^{bc}	5.22±0.41	2.96±0.08	2.28±0.43	1.60±0.39
Mixture	14.83±1.22	37.00±2.42	113.00±6.98	26.67±2.42^{ab}	5.67±0.20	3.03±0.20	2.67±0.16	1.16±0.13
Statistical significance								
Protein level	NS	0.009	NS	<0.001	0.001	<0.001	NS	NS
Supplement	NS	NS	NS	0.002	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

Means in the same column bearing different letters differ significantly (P<0.05). NS=Not significant.

Table VI. Hematological parameters (Mean± SE) of New Zealand White rabbits as affected by protein level and tested feed additives.

	RBCs count (10^6/ml)	Hemoglobin (g/dl)	Hematocrit (%)	WBCs (10^3/ml)	Lymphocytes (10^3/ml)
Protein level effect					
Low	5.40±0.17	10.46±0.19	30.51±0.84	10.95±1.01	5.90±0.66
High	5.70±0.15	11.12±0.29	32.52±0.91	10.75±0.76	5.54±0.47
Supplement effect					
Control	5.28±0.41	10.70±0.44	30.77±1.99	12.87±1.90	6.46±0.65
Pyridoxine	5.40±0.11	10.40±0.24	30.25±0.87	9.37±1.22	5.07±0.68
ZnO	5.51±0.09	10.48±0.16	30.65±0.47	10.68±1.01	5.87±0.59
Protease	5.62±0.16	10.75±0.38	31.62±1.28	10.62±1.10	6.13±1.26
Mixture	5.93±0.33	11.62±0.59	34.28±1.73	10.73±1.64	5.07±1.23
Statistical significance					
Protein level	NS	NS	NS	NS	NS
Supplement	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS

Means in the same column not bearing different letters differ significantly. NS = Not significant.

et al. 2011). Nevertheless, in rabbits, growth performance was not improved in response to PR in most trials (García et al. 2005, Falcão et al. 2007, Falcão-e-Cunha et al. 2004).

PY is an important regulator of the metabolism of homocysteine in the body (Cuskelly et al. 2001). Additionally, a strong correlation has been proven between the growth performance and levels of homocysteine in growing Pekin ducks (Xie et al. 2007). In the present study, the enhancement in LBW of rabbits given the additional Zn may be due to the important role of Zn in the polymeric organization of macromolecules like DNA which are responsible for the growth and synthesis of body protein (García-Contreras et al. 2011). In addition, Zn is one of the essential elements for the action of more than 200 metalloenzymes and for biological functions of all living matter. Hence, Zn is necessary for growth, appetite, skin integrity and mental activities (Shinde et al. 2006). Additionally, herein, improved body weight by supplementation of PR sources may be attributed to higher availability of amino acids for the rabbit (Rehman et al. 2017).

The findings of PY here are corroborated with those of Irani et al. (2015) in broilers regarding feed conversion ratio**.** The inhibition effects of PY against oxygen radical generation, lipid peroxidation, and mitochondrial membrane damage might be responsible for the improved feed conversion ratio the supplemented group (Kannan & Jain 2004, Irani et al. 2015). Also, Zn supplementation resulted in enhancement of

feed conversion ratio without significant effect on feed intake in various previous studies (Ayyat & Marai 2000, El-Rahim et al. 1995, Nessrin et al. 2012, Chrastinová et al. 2015, 2016) owed to its beneficial role metabolism of energy, protein and nucleic acid (Tabatabaie et al. 2007). Also, the addition of PR has been documented to improve feed conversion ratio probably due to the reduction of the ileal flow (García et al. 2004, 2005).

A retardation of nutrients digestibility following supplementation of LP diets was clear in this investigation. Parallel with the former findings, previous studies found that decreasing dietary protein level leads to the decreasing values of apparent digestibility (Dahlman et al. 2002). Some studies in early-weaned piglets have shown that LP diets led to an atrophy of intestinal mucosa and to a reduction in its absorptive and immunological capacity (Gu & Li 2004). On the other hand, an outstanding improvement of the digestibility of DM, OM, and CF besides nutritive values as TDN and DE due to PY and MIX supplementation was obvious. It was suggested that such favorable effect of PY could be linked to its potency in stabilizing cell membranes through interaction with membrane

Table VII. Carcass and some internal organs weights (g; mean± SE) of growing New Zealand White rabbits as affected by protein level and tested feed additives.

	Pre-slaughter weight (g)	Carcass weight (g)	Dressing (%)	Liver weight (g)	Kidney weight (g)	Heart weight (g)	Lunges weight (g)	Spleen Weight (g)
Protein level effect								
Low	1984±26.80 [b]	1136±19.07 [b]	57.25±0.32	62.49±1.47	12.95±0.63 [b]	5.99±0.18	14.30±1.12	1.29±0.07
High	2243±30.10 [a]	1314±24.25 [a]	58.56±0.72	65.43±1.55	14.25±0.35 [a]	6.38±0.24	14.18±0.86	1.45±0.09
Supplement effect								
Control	1991±71.26 [b]	1135±47.08 [b]	56.97±0.52	62.97±1.73	12.39±1.01	5.86±0.23	15.55±1.46	1.29±0.07
Pyridoxine	2169±60.15 [a]	1293±48.61 [a]	59.59±1.47	66.48±1.88	13.12±0.71	5.93±0.46	12.00±0.87	1.53±0.12
ZnO	2145±73.92 [a]	1237±53.13 [a]	57.59±0.74	66.02±3.88	14.96±0.65	6.28±0.28	17.01±2.11	1.29±0.13
Protease	2119±71.52 [a]	1215±46.31[ab]	57.33±0.70	61.22±2.67	14.32±0.69	5.98±0.26	13.30±1.16	1.34±0.12
Mixture	2144±70.01[a]	1245±48.11 [a]	57.90±0.81	63.11±1.25	13.21±0.91	6.16±0.28	13.33±1.44	1.41±0.19
Statistical significance								
Protein level	<0.001	<0.001	NS	NS	0.048	NS	NS	NS
Supplement	0.049	0.022	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

Means in the same column bearing different letters differ significantly (P < 0.05). NS = Not significant.
Analysis of covariance showed that pre-slaughter weight affected significantly (P < 0.001) carcass and live weights.

phospholipids and accordingly increase the digestibility of several nutrients (Irani et al. 2015).

Regarding Zn impacts on nutrient's digestibility, the current results are in agreements with those reported by (Gad Alla 2001) who found that the apparent digestibility of DM, OM and EE was significantly (P<0.05) better because of Zn supplementation, but CF and CP tended to be insignificantly higher. Also, Hafez et al. (2002) found that rabbit's diets supplemented with Zn recorded higher digestibility of nutrients. Previous research indicated that feeding 3000 mg/kg Zn to piglets had a positive effect of producing deeper crypts and a trend for longer villi in the duodenum (Carlson et al. 1998). The improved digestibility and nutritive values following Zn supplementation could be directly linked with the higher absorptive capacity of the mucous membrane. Also, increasing the digestive ability of rabbit by Zn supplementation could be connected with increased activity of some enzymes associated with the digestion of carbohydrates, fat, and protein such as amylase, lipase, chymotrypsinogen, trypsinogen, and some peptidases, since these enzymes are known to be Zn-dependent enzymes (Banerjee 1988). Besides, Zn supplementation affects carbohydrate and protein metabolism, which found in many highly purified enzymes functioning in carbohydrate and protein digestion (Suttle 2010).

Supplemental exogenous PR have been previously reported to improve nutrients digestibility in broilers (Angel et al. 2011). Commercial exogenous PR may enhance endogenous peptidases via improving the digestibility of dietary protein and hydrolyzing proteinaceous anti-nutritional factors such as antigenic proteins, trypsin inhibitors and lectins (Douglas et al. 2000). Moreover, increased nutrient digestibility for animals fed PR may be returned to direct impacts on the digestion of nutrient substrates and decreased endogenous nutrient loss (Cowieson & Ravindran 2008). Also, PR can augment endogenous digestive enzymes (Ritz et al. 1995) and decrease endogenous amino acid losses via disturbing the generation of pancreatic enzymes (Jiang et al. 2008) and mucin secretion (Cowieson & Bedford 2009).

The blood biochemistry of farm animals is affected by several factors, one of which is nutrition (Ajao et al. 2013). The reduction in urea, total protein and albumin concentrations and ALT activity following LP supplementation could be linked to low availability of amino acids metabolic products (Rehman et al. 2017). While the increase in urea level following fortification of diet with the tested additives might probably be associated with the increase of DCP. Dietary feed supplements did not show any significant differences in evaluated blood parameters. Similarly, a trial in pigs fortified with $ZnSO_4$ at 84.3 mg/kg of diet or Zn-Met at 40.9 mg/kg of diet did not indicate any significant effect in albumin and total protein levels (Rupić et al. 1998). In contrast, in female Holstein calves, serum albumin level was improved due to ZnSO4 supplementation with 20, 40, and 80 ppm (GuangZhou et al. 1995). Nevertheless, this variance might be linked with the animal species used.

Slaughter weight (SW) and carcass weight were significantly (P<0.001) declined with rabbit fed LP diets compared to HP diets. This could be strongly associated with the decreased LBW and DWG observed here. Comparable findings were previously recorded (Sankhyan et al. 1991). Dietary supplementation of PY, Zn, PR or MIX did not have any significant effects on carcass weight, dressing percentage and organs weights (g/kg SW) compared to non-supplemented rabbits. Similar results were recorded with PR supplementation (Rada et al. 2013).

In conclusion, the growing rabbit responded positively to PY, PR, Zn, or MIX supplemental of low (14.76%) or high protein (18.53%) diet, in terms of significant enhancement in growth performance, feed utilization and nutrient digestibility of growing rabbits. Regardless of dietary protein level, the maximum improvement was recorded with PR (5 mg/kg diet) supplementation. Additionally, the previous feed additives had no adverse effect on blood biochemistry so it could be used safely. Thus, from both health and an economic point of view, several benefits might be gained by adding these additives to the diet of commercial rabbits, especially with low protein diets.

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