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# **PROFITABILITY OF DIETS, NUTRITIVE VALUE, PERFORMANCE AND** CECAL ACTIVITY OF GROWING RABBITS FED BEAN VEIN HAY

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Supporting Information

ABSTRACT: A feeding trail was conducted to study effect of bean viens hay (BVH) on the growing rabbit's performance, nutritive value and cecum activity and economic return of experimental diets that. Seventy two New Zealand White (NZW) rabbits (6 week of age) were divided into 6 groups and were fed 6 experimental diets inclusion BVH and Galzym® enzyme contains cellulase, xylanase, lipase, amylase, protease, pectinase, arabinase, phytase, α-galactosidase, and β-glucosidase additives. Group 1 fed negative control (basal diet without both BVH and Galzym®) and group 2 fed positive control diets (basal diet without BVH and with Galzym®). The 3rd and 4th groups fed 25% BVH substitution of clover hay without Galzym® (T3) and with Galzym® (T4). The 5th and 6th group's rabbits fed 50% BVH substitution of clover hay without Galzym® (T5) and with Galzym® (T6). The crude fiber, NDF, ADF, ADL and cellulose were higher in BVH than those in clover hay while, CP% and digestible energy (Kcal/kg) were lower in BVH than those in clover hay. Results of interaction between BVH and Galzym® additives (treatment effect) had significant (P<0.05) effect on rabbits productive performance, all nutrients digestibility except EE and DCP%, blood biochemical (TP, albumin, globulin, albumin/globulin ratio, cholesterol, ALT, and urea), and cecum activity (TVFA's and NH<sub>3</sub>), Moreover, main effect of BVH was significantly (P<0.05) improved of rabbits productive performance, nutrients digestibility (DM, OM, CP, CF, and NFE%), blood constituents (TP, albumin, globulin, and ALT), and TVFA's in cecum. Enzyme main effect decreased (P<0.05) FI and formation of NH<sub>3</sub> in rabbit's cecum. In conclusion, the dietary BVH improved the productive performance of growing rabbits without negative effect on health status. Enzyme addition increases the BVH utilization and diets profitability.

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Abbreviation: BVH: bean veins hay; FBW; final body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract; DCP: digestible crude protein; TDN: total digestible protein; DE: digestible energy; TP: total protein; alb. Albumin; ALT: aspartate aminotransferase; AST: alanine aminotransferase; TVF's: total volatile fatty acids; g: gram; mg: milligram; U: unit; dl: deciliter; NH3: ammonia.

# INTRODUCTION

Alfalfa hay considers the best fiber source use in rabbit diets that incorporated in to diets up to 40% (Gidenne et al., 2017). One of perfect solution to reduce the cost is untraditional feedstuffs (Molina et al., 2018) and wide global orientation to reduce the feed cost of feeding programs (Gidenne et al., 2017). Using the agricultural by-products in rabbit formulation diets lead to reduction of nutritional cost without any adverse effect on productive performance (Mennani et al., 2017). The chemical composition of legumes hay is close to clover hay (Feedipedia, 2016).

The nutritional value of leguminous proteins may be limited due to anti-nutritional factors (Nalle, 2009). Legumes is a good source of starch, protein dietary fiber, oligosaccharides, phytochemicals, and minerals, also, contribute to many health benefits to the human beings (Hangen and Bennink, 2002). In Egypt, the annual production of green bean (Phaseolus vulgaris) is estimated about 287575 ton (FAOSTAT, 2016) and bean green by-product was around 70% (Aparicio et al., 2010). A wide common bean straw (haulms) contains on DM basis about 5-11% protein, rich fiber 38-45%, 51.1-86.4% NDF, 37.3- 56.9% ADF and 5.4-9.3% lignin (Feedipedia, 2016).

The calcium and phosphorus contain were 0.68-1.15% and 0.09-0.13%, respectively. Although legumes by products are rich in protein, the nutritional value of their proteins may be limited by the presence of anti-nutritional factors (Tadele, 2015). Indigestible components presence in some feedstuffs and reduce of specific enzymes releasing from animal bodies lead to reducing the digestion about 15-25% of the diet that animals fed (Konietzny and Greiner, 2002). Enzyme supplementation to animal diets increased the nutritive value of diets due to complementary role with endogenous enzymes for young animals not mature. Also, increase availability of some nutrients for feedstuffs contains ani-nutritional factors (Cachaldora et al., 2004).

The current study aimed to investigate the influence bean veins hay as an alternative feedstuffs of clover hay in New Zealand White (NZW) growing rabbits diets on productive performance, cecum activity of growing NZW rabbits and economic profit.

## **Experiment and ethical approval**

This experiment was approved under the guidelines of ethical from Cairo University, Institutional Animal Care and Use Committee (CU-IACUC, CU-II-F-6-22) and conducted in Rabbits Experimental Unit and the laboratories analyses were carried out at Department of Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

## Preparation of bean veins hay and diets

The cultivated fresh bean veins hay (BVH) was obtained from fields in Giza governorate, then sun-dried, and was grinded by hammer mill chemical analysis composition (AOAC, 2000) and determination of tannins (Burn, 1971), saponin (Shany et al., 1970), and phytic acid (AOAC, 2000). The ingredients were blended with BVH and pelleted in Feed Processing Unit, Faculty of Agriculture, Cairo University. A commercial enzyme named Galzym<sup>®</sup> (Tex Biosciences Ltd., India) was added to the experimental diets. The recommended dose by the producer is 0.5 g/kg diet. Galzym<sup>®</sup> contains cellulase (10000000 U), xylanase (1500000 U), lipase (10000 U), amylase (1000000 U), protease (400000 U), pectinase (30000 U), arabinase (7000 U), phytase (500000 U),  $\alpha$ -galactosidase (10000 U), and  $\beta$ -glucosidase (10000 U). Six experimental diets were: T1-basal diet (negative control, NC, diet without BVH and Galzym<sup>®</sup>), T2 (positive control, PC, without BVH and with the Galzym<sup>®</sup>), T3 (25% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galzym<sup>®</sup>), T6 (50% of clover hay in NC replaced by BVH without Galz

	Experimental diets	Control	25% BVH	50% BVH
Items				
	Without Galzym®	T1	Т3	T5
	With Galzym <sup>®</sup>	T2	T4	T6
Feed ingredients				
Soybean meal (44%)	CP)	16.3	16.8	18.0
Yellow corn		13.8	13.8	13.8
Barley		13.0	13.0	13.0
Wheat bran		16.7	16.2	15.0
Clover hay		34.0	25.5	17.0
Bean vein hay (BVH)		0.0	8.5	17.0
DI- methionine		0.2	0.2	0.2
Di-Cal-phos		2.2	2.2	2.2
NaCl		0.3	0.3	0.3
Min & Vit Mix1		0.3	0.3	0.3
Anti-coccidia & fungi	i	0.2	0.2	0.2
Molasses		3.0	3.0	3.0
Total		100	100	100
Chemical analysis (DM bas	is)			
Dry matter (DM, %)		82.80	83.30	83.77
Organic matter (OM,	%)	85.64	84.82	84.08
Crude protein (CP, %	)	17.20	17.03	17.07
Crude fiber (CF, %)		13.90	14.67	15.44
Ether extract (EE, %)		2.20	2.24	2.29
Nitrogen free extract	t (NFE, %)	55.34	54.35	53.23
Ash (%)		5.08	5.52	5.97
Digestible energy (D	E, Kcal/kg)2	2612	2608	2612
Calcium (%)		1.00	0.99	1.02
Total phosphors (%)		0.50	0.50	0.50
<sup>1</sup> Commercial vitamin and mir 2000 mg, Vit. B1 1000 mg, Vit acid 1000 mg, Biotin 75 mg, C and Cobalt 100 mg. <sup>2</sup> DE= Dige	neral premix contained (per t. B2 5000 mg, Vit. B6 1500 Copper 4000 mg, Manganese stible Energy (kcal/kg) = 4.3	3 Kg premix) Vit. A 1200 mg, Vit. B12 10 mg, Pan e 80 000 mg, Zinc 50 00 6-0.049 × [28.924 + 0.6	00 000 IU, Vit. D3 3000 000 IL tothenic acid 10 000 mg, Nico 00 mg, Iron 30 000 mg, Iodine 57 (CF %)] according to Cheeke	J, Vit. E 10 000 mg, Vit. K3 tenic acid 30 000 mg, Folic 500 mg, Selenium 100 mg e, (1987).

# Table 1 - Experimental diets formulation and chemical composition (DM% basis).

# Animal and management

A total of 72 New Zealand White (NZW) rabbits aged 42 days weighing about 744.91 ± 5.27g were randomly allocated to 6 groups (12 rabbits/group). The experiment lasted 8 weeks (6-14 weeks of age). Rabbits housed in metal

battery cages (30×35×40 cm) supplied with separated feeders. The animals were provided *ad-libitum* access to automatic nipple fresh water drinkers and pelleted feed throughout the experimental period. All animals were kept under the same hygienic conditions and management. Also, all rabbits were vaccinated against diseases.

## **Animal performance measurements**

Rabbits and feed weights were taken every week. Rabbit's body weight were weighed individually and averaged by cage for statistical analysis. Mortality was recorded daily. Body weight (BW), body weight gain (BWG), feed intake (FI), mortality, and feed conversion ratio (FCR, g.feed : g.gain) were determined on a per cage basis, and then averaged by treatment.

## **Digestion trial**

Rabbits divided into 6 groups of 5 rabbits each for digestion trial execution (Perenz et al., 1995). Rabbits were placed in individual metabolism cages (56×38×28 cm). Feces were collected daily before the morning meal and weighed freshly and dried at 60 °C for 24 hour in air-drying oven. The BVH, experimental diets and feces were prepared to analyze moisture, ash, nitrogen, ether extract (EE), and crude fiber (CF). Data of quantities and chemical analysis (AOAC, 2000) of feed and feces were used to calculate the nutrient digestion coefficients, nutritive value (Fekete, 1985) and digestible energy (DE Kcal/kg; Schneider and Flatt, 1975) for each dietary experimental treatment.

## **Blood constituents**

Five rabbits from each treatment were randomly selected to collect the blood samples at the end of growing period. During slaughtering time, blood samples were collected in heparinized glass tubes and centrifuged at 3000 rpm for 20 minute, then samples were transferred and stored in deep freezer at -20°C till biochemical analysis kits assay (Purchased: Bio-diagnostic Co, Egypt). All plasma biochemical measurements; total protein (Gornall et al., 1949), albumin (Doumas and Waston, 1971), plasma globulin concentration (the difference between total protein and albumin), creatinine and urea (Folin, 1934; Tabacco et al. 1979), and aspartate aminotransferase and alanine aminotransferase (Henry, 1964) were assayed by colorimetric methods and performed according to manufacturer's instruction.

## **Cecum activity**

All rabbits slaughtered (after 16 hour fasting) for blood constituents collection at end of 14<sup>th</sup> week also had ceca collected to determine cecum characteristics. Each sample of cecum content was strained through 4 folds of gauze and divided into 2 portions. The 1<sup>st</sup> portion was used immediately for measuring the pH value and ammonia nitrogen concentration (Conway, 1958). The 2<sup>nd</sup> portion was preserved by the addition of 1ml HCl (N/10) and 2 ml orthophosphoric acid to each 2 ml of cecum contents juice for total volatile fatty acids (TVFA's) determination (Eadie et al., 1967).

# **Economic profit**

The calculation of economic efficiency is dependent on total cost and selling price of live body weight (2.43\$) at the time of experiment execution.

## **Statistical analysis**

Data were analyzed using general linear model SAS software, version 9.2. (SAS Institute, 2004, USA). Duncan's multiple range test was performed to detected significant differences between means when F-test is significant. The significant was accepted at  $P \le 0.05$ .

## **RESULTS AND DISCUSSION**

## Nutritional composition and phytochemicals of bean veins hay (BVH)

The estimated chemical analysis contents (DM, and CF%) of BVH were higher than those in clover hay, while, crude protein (CP%), ether extract (EE%) and nitrogen free extract (NFE%) and digestible energy (DE kcal/kg) were lower than those in clover hay (Table 2). Organic matter (OM%) and ash (%) content of BVH were similar to clover hay. The cell wall contents (NDF, ADF, ADL, hemicellulose and cellulose) of BVH were increasing when comparable of clover hay except hemicellulose was decreasing. The mineral contents (calcium and total phosphors) in BVH were lower than those in clover hay (1.43 and 0.20% vs 1.60 and 0.35%, respectively). As the same trend, the CF and CP of BVH on DM basis ranged from 38-45% and 5-11%, respectively (Feedipedia, 2016). However, the approximate analysis for BVH were 87.78% DM, 80.43% OM, 22.37% CP, 29.00% CF, 4.37% EE, 24.69% NFE, 19.57% ash, and 2000 Kcal DE/kg. Moreover, BVH cell wall contents from NDF, ADF, ADL, hemicellulose and cellulose were 47.98, 35.88, 12.45, 12.10, and 23.43 %, respectively (Lounaouci-ouyed et al., 2014; Abou El-Fadel et al., 2019). The values of phytochemicals (phytic acid, tannins, and saponins) were 1.00 g/100g DM, 1.85g/100g DM and 1.23%, respectively (Table 2). Similarly, phytic acid content of BVH was 0.5-1% (Vasić et al., 2012) and phytic acid, tannin and saponins concentration of BVH were 1.00, 1.85, and 1.23%, respectively (Mohamed Doaa, 2020). Tannins have been positive effects on animal healthy by reducing the gastro intestinal pathologies in mammals (Min et al., 2005).

Table 2 - Chemical analysis and phytochemicals of clover hay and bean vein hay

Items	Clover hay	Bean vein hay (BVH)
Dry matter (DM, %)	91.95	92.70
Organic matter (OM, %)	87.50	87.27
Crude protein (CP, %)	13.45	9.67
Crude fiber (CF, %)	26.00	39.70
Neutral detergent fiber (NDF, %)	42.50	56.85
Acid detergent fiber (ADF, %)	29.50	49.40
Acid detergent lignin (ADL, %)	5.50	12.00
Hemicelluloses%1	13.00	7.45
Celluloses% <sup>2</sup>	24.00	37.40
Ether extract (EE, %)	4.00	1.05
Nitrogen free extract (NFE, %)	44.05	36.50
Ash (%)	12.50	12.73
Digestible energy (DE, Kcal/kg) <sup>3</sup>	2104.74	1664.66
Calcium (%)	1.60	1.43
Total phosphors (%)	0.35	0.20
Phytochemicals		
Phytic acid (g/100 g DM)	-	1.00
Tannin (g/100 g DM)	-	1.85
Saponins (%)	-	1.23
<sup>1</sup> Hemicellulose% = NDF - ADF, <sup>2</sup> cellulose% = ADF - ADL, <sup>3</sup> DE (kcal/g) = 4.	36 - 0.049 x [28.924 + 0.657 (C	F %)] according to Cheeke, (1987).

#### **Productive performance**

As presented in Table 3, there were significant differences (P<0.05) interaction between BVH level and Galzym<sup>®</sup> addition on final BW (P<0.0001) and daily BWG (P<0.0001). Final BW and daily BWG were significantly (P<0.05) increased with all groups fed dietary BVH compared to control groups, with no significant differences between 25 and 50% replacement BVH levels. Final BW and BWG significantly affect (P<0.0001) by substitution level of BVH, but Galzym<sup>®</sup> addition had no effect (P=0.52 and P=0.50, respectively). There were significant effect of dietary BVH levels (P<0.0001) and Galzym<sup>®</sup> addition (P=0.05) on average FI (Table 3) and a significant interaction was noted between BVH level and Galzym<sup>®</sup> addition (P<0.0001). Rabbits in negative and positive control groups consumed lower (P<0.05) than those in other groups. The rabbits fed two level substitution of BVH without enzyme consumed higher (P<0.05) than rabbits fed BVH with enzyme groups. The crude fiber content (Table 2) in BVH (39.70%) higher than clover hay (26.00%) due to this increase the fiber content in dietary BVH increased the consumed feed (Blas and Mateos 2020). A significant interaction effect was observed between BVH level and Galzym<sup>®</sup> addition (P=0.07) due to enzyme addition. Positive control had lower FCR value (2.24) followed by T6 (2.99) than other treatment groups, while negative control group had the highest FCR value (3.41). Nevertheless, the negative control rabbits was not significant difference in FCR with those fed 25 and 50% BVH without enzyme addition (T3 and T5).

As the same trend, rabbits fed high level of dried waste green bean at 30% were significantly (P<0.05) increased in FBW and BWG. While, not significant differences (P>0.05) were observed in consumed feed and improving FCR (Abou El-Fadel et al., 2019). The improvement in FBW, daily BWG and FI due to phytochemicals (tannins, saponins and phytic acid) content of bean veins hay. The tannins can protect intestinal mucosa against oxidative damage and pathogens and limit peristaltic activity in digestive disorders preventing diarrhoea (Kermauner and Lavrenčič, 2008). Using the saponins as phytochemicals feed additives in pig diets caused improving the body weight, daily weight gain and feed intake Bartoš et al. (2016). Multi-enzyme addition (Natuzym; cellulase, xylanase,  $\beta$ -glucanase,  $\alpha$ -amylase, protease and lipase) not affected (P>0.05) on productive performance of cross-breeds growing rabbits (Ayodele et al., 2016).

In contrast, amylofeed® enzyme (amylase, B-glucanase and B-xylnase) affected (P<0.05) on productive performance of growing Algerian white rabbit was not significantly difference (P>0.05) when compared rabbits fed completely replacement of protein source in a basal soybean meal diet by 26% filed bean group with rabbits fed control diet group (Lounaouci-ouyed et al., 2014). Also, final BW BWG, and FCR were not significantly differences (P>0.05) between rabbits fed different levels of bean waste up to 30% and those fed control diet (Hervé et al., 2019). Growth rabbit's performance improved (P<0.05) by enzyme addition from 25-39 days of age (Gutierrez et al., 2002), protease addition (Al-Sagheer et al., 2020), and exogenous enzyme ZAD® (a biotechnical product made from natural sources) and ZAD® combined with *Lactobacillus acidophilus* (Abdel-Aziz et al. 2014). Final BW and BWG not significantly differences (P>0.05) between broiler fed green bean up to 16% while, broilers fed green bean with Benzyme-A (commercial enzyme contains cellulase 700000 U, amylase 100000 U, pectinase 60000 U, phytase 100000 U, xylanase 100000 U, and carrier) were significantly (P<0.05) increased final BW and BWG (Abbel-Monein, 2013). The enzyme addition did not affect growth performance parameter that may be due to lower enzyme dose and amount of substrate in experimental diets (Suliman, 2012).

# Table 3 - Effect of tested diets on productive performance of NZW growing rabbits

Itoma	IBW	FBW	BWG	FI	FCR
items	(g)	(g)	(g/day/rabbit)	(g/day/rabbit)	(gain: feed)
T1 (negative control)	746.11	2064.69 <sup>b</sup>	<b>23.54</b> ⁵	79.28°	<b>3.41</b> ª
T2 (positive control)	743.89	<b>2165.81</b> <sup>b</sup>	25.39 <sup>b</sup>	<b>81.39</b> °	<b>2.24</b> <sup>b</sup>
тз	742.78	2369.91ª	29.06ª	90.31ª	3.12 <sup>ab</sup>
T4	746.11	2334.04ª	28.36ª	86.33 <sup>b</sup>	3.08 <sup>b</sup>
Т5	747.22	2335.56ª	28.36ª	90.73ª	3.22 <sup>ab</sup>
Т6	742.78	2350.96ª	<b>28.72</b> ª	85.19 <sup>b</sup>	2.99 <sup>b</sup>
SEM	0.40	20.97	0.37	0.63	0.042
P-value					
Treatment effect	0.34	<0.0001	<0.0001	<0.0001	0.05
Level substitution of BVH	0.21	<0.0001	<0.0001	<0.0001	0.03
Galzym <sup>®</sup> additive effect	0.17	0.52	0.50	0.05	0.07

Mean values with different superscript letters in a column are significantly different (p<0.05). T1: negative control diet without BVH and Galzym<sup>®</sup>; T2: positive control diet without BVH and with the Galzym<sup>®</sup>; T3: 25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T5: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake; FCR; feed conversion ratio.

## Table 4 - Effect of tested diets on nutrients digestibility coefficients and nutritive value of tested diets

ltomo	Digestion coefficients (%)							Nutritive value		
items —	DM	OM	CP	CF	EE	NFE	DCP%	TDN%	DE Kcal/kg	
T1(negative control)	60.15 <sup>b</sup>	63.70°	<b>75.15</b> ⁵	44.92 <sup>b</sup>	75.37	73.83°	13.22	65.32°	2893.70°	
T2 (positive control)	64.96ª	66.90 <sup>b</sup>	75.69 <sup>b</sup>	45.57 <sup>b</sup>	75.79	78.61 <sup>ab</sup>	13.43	68.12 <sup>ab</sup>	3017.85 <sup>ab</sup>	
Т3	66.57ª	68.74ª	77.79ª	50.14 <sup>ab</sup>	76.99	80.30ª	13.51	68.29ª	3025.17ª	
T4	65.89ª	67.28 <sup>ab</sup>	77.81ª	51.31ª	77.37	77.69 <sup>b</sup>	13.40	66.81 <sup>ab</sup>	2959.68 <sup>ab</sup>	
T5	65.09ª	66.72 <sup>b</sup>	76.11 <sup>b</sup>	51.57ª	77.05	77.61 <sup>b</sup>	13.63	66.65 <sup>bc</sup>	2952.74 <sup>bc</sup>	
Т6	64.71ª	66.67 <sup>b</sup>	77.98ª	53.50ª	76.77	77.43 <sup>b</sup>	13.55	67.41 <sup>ab</sup>	2986.51 <sup>ab</sup>	
SEM	0.48	0.36	0.30	0.90	0.38	0.47	0.05	0.003	0.003	
P-value										
Treatment effect	<0.0001	0.0002	0.003	0.009	0.650	0.0001	0.300	0.003	0.003	
Level substitution of BVH	0.002	0.005	0.001	0.005	0.190	0.049	0.110	0.460	0.460	
Galzym <sup>®</sup> additive effect	0.200	0.460	0.190	0.500	0.820	0.500	0.590	0.200	0.200	

Galzym<sup>®</sup>; T2: positive control diet without BVH and with the Galzym<sup>®</sup>, T3:25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T5: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: ether extract; NFE: nitrogen free extract; DCP: digestible crude protein; TDN: total digestible nutrients; DE: digestible energy.

### Nutrients digestibility coefficients and nutritive value

As presented in Table 4, there were significant differences in digestibility coefficients of DM (P<0.0001), OM (P=0.0002), CP (P=0.003), CF (P=0.009), and NFE (P=0.0001) due to interaction between BVH level and Galzym<sup>®</sup> addition, but EE digestibility not affected (P=0.65). The negative control group (T1) was significantly (P<0.05) decreased in DM%, OM%, and NFE% digestibilities in comparison with other experimental groups. The CP% and CF% digestibility coefficients improved (P<0.05) with different levels of BVH except CP% of T5 not improved. The NFE% digestion coefficient recorded best value (P<0.05) with T3 group compared with all experimental groups except positive control group. The effect of BVH level substitution did statistically (P<0.05) affected on all nutrients digestion except EE digestion was not effect, while enzyme addition was not effect (P>0.05). The inclusion of dietary filed bean in rabbits not affected (P>0.05) on nutrient digestion coefficient (Lounaouci-ouyed et al., 2014). For nutritive value (Table 4), there were significant interaction between BVH level and enzyme addition on total digestible nutrients (TDN%; P=0.003) and digestible energy (DEKcal/kg; P=0.003), while digestible crude protein (DCP%) not affected significantly (P=0.300). The TDN% and DE were significantly (P<0.05) improved with all trial groups when compared to negative control group (T1) except T5 recoded not significantly. The main effects of BVH levels substitution and enzyme addition did not effect on nutritive value.

Similar results were reported for digestibility of DM, OM, NDF and ADF% of growing rabbits did not affected (P>0.05) by Natuzyme addition (Ayodele et al., 2016). However, the nutrients digestibility and nutritive value (DCP%, TDN% and DE kcal/kg) improved by protease addition to growing rabbit diets (Al-Sagheer et al., 2020). Also, nutritive value of nutrients by enzyme addition (ZAD®) and mixed it with *Lactobacillus acidophilus* (Abdel-Aziz et al., 2014). The nutrients digestion coefficients of dietary dried green bean and nutritive value (DCP% and DE kcal/kg) were significantly (P<0.05) differences (Abou El-Fadel et al., 2019). The improvement of nutrients digestion and nutritive value for BVH dietary may be due to phytochemicals content (Tannins, saponnin and phytic acid) that have been antioxidant property (Dost and Tokul, 2005). It was enhanced by protecting against oxidation and improving nutritional value (Corino and Rossi, 2021).

Table 5 - Effect of tested diets on blood measurements of growing NZW rabbits									
<b>H</b>	TP	Alb.	Globulin		Cholesterol	Liver function		Kidneys function	
items	(g/dl)	(g/dl)	(g/dl)	Al/ G ratio	(mg/dl)	ALT	AST	Creatinine	Urea
						(U/I)	(U/I)	(mg/dl)	(mg/dl)
T1 (negative control)	6.70 <sup>cb</sup>	4.30 <sup>b</sup>	2.40 <sup>b</sup>	1.79 <sup>bc</sup>	<b>110.00</b> ª	34.05ª	12.17	1.00	21.82ª
T2 (positive control)	6.35°	4.00 <sup>c</sup>	2.35 <sup>b</sup>	1.70 <sup>cd</sup>	100.00 <sup>b</sup>	29.40 <sup>b</sup>	11.54	0.95	22.50ª
Т3	6.65 <sup>bc</sup>	4.30 <sup>b</sup>	2.35 <sup>b</sup>	<b>1.83</b> ab	108.00ª	24.30 <sup>c</sup>	11.09	0.95	20.50 <sup>b</sup>
T4	6.60 <sup>bc</sup>	4.30 <sup>b</sup>	2.30 <sup>b</sup>	<b>1.87</b> <sup>ab</sup>	107.50ª	24.20 <sup>c</sup>	11.75	1.20	<b>22.17</b> ª
T5	7.25ª	4.75ª	2.50 <sup>ab</sup>	<b>1.90</b> ª	112.50ª	24.25°	11.78	1.00	<b>21.67</b> ª
Т6	6.85 <sup>b</sup>	4.25 <sup>b</sup>	2.60ª	<b>1.64</b> <sup>d</sup>	<b>112.50</b> ª	24.25 <sup>c</sup>	12.52	1.00	20.50 <sup>b</sup>
SEM	0.078	0.059	0.032	0.0005	1.25	0.90	0.159	0.29	0.22
P-value									
Treatment effect	0.003	0.0001	0.030	0.0005	0.010	0.0001	0.120	0.090	0.006
Level substitution of BVH	0.005	0.030	0.003	0.210	0.370	0.0001	0.180	0.370	0.110
Galzym <sup>®</sup> additive effect	0.080	0.200	0.990	0.030	0.170	0.610	0.420	0.260	0.390
Mean values with different superscript le	tters in a columi	n are significantly	different (p<0.05)	). TP: total protein	n; Alb.: albumin; Al	/G: albumin globuli	n ratio; AST: aspar	tate aminotransfe	rase: ALT: alanine

aminotransferase; T1: negative control diet without BVH and Galzym<sup>®</sup>; T2: positive control diet without BVH and with the Galzym<sup>®</sup>; T3: 25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T5: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50

Table 6 - Effect of tested diets on cecum activity of growing NZW rabbits			
Items	рН	TVFA's (mleg./100 ml)	NH₃ (mg/100 ml)
T1( negative control)	5.94	5.23°	6.84 <sup>b</sup>
T2 (positive control)	6.00	5.30°	9.37ª
T3	6.87	6.17 <sup>b</sup>	7.55 <sup>₅</sup>
T4	5.97	6.40 <sup>b</sup>	<b>9.92</b> ª
T5	6.00	6.80ª	7.70 <sup>b</sup>
T6	6.03	6.40 <sup>b</sup>	9.15ª
SEM	0.03	0.13	0.26
P-value			
Treatment effect	0.64	0.0001	0.0001
Level substitution of BVH	0.37	0.0001	0.62
Galzym® additive effect	0.26	0.90	0.0001

Mean values with different superscript letters in a column are significantly different (p<0.05). TVFA's: total volatile fatty acids; NH<sub>3</sub>: ammonia production; T1: negative control diet without BVH and Galzym<sup>®</sup>; T2: positive control diet without BVH and with the Galzym<sup>®</sup>; T3:25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T5: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>.

## **Plasma constituents**

As presented in Table 5, the effect of interaction between BVH level and Galzym® addition were affected on total protein (TP g/dl; P=0.003), albumin (g/dl; P=0.0001), globulin (g/dl; P=0.030), albumin/globulin ratio (P=0.0005), cholesterol (mg/dl P=0.010), ALT (U/I: P=0.0001), and urea (mg/dl: P=0.006), While, no significant interaction was found regarding to AST (U/I; P=0.120) and creatinine (mg/dl; P=0.090). The TP and albumin increased (P<0.05) with T5 when compared to all tested groups included the control groups. The rabbits fed T6 and T5 diets gave the highest concentration of globulin (2.60 g/dl) and albumin/ globulin ratio (1.90), respectively. The lowest value (P<0.05) of cholesterol (100 mg/dl) had recorded with rabbits fed positive control diet (T2). The ALT concentration was significantly (P<0.05) decreased with different levels of dietary BVH groups (without or with Galzym®) compared to negative or positive control groups. The urea concentration was lowest (P<0.05) with T3 (20.50mg/dl) and T6 (20.50mg/dl) groups. Moreover, there were significant differences in total protein (P=0.005), albumin (P=0.030), globulin (P=0.003), and ALT (P=0.0001) due to main effect of BVH levels substitution. Abou El-Fadel et al. (2019) observed that the rabbits fed different level of dried green bean up to 30% had significantly differences in albumin, ALT, urea, total cholesterol. However, the alternative crude fiber source caused significant effect on urea, AST and cholesterol due to lipids metabolism in growing rabbits (Petkova et al., 2011). The main effect of Galzym<sup>®</sup> addition had not effect (P>0.05) on all plasma parameters except albumin/globulin (P=0.030). As same trend, TP, albumin, glubulin, cholesterol, ALT, and AST not influence (P>0.05) by multi-enzyme addition (xylanase, cellulose, beta-glucanase, pectinase, amylase, protease, lipase, phytase, glactosidase, and mannanase) in growing rabbit diets (Sherif, 2018). Moreover, Abd El-Ghani et al. (2018) found that the enzyme additive significantly affect (P<0.05) on AST and AST/ALT ratio concentration of growing rabbit blood.

### **Cecum activity**

As presented in Table 6, there were significant interactions between BVH level and enzyme addition on TVFA's (mleq./100ml; P=0.0001) and NH<sub>3</sub> (mg/100ml; P=0.0001), however, no interaction effect on pH (P=0.640) in cecum contents. Little effect showed in pH value with rabbits fed all dietary BVH (P=0.370) and enzyme addition (P=0.260). The TVFA's was significantly (P<0.05) increased with rabbits fed dietary BVH without or with enzyme compared to those in control groups. The NH<sub>3</sub> was significantly (P<0.05) increased in cecum for groups fed all diets contain enzymes compared the groups fed diets without enzyme including positive control group. Probability value had significant with main effect of BVH levels substitution for TVFA's (P=0.0001), while NH<sub>3</sub> was not affected (P=0.620). Main effect of enzyme addition had not affected on TVFA's (P=0.900), while NH<sub>3</sub> had significant (P=0.0001). The improved in cecum activity explained by Petkova et al. (2011) found that alternative crude fiber sources, such as meadow hay or straw make caused a best environmental condition for benefit microbes growth in cecum which lead to better fermentation. Also, enzymes additives enhanced on microflora growth in rabbit's gut and cecum caused increasing in volatile fatty acids (VFA's) production (Abd El-Latif et al., 2008).

# **Economic profit**

Data concerning of profitability and economic efficiency of trial diets are shown in Table 7. According the results of performance (Table 3) showed improving in FBW (g) of rabbits fed all diets contains BVH. Also, FCR improved with enzyme addition diets. The economic efficiency increased with groups fed enzyme diets including the positive control group (T2) also, this improving increased with the several levels of BVH. The net revenue and economic efficiency improved with BVH dietary without or with exogenous enzymes. The best economic efficiency observed with T6 group followed by T4, T3 and T5. Similarly, the economic profit increased with feeding growing rabbit on diets containing peanut veins hay diets (Omer et al., 2017). The economic efficiency of dietary dried waste green bean also increased due to increase the level of dried waste green bean (Abou EI-Fadel et al., 2019). Inclusion of dietary bean offal in growing rabbit reduced the total feed cost (Hervé et al., 2019).

Table 7 - Effect of tested diets on economic profit								
Experimental treatments Items	T1	T2	тз	T4	T5	T6		
Total average weight (kg)	1.32	1.42	1.63	1.59	1.59	1.61		
Price of one kg body weight (\$)	2.43	2.43	2.43	2.43	2.43	2.43		
Selling price/rabbit (\$)(A)	3.21	3.46	3.96	3.86	3.86	3.91		
Total feed intake (kg)	4.44	4.56	5.06	4.83	5.08	4.77		
Price/kg feed(\$)	0.23	0.23	0.23	0.23	0.23	0.23		
Total feed cost/rabbit (\$)(B)	1.02	1.06	1.15	1.11	1.16	1.10		
Net revenue (\$) <sup>1</sup>	2.19	2.40	2.80	2.75	2.70	2.81		
Economic efficiency <sup>2</sup>	2.14	2.27	2.43	2.47	2.33	2.56		

<sup>1</sup>Net revenue: A – B; <sup>2</sup>Economical efficiency (%): (Net revenue / B) × 100. T1: positive control diet without BVH and Galzym<sup>®</sup>; T2: negative control diet without BVH and with the Galzym<sup>®</sup>; T3: 25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T4: 25% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH without Galzym<sup>®</sup>; T6: 50% of clover hay in basal diet replaced by BVH with Galzym<sup>®</sup>; LE= 0.064\$

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# CONCLUSION

It could be concluded that BVH can be fed the New Zealand White growing rabbits from 6 to 14 week of age at 25% and 50% substitution of clover hay in basal control diet without adverse effects on performance, health status, blood biochemical parameters, and both liver and kidney functions.Bean veins hay increased the market weight of growing rabbits and improved the nutritive value. Also, bean veins hay with Galzym<sup>®</sup> enzyme addition can improve feed conversion ratio. Enzyme addition was effectively in protein and fiber digestion and ammonia production in cecum by microflora. Enzyme addition had positive effect on cost return of diets.

### DECLARATIONS

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#### Authors' contribution

Dr. M.A. Suliman designed the experiment, and drafted the manuscript. Dr. D.M. Saber performed the practical part, collaborated the chemical analyses and the statistical analysis, tabulation of the experimental data. Dr. M.A. Manylawi and Dr. M.R. Ibrahim collaborated in the main idea and participated in manuscript review.

#### **Conflict of interests**

The authors have declared that no competing interest exists.

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