

***Phaseolus vulgaris* Straw as a Substitute for Clover Hay in Rabbit Diets with Prebiotic Supplementation and Feed Restriction Interaction: Influence on Nutrient Utilization, Caecal Activity, Carcass Yield and Blood Plasma Constituents**

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Abstract: *Phaseolus vulgaris* is one of the most important leguminous crops cultivated in Egypt. It is rich in protein, dietary fibers, vitamins (A, B1, B & C), minerals (Ca, P, Fe, K, Mg & Mn) with high amino acids. Nutrient utilization, caecal fermentative and microbial activity, carcass yield and blood plasma constituents of 54 weaned NZW rabbits were investigated in this study. These parameters were evaluated under the conditions of *Phaseolus Vulgaris* straw (PS) a substitute of clover hay with prebiotic (Mannan oligosaccharides, MOS) supplementation and feed restriction interaction. For this purpose, 54 six-weeks old NZW rabbits were randomly assigned into six groups (9 rabbits/ group) reared in metallic batteries under similar conditions. Six pelleted diets were formulated as following: control 1 & 3 (basal diet 100% clover hay), diets 2 & 4 (100% *Phaseolus vulgaris*, PS as a substitutive of clover hay), diet 5 (basal diet supplemented with prebiotic; mannan oligosaccharids, MOS) and diet 6 (100% PS supplemented with prebiotic; mannan oligosaccharids, MOS). Feed restriction was achieved by reducing the daily feed intake by 30% in groups 3, 4, 5 & 6. Results showed that *Phaseolus vulgaris* straw substitute had no adverse effect on the measured data. Rabbits subjected to feed restriction exhibited significant decrease ($P \leq 0.05$) in nutrient digestibility, caecal pH values, caecal microbial load and stress hormonal levels of T3 and cortisol. Meanwhile, dietary supplementation of MOS prebiotic restored the nutrient digestibility and nutritive values to the level of the control ones and supported carcass yield with no detrimental influence on other measurements. Therefore, *Phaseolus vulgaris* straw can safely be a substitute for clover hay in growing rabbits and MOS inclusion in rabbit diets is recommended.

Key words: Rabbit • *Phaseolus vulgaris* Straw • MOS (Mannan-Oligosaccharids) • Nutrient Utilization • Carcass Yield • Stress Hormones

INTRODUCTION

Shortage of feed and its high cost are the major causes of lack of animal proteins for human in the developing countries at the present time. Egypt, like other developing countries, is also facing a deficiency of animal protein sources. The rabbit as a non – ruminant herbivore can efficiently use different sources of roughage.

Many seed legumes are cultivated in Egypt for human consumption. Their by-products such as dried seed legumes straws are available in large amounts with no efficient utilization in animal feeding.

In rabbit, as for other livestock production, feeding represents about 60-70 % of the total production costs. The challenge for the feed formulation is to obtain the least cost diets that fully match animal requirements [1].

Clover hay is the most common source of fiber used in rabbit diets, accounting for 30-40 % of the commercial diets. So, substituting clover hay by other fiber sources such as locally-grown vegetable crops residues can reduce the feeding costs [2]. In many areas of developing countries, rabbit production could be an effective mean of converting forages and harvest by-products into high quality animal protein [3].

In Egypt, *Phaseolus vulgaris* is cultivated over a total area of about 94, 231 feddans through 2012, producing about 300, 425 tons green horns and 52, 438 tons of seeds as well as similar amount of by-products. Feed processing for growing rabbits should aim to reduce the incidence of enteritis that leads to animal losses. Nevertheless, feed restriction can be used at different periods [4, 5].

The control of feed intake is widespread in animal breeding, such as for adjusting the ration to the nutrient requirements, or to manage the fattening and the meat quality. In recent years, there has been an increased interest in studying feed restriction in rabbits as a mean of reducing the cost of production. Early feed restriction also helps to address problems associated with early-life fast growth rate such as increased body fat deposition, high incidence of metabolic disorders and high mortality [6, 7].

The feed supplemented with antibiotics are the most common means for prevention of digestive problems in rabbits. But, there is a great demand to find alternatives due to the prohibition of antibiotic growth promoters by the European Union. Prebiotics, are non-digestible feed ingredients as alternatives to antibiotics. They beneficially affect growth and activities of gut microflora by selective stimulating the growth or metabolic activity of some gut microflora [8]. Prebiotics such as fructose oligosaccharides (FOS) and Mannan oligosaccharides (MOS) are derived from yeast and bacteria cells. They are not easily hydrolyzed by the endogenous digestive enzymes and cannot be absorbed by the host animal. The addition of MOS to a diet of early weaned rabbits (between 28 and 35 days of age) reduced caecal PH and mortality and activated villi development and caecal volatile fatty acid concentration [9, 10]. Therefore, The main targets of the current study were to evaluate the effects of feed restriction and dietary inclusion of *Phaseolus vulgaris* straw (PS) as a substitute for clover hay in presence or absence of added prebiotics (Mannan oligosaccharides, MOS) on nutrients' digestibility, caecal microbial activity (fermentative activity and microflora activity), carcass characteristics and some blood constituents in New Zealand White rabbits.

MATERIALS AND METHODS

The experimental work of this study was carried out at the Poultry Station, Agriculture Research and Experimental Center, Faculty of Agriculture, Mansoura

University during the period from 15 October till 5 December 2012, while the chemical analyses were done at the Poultry Production Department. This study was conducted to investigate the feasibility of replacing 100% of clover hay by *Phaseolus vulgaris* (PS) straw in the pelleted diets to study its effect on nutrients digestibility, caecal activity, carcass yield and blood constituents of NZW rabbits from the weaning age (6 weeks) to 12 weeks old. In addition, the effects of prebiotic containing Mannan oligosaccharides (MOS) supplementation to the diet contained 100% PS with or without feed restriction were also investigated.

Experimental Animals and Management: A total of 54 NZW rabbits, weaned at 45 days of age were collectively caged in galvanized wire cages (50×50×45 cm) supplied with a feeder and stainless steel nipple drinkers as (3 rabbits / cage) and randomly assigned into 6 groups (9 rabbits/group). Fresh water and pelleted diets were offered *ad libitum* during the entire experimental period from 6- 12 weeks of age, no antibiotics were added to feed or water except for anticoccidial drugs. The experimental rabbits were kept under the same managerial and hygienic conditions.

Chemical Analysis of Tested Materials: *Phaseolus vulgaris* are grown in Egypt, during May-September, for human consumption, which based on their seeds (Green and dry). *Phaseolus vulgaris* were obtained from Dakahlia governorate fields. After the end of seed collection season of *Phaseolus vulgaris*, the vines (Stalks, leaves) were harvested, then left to dry in the air and ground for diet manufacturing. The chemical analysis of PS was (16.61, 34.33, 0.52, 9.60, 22.60% of CP, CF, EE, Ash and NFE on DM basis) and 2287.0 DE (kcal/kg) [11]. *Phaseolus vulgaris* straw and clover hay (Third cutting) using duplicate samples were determined according to AOAC [12].

Experimental Diets: Six experimental pelleted diets were formulated (Table 1) to cover the nutrient requirements for rabbits according to NRC [13]. Groups were arranged as following; the control 1 & 3 (basal diet 100% clover hay), groups 2 & 4 (100% *Phaseolus vulgaris*, PS as a substitutive of clover hay), group 5 (100% clover hay supplemented with prebiotic; mannan oligosaccharids, MOS; Y-MOS*, 0.5 mg Kg-1) and group 6 (100% PS supplemented with prebiotic; mannan oligosaccharids,

Table 1: Composition of dietary groups

Groups	Dietary composition
1	100% clover hay
2	100% PS
3	70% clover hay + feed restriction (30%)
4	70% PS+ feed restriction (30%)
5	70% clover hay + MOS + feed restriction (30%)
6	70% PS + MOS + feed restriction (30%)

PS = *Phaseolus vulgaris* straw, MOS = mannan oligosaccharids

Table 2: Ingredients and chemical analysis of the experimental diets fed to NZW rabbit (6-12 wks old)

Ingredients	Clover hay	<i>Phaseolus vulgaris</i> (PS)
Yellow corn	20	20
Soybean meal, 44%	7.8	7.8
Wheat bran	27.3	27.3
Clover hay	40	-----
P. S.	----	40
Molasses	1.5	1.5
Di-calcium phosphate	1.0	0.3
Limestone	1.6	1.6
Premix ¹	0.3	0.3
Salt	0.5	0.5
Total	100	100
Calculated analysis (%) ²		
Digestible energy, kcal/kg	2638	2673
Dry matter, %	88.5	88.7
Crude protein, %	16.56	16.15
Crude fiber, %	13.74	17.5
Ether extract, %	3.0	2.26
Nitrogen free extract,%	57.2	52.79
Ash, %	9.5	11.3
Ca, %	1.44	0.91
P, %	0.73	0.63
Lys., %	0.78	0.43
Meth., %	0.19	0.12
Meth. + Cys., %	0.48	0.27
Determined analysis (%)		
Dry matter, %	89.2	89.8
Organic matter, %	86.4	85.9
Crude protein, %	16.16	16.55
Crude fiber, %	14.04	16.50
Ether extract, %	3.22	2.56
Nitrogen free extract,%	56.69	52.39
Ash, %	9.89	12.00
Price ³	1.9432	1.8321

⁽¹⁾Each 3 Kg vitamin and mineral mixture contained 12000000 IU Vit. A, 2500000 IU Vit. D₃, 10000 mg Vit. E, 2500 mg Vit K₃, 1000 mg Vit B₁, 4000 mg Vit. B₂, 1500 mg Vit. B₆, 10 mg Vit. B₁₂, 10000 mg Pantothenic acid, 20000 mg Nicotinic acid, 1000 gm Folic acid, 50 mg Biotin, 500 gm Choline chloride, 60 gm Manganese, 55 gm Zinc, 100 mg Selenium, 1000 mg Iodine, 35 gm Iron, 10 gm Copper, 250 mg Copalt and Carrier CaCO₃ to 3 kg.

⁽²⁾Calculated (NRC [13] except test materials (Calculated according to its determined values, Table 1).

⁽³⁾Price of 1kg diet was calculated according to the prevailing prices of feed ingredients during the experimental period.

MOS; Y-MOS, 0.5 mg Kg-1). Feed restriction was applied by reducing the daily feed intake by 30% in groups 3, 4, 5 & 6. These additives were added at the level recommended by the manufacturer. All the experimental diets were formulated to be iso-nitrogenous and iso-caloric and to meet all essential nutrient requirements of growing rabbits.

*Mannan-oligosaccharide (Y-Mos, Alltech, Inc., Nicholasville, KY).

Digestibility Trials: During the 12th week of age, the experimental rabbits using the same replicates for each treatment were examined for diets digestibility evaluation using total collection method. Animals were fed their corresponding experimental diets for 4 days, in which feed intake and feces voided, were accurately weighed every 24 hr for each animal. Feces produced daily for each replicate were collected quantitatively in polyethylene bags and stored at -20°C for four consecutive days [14]. Fecal samples were dried in a forced air oven at 70°C for 72 hr (Air-dried samples) then ground and placed in screw-top glass jars until chemical analyses. Dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content of the feces as well as those of feed were determined according to AOAC [12].and expressed on a dry matter basis. Nitrogen free extract estimated as following: (NFE) = 100- (CP+ EE+ CF+ Ash). The apparent digestion coefficient for DM, OM, CP, EE, CF and NFE were calculated.

The nutritive values of the experimental diets were calculated and expressed as TDN, DE and DCP. TDN was calculated as the summation of multiplying the digestible ether extract by the factor 2.25 and each of digestible CP, CF and NFE by the factor 1.0. The Digestible energy (DE) was calculated as follows: DE (Kcal/ Kg diet) = TDN x 44.3 according to Schneider and Flatt [15].

Slaughter Test: At 12 weeks of age, three rabbits from each treatment were randomly taken and fastened for 12 hrs before sacrifice, weighed after being fasted and slaughtered to complete bleeding, then, they were skinned, weighed and eviscerated to obtain carcass characteristics. Carcass Empty percentage was estimated by dividing the weight of hot eviscerated carcass without liver, heart, kidney, spleen, testes and lungs by the live body weight. The internal organs as well as abdominal fat were separated and weighed individually to estimate their relative weights of live body weight.

Determination of Microbial and Fermentation Activities in the Caecum:

After sacrificing, caecal fluid samples were collected from each slaughtered rabbits (3 samples for each treatment) to estimate caecal microbial activity and volatile fatty acids (VFA's) content. The caeca were evacuated by squeezing, the pH value of caecum fluid was estimated immediately using battery operated pH meter. The caecum fluid samples were filtered through two layers of surgical gauze and were used for determinations. Caecal digesta was divided into two portions, one used for determination of VFA's and ammonia-N ($\text{NH}_3\text{-N}$) concentrations according to the method of Vernay and Marty [16]. Briefly, caecal fluid samples were acidified using concentrated orthophosphoric acid and hydrochloric acid (0.1 N), then VFA's were steamed-distilled from a known volume of sample using the micro-kjeldahl apparatus. Distillation rate was adjusted so that 100 ml distillate was collected within 7 to 10 minutes. The concentration of the VFA's was calculated by recording the amount of NaOH (0.01 N) needed to neutralize the VFA's in the distillate. The other portion of caecal digesta was transferred immediately to the laboratory on ice for microbial enumeration. Total bacterial count (TBC), coliforms and Lactobacillus counts were enumerated according to Xia *et al.* [17]. In brief, 1 g of fresh caecal samples were dissolved in 9 ml of phosphate buffered saline solution supplemented with 0.5 g/ L cysteine-hydrochloride. Further decimal dilutions of samples were prepared in 0.1% peptone solution. Pour plate technique on Plate Count Agar, MacConkey Agar and MRS agar were used for counting of TBC, coliforms and Lactobacillus counts, respectively. The plates were incubated aerobically for at 37°C for 24 h for TBC and coliforms counts and for 72 h for lactobacillus counts. The counts were determined according to Maturin and Peeler [18]. Bacterial concentrations were expressed as log₁₀ of viable bacteria per gram of fresh matter.

Measurements of Blood Plasma Constituents: At the end of the study three blood samples per treatment were collected from slaughtered rabbits in heparinized tubes for determination of some blood biochemical constituents. Then blood samples were centrifuged at 3500 rpm for 20 minutes to separate plasma and were stored at -20°C for further analyses. Colorimetric methods using commercial kits produced by Diamond diagnostics were used to estimate both plasma total protein; g/100 ml [19], Albumin; g/100 ml [20], total lipids; g/l [21], total cholesterol;

mg/100 ml [22], alanine aminotransferase (ALT) & aspartate aminotransferase (AST) enzymatic activities; IU/l [23] and creatinine; mg/100 ml [20]. While, globulin concentration (g/100 ml) was calculated by subtraction of albumin values from the corresponding total protein values [24]. Cortisol; µg/dl [25] and triiodothyronine (T3); ng/ml [26] were also determined.

RESULTS

Nutrient Digestibility and Nutritive Values: All rabbits showed good health throughout experimental period and well adapted to the experimental diets. Neither morbidity nor mortality was observed. Average digestion coefficients of DM, OM, CP, CF, EE and NFE and nutritive values (DCP, TDN and DE) of experimental diets for growing rabbits are presented in (Table 3). Our data indicated that there was no significant differences ($P>0.05$) among groups receiving diets 1, 2, 5 & 6 in the digestibility coefficients of DM, CP, CF, EE and NFE. While, rabbits subjected to 30 % feed restriction (groups 3, 4) exhibited significant decrease ($P\leq 0.05$) in nutrient digestibility of the experimental diets compared with the control diets. Dietary supplementation of MOS to rabbits subjected to feed restriction (5, 6) had a positive effect in restoring nutrient digestibility and nutritive values to the level of the control ones.

Caecal Fermentative Activity: Table (3) shows the caecal fermentative activity of rabbits fed different diets. Significant differences ($P\leq 0.05$) of caecal ammonia concentration and pH value among dietary treatments are clear. In feed restricted groups (3, 4), significantly low pH values with high caecal ammonia contents were observed, but TVFA was significantly increased only in rabbit group given diet 3 compared with the control groups. Meanwhile, rabbits fed diets supplemented with MOS (5, 6) had the highest pH values but ammonia and TVFA contents were not affected. Replacing dietary clover hay with PS significantly decreased caecal ammonia content, while pH value and TVFA content remained unaffected.

Caecal Microbial Activity: The influence of different treatments on the microbial load in rabbits' caeca is showed in Table (4). In the present study, rabbits either fed on clover hay or PS with prebiotic (MOS) supplementation or those subjected to feed restriction harbored lower total bacterial counts than control groups.

Table 3: Effect of dietary treatments on nutrient digestibility and nutritive values of 12-week-old NZW rabbits

Dietary Treated groups	Nutrients digestibility (%)					Nutritive value			
	DM	OM	CP	CF	EE	NFE	DCP%	TDN%	DE*(Kcal/Kg)
1	68.85 ^a	70.64 ^a	70.89 ^a	50.68 ^a	75.93 ^a	72.98 ^a	12.15 ^b	61.13 ^c	2708.06 ^c
2	68.88 ^a	70.64 ^a	70.90 ^a	50.69 ^a	76.00 ^a	72.97 ^a	12.26 ^a	62.31 ^a	2760.19 ^a
3	64.99 ^c	66.24 ^b	66.93 ^c	45.86 ^c	72.04 ^c	69.70 ^c	11.96 ^d	58.40 ^e	2587.12 ^e
4	65.79 ^b	66.34 ^b	66.62 ^b	46.5 ^b	72.75 ^b	69.80 ^b	12.04 ^c	60.22 ^d	2667.60 ^d
5	68.87 ^a	70.62 ^a	70.88 ^a	50.68 ^a	75.95 ^a	72.96 ^a	12.14 ^b	61.31 ^c	2716.18 ^c
6	68.88 ^a	70.64 ^a	70.88 ^a	50.68 ^a	75.95 ^a	72.97 ^a	12.24 ^a	61.99 ^b	2746.16 ^b
SEM	0.009	0.007	0.007	0.006	0.002	0.005	0.009	0.074	3.273
Significance	**	**	**	**	**	**	**	**	**

^{a-d} Means in the same column having different superscripts are significantly different ($P \leq 0.05$).

n = 3 for each treatment

DM= dry matter, CP= crude protein, CF= crude fiber, EE= ether extract, NFE= nitrogen free extract, * DE= TDN x 44.3 [15].

Table 4: Caecal fermentative activities of 12-week-old NZW rabbits as affected by dietary treatments

Dietary Treated groups	pH value	NH ₃ -N (mg/100 dL)	TVFA(mmol/100 ml)
1	6.44 ^b	25.33 ^c	8.26 ^b
2	6.42 ^b	24.67 ^d	8.26 ^b
3	6.25 ^c	37.33 ^a	8.48 ^a
4	6.13 ^d	31.65 ^b	8.25 ^b
5	6.54 ^a	25.23 ^c	8.28 ^b
6	6.56 ^a	26.00 ^c	8.26 ^b
SEM	0.016	0.012	0.012
Significance	**	**	**

^{a-d} Means in the same column having different superscripts are significantly different ($P \leq 0.05$) based on Duncan's test.

n = 3 for each treatment

Table 5: Caecal microflora of NZW rabbits at 12 weeks-old as affected by dietary treatments

Dietary Treated groups	TBC	Coliforms count	Lactobacilli count
1	14.54 ^a	2.06 ^c	9.55 ^b
2	14.33 ^b	2.03 ^c	9.93 ^a
3	12.86 ^d	5.42 ^a	6.98 ^f
4	11.99 ^e	4.62 ^b	7.21 ^e
5	13.51 ^c	1.99 ^d	8.55 ^d
6	13.52 ^c	1.92 ^c	8.94 ^c
SEM	0.012	0.013	0.015
Significance	**	**	**

^{a-f} Means in the same column having different superscripts are significantly different ($P \leq 0.05$).

n=3 for each treatment

Table 6: Carcass characteristics of 12-week-old NZW rabbits as affected by dietary treatments

Dietary Treated groups	LBW (g)	Skin+foot(%)	EC (%)	Liver (%)	Heart (%)	Kidneys (%)	Abdominal fat (%)
1	1981.3 ^a	17.12	55.53 ^a	4.05	0.299	1.060	0.267
2	1993.1 ^a	17.22	55.49 ^a	4.03	0.308	1.080	0.263
3	1915.6 ^b	16.84	53.34 ^b	3.92	0.292	1.070	0.270
4	1920.4 ^b	16.91	54.01 ^b	3.96	0.291	1.048	0.273
5	1986.4 ^a	17.16	55.54 ^a	4.01	0.303	1.060	0.273
6	1990.0 ^a	17.18	55.63 ^a	4.01	0.304	1.059	0.272
SEM	0.026	0.062	0.116	0.029	0.004	0.015	0.006
Significance	**	NS	**	NS	NS	NS	NS

^{a-b} Means in the same column having different superscripts are significantly different ($P \leq 0.05$).

LBW= live body weight, EC= empty carcass, NS= Not significant

n=3 for each treatment

Table 7: Blood plasma parameters of 12-week-old NZW rabbits as affected by dietary treatments

Dietary treated groups	Total protein	Albumin	Total lipids	Cholesterol	ALT	AST	Creatinine	Cortisol	T3
1	4.95	2.56	7.55	86.76	19.95	29.87	1.480	1.0 ^b	2.55 ^c
2	5.08	2.54	7.53	87.90	19.72	29.88	1.401	1.1 ^b	2.69 ^c
3	4.96	2.46	7.45	84.81	20.00	30.01	1.512	1.2 ^b	1.70 ^d
4	5.03	2.44	7.51	84.60	19.68	30.32	1.543	1.3 ^b	1.70 ^d
5	4.97	2.55	7.47	85.80	19.41	29.68	1.553	2.0 ^a	4.05 ^b
6	5.06	2.52	7.49	85.52	19.53	29.56	1.492	1.9 ^a	6.06 ^a
SEM	0.045	0.023	0.038	0.975	0.318	0.327	0.013	0.019	0.041
Significance	NS	NS	NS	NS	NS	NS	NS	**	**

^{a-b} Means in the same column having different superscripts are significantly different ($P \leq 0.05$).

NS= Not significant, n=3 for each treatment

Dietary inclusion of MOS with clover hay or PS resulted in significantly low ($P \leq 0.05$) coliform counts in rabbits' caeca, with greater reductions in feed restricted MOS supplemented groups (5, 6). Meanwhile, the highest counts were recorded in groups (3, 4). The population of Lactobacillus species in the wet caecal contents of rabbits were significantly increased ($P \leq 0.05$) by dietary substitution of PS; group (2) followed by group (1) where rabbits fed clover hay and with marked increase in groups (5 & 6) supplemented with MOS prebiotic. Meanwhile, control rabbits recorded lower Lactobacillus counts.

Carcass Yield: As presented in Table 5, significant differences were observed among different treatments in live body weight and empty carcass of the experimental rabbits. There was a trend of improvement of live body weight in groups (1, 2, 5, 6). Conversely, the experimental groups of rabbits subjected to feed restriction (3 & 4) achieved significantly lower live body weight and lower relative weight of empty carcass, while percentage of skin plus foot, liver, heart, kidneys and abdominal fat were not affected. The adverse effect of feed restriction on live body weight and relative weight of empty carcass was absent by dietary prebiotic (MOS) supplementation. Replacing dietary clover hay with PS did not adversely affect carcass characteristics of rabbits.

Blood Plasma Constituents: With respect to the effect of dietary treatments on blood plasma constituents throughout the experimental period, differences of total protein, albumin, total lipids, cholesterol and creatinine and activity of ALT and AST among treatment groups was not significant (Table 7). However, hormonal levels were influenced by dietary treatments as shown. T3 and cortisol in groups (5 & 6) were both significantly higher ($P < 0.05$), than those of other dietary treatments. Meanwhile, the lowest levels of T3 and cortisol were found in feed restricted groups with no additives (3&4).

DISCUSSION

Nutrient Digestibility and Nutritive Values: Results of this experiment indicated that *Phaseolus vulgaris* Straw (PS) may replace clover hay in rabbit diets fed from d 45 to d 60 with no negative impact on either nutrient digestibility or nutritive value of the diets. This may confirm that this feed ingredient is safe and its content of anti-nutrition factors is tolerant to the growing rabbits. Rabbits in feed restricted groups with no additives (3, 4) showed the lowest digestibility coefficients of DM, OM, CP, CF, EE and NFE and nutritive values (DCP, TDN and DE). Clear improvements of nutrient digestibility and nutritive values were achieved by MOS inclusion to diets (5, 6). These higher values can be attributed to more efficient nutrient utilization in the intestinal tract. Our data go in accordance with those reported by Pinheiro *et al.* [10] recorded higher feed digestibility in MOS supplemented rabbit groups and suggested that improvement of intestinal morphology, as MOS may increase villi height: crypt depth at the ileum, which justify the higher feed utilization by expanding the intestinal absorption surface area. The positive effect of MOS inclusion with feed restriction on nutrient digestibility of the experimental diets, observed herein, may be related to some changes in gut morphology. In this regard, Pote *et al.* [2] and Tumova *et al.* [27] found that the gastrointestinal tract is longer in feed restricted animals, which might explain improved nutrient digestibility. The beneficial effect executed by dietary prebiotic on digestibility of nutrients of the experimental rabbits may be due to its stimulating action on caecal microbial activity and thus improving the digestion efficiency. The present results are in harmony with the findings obtained by Abo-Egla *et al.* [28] who found that adding prebiotic (MOS) to NZW rabbit diets led to an improvement in nutrient digestibility and nutritive value of diets.

The high digestibility coefficients and nutritive value may be an indication to good acceptability of diets and/or higher caecal activity [29]. In this respect, Sarhan [30] found that the digestible crude protein of diet contained 30% pea pods hulls was significantly ($P \leq 0.05$) higher than that of the control diet. On the other hand, Gidenne [31] attributed the decrease of nutrient digestibility of rabbits fed lemon and orange pulps to their high contents of fiber especially pectin. In addition, Hussien [32] observed that apparent digestibility of EE was not affected with either pea vines, green bean vines or squash vines from 0 to 35 % of growing rabbit diets.

There were significant increases ($P \leq 0.05$) in DCP value for rabbits fed diet 5, containing 100% PS and the group fed 70 % from voluntary daily feed intake plus prebiotic addition, compared to those fed clover hay ad libitum. In this issue, Tag El-Din *et al.* [33] concluded that TDN of rabbit diets contained 10, 20 or 30 % turnip (Roots and leaves) was gradually improved as the level of dried turnip increased in diet. On the contrary, Ibrahim [34] reported that substituting clover hay with peanut hay partially or completely in rabbit diets numerically increased DCP and TDN values.

Caecal Fermentative Activity: In the current study, two methods were used in combination to evaluate the caecal microbial activity; an analysis of fermentation end products and of caecal microflora activity. At 21 days of age, the young rabbit begins to eat solid feed [35] resulting in maturation and development of the caecal flora. Drastic changes of low pH value and high ammonia content in feed restricted rabbits were recorded and this is may be due to slower rate of passage of digesta in the gut. Conversely, the higher pH values of rabbits given prebiotic (MOS) supplemented diets may be related to higher fermentation rate of microflora in the caecum.

Despite dietary CF level increased from 14.0 to 16.05 when clover hay was replaced by PS, in the present study, no change was detected in concentrations of TVFA of rabbits. In agreement with the present results, Gidenne *et al.* [36] reported that fibrolytic capacity of bacteria was not affected by level of fiber intake. Conversely, Hussien [32] found that rabbits fed 25 or 75 % PV did not affect caecal pH value but 25 % GBV had lower pH value compared with the control group with significant increases in caecal ammonia and VFA concentration in response to feeding vegetable crop wastes of rabbits.

Caecal Microbial Activity: The main site of fermentative activity in rabbits is the caecum, which characterized by the wide diversity of microbial flora community [37], when the rabbit feed shifting from milk to dry feed at 3 weeks of age; the microbial flora develops significantly [38], markedly affecting nutritional, physiological, immunological and protective processes in the host animal [39]. The type and level of dietary fiber contribute to determine the extent and diversity of caecal microbial community in the rabbit [40].

Alterations in livestock gut microflora in relation to dietary constituents have been studied by Gidenne *et al.* [36]. Our results agreed with those reported by Robertson [41] who recorded lower microbial counts in caecal contents of rabbits fed diets with high lignin and insoluble non-starch polysaccharides fraction reduced by increasing the digesta transit rate. Significant reductions in total anaerobic and coliforms counts within caecal samples from rabbits fed on diet containing caprylic acid, which is short-chain fatty acid [42]. In the same respect, Oso *et al.* [43] recorded lower microbial counts in caecal contents of rabbits fed with 300 g/kg sorghum milling waste which could indicate the increased digesta transient rate caused by high dietary fiber. The reduction in caecal microbial counts in the caeca may be more obvious with rabbits offered restricted diets and this can be attributed to lower digesta contents and therefore slower digesta passage. The numerous physiological functions related to food intake limitation depending on its intensity and duration [44]. However, a short-term intake limitation might improve digestive functions and health in young animals. For instance, the endocrine and immunological status of the young fowl was modified after a short-term intake restriction [45] and also the development and characteristics of its digestive organs [46]. Our results coincide with those investigated by Gidenne and Feugier [47] who found a higher caecal fermentative activity and a longer transit in weaned rabbits exposed to a short-term restriction of the intake for 3 weeks, associated with a lower incidence of post-weaning digestive troubles [48]. Meanwhile, caecal biodiversity of the bacterial community in rabbits did not change after 16 days of diet restriction [49].

In rabbits, results on the role of prebiotics are clear and effective in reducing total bacterial and coliforms counts and at the same time favoring the growth of *Lactobacillus* in the caeca. In agreement with this study, Xu *et al.* [50] demonstrated that supplementation of 0.02%

fructooligosaccharides increased Bifidobacterium and Lactobacillus in growing pigs. Fructo-oligosaccharides showed some protective effects against experimental inoculation of *E. coli* [51, 52], but in field conditions their positive effects on health have not been strongly demonstrated. However, some authors reported increased growth rate or lower feed conversion rate [53, 54]. The effect of inclusion of probiotics or prebiotics on gut microflora was reported in previous findings [55]. Rabbits fed diets containing various additives in the present study showed lower coliform count in their caecal content when compared with those fed the control diet. Investigation on the mode of action of mannanoligosaccharide pointed out that MOS is able to bind to mannose-specific lectin of gram-negative pathogens those express Type1-fimbriae such as Salmonella and *E. coli*, resulting in their excretion from the intestine [56]. Rabbits fed diets containing MOS however showed the least lactobacillus counts. In vivo trials with pigs also showed synergistic effect of synbiotic in the reduction of food-borne pathogenic bacteria populations [57]. The reduced caecal lactobacillus count obtained from rabbits fed synbiotics when compared to those fed probiotics could be implicative of positive health status [58]. Rabbits fed diets containing oxytetracycline showed higher total bacterial and caecal coliform counts than those fed diets included with prebiotics and probiotics [59].

Carcass Yield: Feed restriction had a more pronounced impact on carcass traits compared to the other groups. According to Ouhayoun [60] feed restriction caused reduced carcass weight and, consequently, by the increase in the relative weight of the full digestive tract. These data go in line with those found by Szendrő *et al.* [61] who recorded decreasing body weight at 12 weeks of age with diet restriction. The other studies have shown that when feed restriction was greater than 85% of the ad libitum rabbits' growth, feed efficiency, carcass adiposity and lipid content decreased [62, 63]. Reviewing several experimental results, Xiccato [64] found that the dressing out percentage decreased due to the feed restriction. This impact of feed restriction was corrected by prebiotic, MOS addition, which caused improvements in live body weight. These results are in agreement with data reported by Piccolo *et al.* [65] and Bovera *et al.* [66] and attributed that to the fact of inclusion of an antibiotic growth promoter in rabbit diets resulted in a reduction in the

enteric mass. In contrast, MOS inclusion in rabbit diets has no effect on enteric mass of rabbits, in spite of MOS increase motility and resulted in increased luminal muscularis. In accordance with our results, Khashaba *et al.* [67] observed an improvement in hot carcass weight and dressing percentages of rabbits fed diets containing 66% pea by-products in place of clover hay. Conversely, Zeweil [68] found that the inclusion of pea residues to Flanders rabbit's diets up to 25 % had no effect on slaughter yield.

Blood Plasma Constituents: Feed restriction significantly lowered stress response (Cortisol and T3 levels) in rabbits. It has been indicated that animals could cope with feed restriction (FR) challenge by decreasing the transformation of thyroxin to triiodothyronine (T3) or increasing catabolism of triiodothyronine and by decreasing the basal metabolism and thus allowing the organism to spare energy to deposit protein and fat. What happened in this study is increasing the catabolism of triiodothyronine in response to prolonged feed restriction compared to rabbits fed *ad libitum*. Despite, the lack of feed restriction effect on carcass traits, feed restriction led to increased fat deposition [69]. Plasma concentrations of leptin, insulin and triiodothyronine were decreased as a metabolic adaptation mechanism to fasting together with the activation of the adrenal axis in more severely feed deprived rabbits [70]. By converse, circulating T3 was depressed while T4 increased in feed restricted broilers [71, 72]. Fasting of broiler breeds decreased the abdominal fat deposition and circulating triiodothyronine level in plasma, while thyroxin increased [73, 74].

Animals have the ability to store large quantities of excess energy in liver and abdominal fat tissue. Variations in nutrient intake and status were communicated to the liver and other internal organs by alterations in levels of triiodothyronine in the plasma, which responded acutely to dietary changes and then changed its fat metabolism [73]. Substantial homology exists in mammalian species in the regulation of energy homeostasis in coping with nutritional stress [75, 76]. Conversely, corticosterone was found to decrease under prolonged feed restriction in rabbits, counteracting the excessive catabolic rate of basal metabolism [77]. Regarding the effect of MOS supplementation on stress response, our results indicated significant increase in both T3 and cortisol levels in feed restricted, MOS supplemented groups in comparison to

the other groups. This may be explained as MOS acting as antagonist to thyroid function. L-carnitine is a peripheral antagonist of thyroid hormone action; in particular L-carnitine inhibits both T3 and T4 entry into the cell so leading to accumulation of them in the peripheral blood [78]. These results are going in accordance with those found by Buyse *et al.* [79] who recorded significant increased T3 concentration in chickens fed diets supplemented with L-carnitine.

CONCLUSION

Based on the obtained results, it could be concluded that dietary clover hay can safely be replaced by *Phaseolus vulgaris* Straw for growing rabbits. MOS prebiotic can be a good supplement in diets for growing rabbits, correcting the negative effect of feed restriction on nutrient digestibility, caecal fermentative activity and carcass yield. Moreover, the addition of MOS will produce a protective effect against common caecal microbial pathogens. Several benefits can be gained by MOS inclusion to the diet of growing rabbits under feed restriction conditions. Therefore, the dietary inclusion of MOS is recommended in growing rabbits.

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