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Economic, Productive, and Behavioral Evaluation of Using Dried Olive Cake and Ground Date Palm in Growing Rabbit's Diet: Histological and Gene Expression Impact

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THE OBJECTIVE of this study was to assess the impact of incorporating different percentages (7.5% and 10%) of dried olive cake and ground date palm together with or without bentonite addition (1%) on economic efficiency, growth performance, gene expression, histopathological findings, and behavioral responses in New Zealand and V-line rabbit breeds. A total of 100 weaning rabbits, from both New Zealand and V-line breeds, were used. The groups were categorized in to five groups per breed based on the content of their feed. The inclusion of bentonite at 1% in combination with both DOC and GDP led to enhanced growth performance for both breeds. From an economic perspective, reduced costs per kilogram of feed, overall feed expenses, leading to lower total production costs. Additionally, significantly higher total return, net profit, and economic efficiency measures were specifically observed within the G10%B group. Gene expression revealed non-significant differences in the growth markers. Bentonite addition at 1% increased the expression profile of antioxidant markers. These findings were also established through the changes in histological features, including increased length of mucosal folds, villi, crypt depth in the intestine, and an enlarged hepatic lobule area in the liver. The groups that consumed treated diets displayed enhanced behavior through increased feeding and grooming activities, as well as reduced periods of inactivity. In conclusion, the inclusion of DOC and GDP together at a rate of 7.5% or 10%, along with an additional 1% bentonite in the diet of growing rabbits, has the potential to yield cost-effective production outcomes.

Keywords: DOC, GDP, Bentonite, economic, productive.

Introduction

Rabbits are a practical choice for small-scale farmers looking to produce meat and generate income. They are medium-sized animals known for their quiet nature and easy manageability [1]. In Egypt, rabbit rearing has the potential to alleviate meat scarcity as a result of its fast growth rate and effective conversion of forage into meat [2]. Rabbits are recognized as a valuable source of palatable protein with desirable taste qualities, as well as low fat and calorie content. Additionally, they offer high mineral content and a significant ratio of edible meat to bone due to efficient dressing out percentage [3]

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The livestock economy is currently facing a significant challenge due to the substantial gap that exists in the global demand and supply of conventional feed resources for feeding livestock [4].

The imbalance in the availability and cost of feed ingredients can have detrimental effects on animal performance, including decreased growth rates, impaired reproductive efficiency, and increased morbidity and mortality. This issue highlights the necessity to explore new technologies for utilizing non-conventional feed resources as a potential solution that could simultaneously enhance economic viability while mitigating environmental concerns [5] and [6].

The Mediterranean region is known for its significant role in the olive and olive-derived industries. The chemical composition of olive cake varies greatly depending on factors such as the characteristics of the olives, climate conditions, and manufacturing processes involved [7].

During the 1990s, a new method for extracting olive oil was introduced. This process resulted in the production of a by-product known as two-stage dried olive cake, which is composed of olive skins, stones, and pulp. Approximately 80% of the entire number of olives used for oil extraction ends up as dried olive cake. According to various sources [8, 9], this by-product contains a high proportion of crude fibers and significant amounts of protein. Additionally, it contains high-quality oil with good concentrations of oleic acid. Consequently, rabbit's diets can successfully incorporate substantial quantities of these olive oil by-products [10, 11] .

In Egypt, the primary crop in dry desert and semi-dry areas of newly cultivated land is date palm trees [12,13, 14].

There are about 11.2 million date palm trees that yield around 1.17 million tons of date, leading to an increase in the supply of date by-products about, which account for about 20-25 % of the production [15, 16], including cull dates, immature dates, date pedicels, date seeds, date press cake, and date molasses. All these are sources for feeding livestock. Cull dates are fruits that are too hard, too small, blemished, of poor appearance, containing foreign matter, suffered from drought or were unfertilized due to a lack of pollen, and can be used for feeding rabbits [14, 17].

Bentonites are a type of feed additives that can be included in animal diets to enhance pellet quality, specifically their durability, particularly when the diets contain sources of lipids [18].

According to [19], including bentonite in the diet at levels ranging from 0.5 to 3% is considered essential for ensuring high-quality pellets that result in improved growth performance, enhanced digestibility, and overall better health for rabbits [20, 21].

Hence, the objective of this study was to assess the impact of incorporating different percentages (7.5% and 10%) of dried olive cake (DOC) and ground date palm (GDP) together with or without bentonite addition (1%) on economic efficiency, growth performance, gene expression, histopathological findings, and behavioral responses in New Zealand and V-line rabbit breeds.

Material and Methods

Experimental Design and management

Housing, management, and all rabbit-related procedures were conducted according to the guidelines of the Mansoura University Animal Care and Use Committee (No. R/149, 2022)

This study included a total of 100 unsexed weaning rabbits, aged 5 weeks, with an average body weight of 659±20.93 gm, from both New Zealand (NZB) and V-line (VB) breeds. They were then individually housed and randomly assigned into 2 replicates per group, 5 treatment groups per breed as illustrated in table 1, with each group consisting of 10 rabbits (resulting in a total of 50 rabbits per breed). The rabbits were housed in 20 fabricated wired rabbit hutches with the same dimensions (200 cm L × 80 cm W × 80 cm H) and under identical management and environmental conditions. Each hutch was divided into five equal cage units, each equipped with feeders and drinkers. Food and water were available *ad libitum* from weeks 5 to 11 of age throughout the entire duration of the experiment.

The DOC and GDP used in this experiment were obtained from a factory specializing in drying feed wastes located in Cairo. Prior to their inclusion in the diets, they underwent chemical analysis to determine their dry matter, crude protein, crude fat, and crude fiber content according to [21] and [22] guidelines. The results of the analysis are presented in Table 2. The feeding trial utilized diets with the price per ton listed in Table 3, where all diets provided to the growing rabbits were carefully formulated based on guidelines for essential nutrient requirements [23].

These diets included ground date palm and dried olive cake at levels of either 0%, 7.5%, or 10%, with an optional addition of bentonite by 1%. These percentages were selected based on previous studies [24, 25], that demonstrated improved productivity and economic efficiency when using each of DOC and GDP alone at 10%. The decision to use a 7.5% percentage was made in order to compare it with the higher value of 10%, as previous research did not recommend surpassing this percentage. The experimental diet composition was then adjusted to resemble the chemical makeup of the control diet.

Evaluation of growth performance

The weights of the rabbits were measured weekly, and their initial (weaning) and final (11th weak) body weights (BW) along with the body weight gain (BWG) for each rabbit within groups were recorded. Body weight gain was calculated by subtracting the end body weight from the start body weight during each recorded period. The diets were consistently provided twice daily at 6 a.m. and 3 p.m. The daily feed intake was then calculated based on these regular feeding times. The total feed intake (FI) was measured after the experiment, and subsequently, the total feed conversion ratio (FCR) for each rabbit in each group was calculated following the methodology outlined by [26, 27], where FCR is equal to the feed intake divided by the body weight gain. To determine the relative Growth Rate (RGR), Broody's formula [28] was utilized, where RGR involves dividing the difference between the final weight and initial weight by half of their sum, multiplied by 100. Additionally, we computed the performance index % (PI) using the formulas proposed by [29, 30], as PI (%) = final live body weight (Kg) / FCR x 100.

Economic evaluation parameters

Costs of production

Cost parameters, as defined by [31, 32], can be categorized into total variable cost (TVC) and total fixed cost (TFC). The sum of TVC and TFC is known as the total cost. TFC includes labor costs, veterinary care expenses such as vaccines, drugs, supervision fees, litter, electricity and water; miscellaneous costs, and rent value **[33]**

TABLE 1. Experimental design of the study.

New Zealand rabbit breed (NZB)		V-line rabbit breed (VB)
1	0% DOC & 0% GDP without bentonite (G0%)	6
2	7.5% DOC & 7.5% GDP without bentonite (G7.5%)	7
3	10% DOC & 10% GDP without bentonite (G10%)	8
4	7.5% DOC & 7.5% GDP with bentonite 1% (G7.5%B)	9
5	10% DOC & 10% GDP with bentonite 1% (G10%B)	10

TABLE 2. Analyzed chemical composition of ground date palm and dried olive cake that incorporated in experimental diets

Item %	DOC	GDP
DM	88.9	86.8
CP	8.4	8.1
CF	11.55	14.6
EE	14.6	2

DM = dry matter, CP = crude protein, CF = crude fiber, EE = ether extract.

TABLE 3. The percentage of ingredients and chemical composition found in the experimental diets, along with their corresponding price per ton.

Ingredient (%)	Control (G 0%)	(G 7.5%)	(G10%)	(G 7.5% B)	(G 10% B)
Yellow corn	10	6.5	7	6.5	7
Soya bean meal 44%	15	14.5	14	14.5	14
wheat bran	17	15	16.5	15	16.5
Alfalfa	35	28	24.5	28	24.5
Barely	20	18	15	18	15
Dried olive cake	0	7.5	10	7.5	10
Ground date palm	0	7.5	10	7.5	10
Di-calcium phosphate	1.20	1.20	1.20	1.20	1.20
Ground limestone	1	1	1	1	1
Common salt	0.50	0.50	0.50	0.50	0.50
premix	0.30	0.30	0.30	0.30	0.30
Bentonite	0	0	0	1	1
Chemical analysis					
DM%	89.32	88.74	87.56	88.74	87.56
CP%	17.88	17.21	17.14	17.21	17.14
CF%	13.50	13.21	13.94	13.21	13.94
EE%	2.42	3.27	3.65	3.27	3.65
TON PRICE (LE)	12912.5	12167.5	11897.5	12171.5	11901.5

DM = dry matter, CP = crude protein, CF = crude fiber, EE = ether extract. The vitamin and mineral premix contain essential nutrients per kilogram of diet: including 12,000 IU of vitamin A, 2,500 IU of vitamin D, 12mg of vitamin E, 2.5 mg of vitamin K, 1.2 mg of vitamin B1, 6 mg of vitamin B2, 12mg of pantothenic acid, 1.2mg of folic acid, 36mg of niacin, 2mg of pyridoxine, 0.01 mg of vitaminB12, 36mg of iron,5mg of copper,72 mg of manganese, 60 mg of zinc, 0.45mgs of Iodine and 0.12mg of selenium.

On the other hand, the price of purchased weaned rabbits and feed costs are considered as TVC. The cost of composite diets was determined by the prices of the ingredients used and their prices at the time of the experiment. This included assessing the cost of feed intake per rabbit as well as the reduction percentages of feed costs of the experimental groups compared to the percentages of the control group [34].

In addition to evaluating the cost of feed intake per kilogram (kg) of body weight gain, an assessment is conducted to determine the savings in feed costs per kilogram of meat gained when compared with a controlled group [35].

Return from production:

The return of weight gain was determined by multiplying the total weight gain by the price of live body weight per kilogram. To assess the percentage of change in return from weight gain for each experimental group, it was compared to that of the control group [34, 36] and calculated according to methods by [37, 38] as the percentage of change = ((return of treated group – return of control group) / return of control group)*100. The total return (TR) is calculated as the sum of the sales of final body weights and litter [32]. The net profit (NP) calculated according to [39] as the difference between TR and TC.

Economic efficiency measures

Efficiency measures were determined as ratio of total return to total costs (TR/TC) and net profit to total cost (NP/TC), consistent with the methods described by [31, 39].

Furthermore, the percentage of economic efficiency was determined by calculating the ratio between the return from total weight gain and the total cost of feed consumed throughout the duration of the experiment [31, 34, and 40]

RNA extraction, reverse transcription, and quantitative real time PCR

RNA was isolated from rabbit's muscle, liver, and spleen tissues using a Trizol reagent (Direct-zolTM RNA MiniPrep, catalogue No. R2050), according to the manufacturer's instructions. The quantity and purity of the isolated RNA were determined using a Nanodrop (UV-Vis spectrophotometer Q5000/USA), while gel electrophoresis was utilized to assess its integrity. For each sample, cDNA was generated using the SensiFastTM cDNA synthesis kit, Bioline, catalogue No. Bio- 65053. A reaction mixture was prepared by combining 20 µl of total RNA up to 1 µg, 4 µl of 5x Trans Amp buffer, 1 µl of reverse transcriptase, and 20 µl of DNase-free water. To complete the reaction, a thermal cycler was employed with the following protocol: primer annealing at 25 °C for 10 minutes, reverse transcription at 42 °C for 15 minutes, and inactivation at 85 °C for 5 minutes. Then samples were kept at a temperature of 4 °C.

The mRNA levels of growth performance and antioxidant markers were relatively quantified using the SYBR Green PCR Master Mix and real-time PCR method (2x SensiFastTM SYBR, Bioline, catalogue No. Bio-98002). Using the Oryctolagus cuniculus genome from PubMed, the sense and antisense primer sequences were generated (Table 4). The internal control for this study was the housekeeping gene β-actin. The reaction mixture, which had a total volume of 20 µl, consists of 0.8 µl of each primer, 5.4 µl of distilled water, 10 µl of SensiFast SYBR, 3 µl of cDNA, and an additional 10 µl of 2x SensiFast SYBR. The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 2 minutes, followed by 40 cycles at 94 °C for 15 seconds, annealing for 20 seconds, and extension at 72 °C for an additional 20 seconds. To ensure the specificity of the amplified PCR product, a melting curve analysis was performed. Subsequently, using the $2^{-\Delta\Delta Ct}$ method as described by **[41],** the relative expression levels of each sample's gene compared to those of the β-actin control gene.

Histological Examination

Following the completion of the experiment, specimens from the liver, intestine (specifically the duodenum and cecum) were surgically obtained from each rabbit. The collected tissue samples were promptly fixed for histopathological analysis. Subsequently, standard histological techniques such as paraffin embedding, sectioning, and staining with hematoxylin and eosin (H&E) were employed to process the fixed tissue samples. Following the preparation of slides, a light microscope was used to examine them according to methods described by [42]. The morphology of

TABLE 4. Oligonucleotide forward and reverse primers for growth, immune, and antioxidant genes under investigation used in real time PCR.

Investigated marker	Source	Primer	Product size (bp)	Annealing Temperature (°C)	GenBank isolate
IGF-I	Liver	F5′- TGTGATCTGAGGAGGCTGGA -3′ R5′- ACTTGTGTTCTTCAAATGTACTTCC -3′	181	58	NM_001082026.1
PGAM2	Muscle	F5- GCCACCATGGCCACCCACCG -3′ R5′- TCGGCCCCCTTCTCGCTCAG -3′	113	58	XM_002713845.3
SOD	Liver	F5′- GTCAGCGGCTTGGTAGTCTT -3′ R5′- GCCCTCCAAGTCGAAGAAGG -3′	72	56	NM_001082632.1
CAT	Liver	F5′- GGCAACTACCCCTCTTGGAC -3′ R5′- TTGGGTCAAAGGCCAACTGT-3′	196	58	XM_002709045.4
GPX	Liver	F5′- TGCTCTTCCAGAAGTGCGAG-3′ R5′- TCCAGGAAACGTCGTTACGG -3′	159	58	NM_001085444.1
ß. actin		F5′- GATTCACCATGGATGATGATA -3′ R5′- ACTAGTGATTCGTGCTCGATG-3′	238	56	AF404278.1

IGF-I= Insulin-like growth factor-1; PGAM2= Phosphoglycerate mutase 2; SOD= Super oxide dismutase; CAT= Catalase; GPX= Glutathione peroxidase; and ß. actin= Beta actin cells, the architectural integrity of tissues, and any possible abnormalities were assessed in sections representing the liver and intestine.

Various histological parameters were assessed in this study. The liver was evaluated based on parameters including the area of hepatic lobules and central veins [43, 44], while for the intestine, measurements like villi length, crypt depth, mucosa thickness, and muscularis thickness were taken into account [45]. All images were analyzed using Image J software.

Behavioral observations

In each group, the unaltered behavior of rabbits was assessed for a period of 10 minutes using an instantaneous scan sampling method with a 30 sec sample interval and video recording, followed by direct observation for three consecutive days every week from the 5th to the 11th weeks of the rearing cycle. Observations were conducted from 7:00 to 8:40 a.m. and 16:00 to 17:40 p.m. each day. Before each observation, there was direct observation of the animals by one trained operator and a period of 5 minutes for them to adapt to the observer's presence. An ethogram based on [46] has been developed after three days of observation of rabbit behavior. The following behaviors were noted: intake maintenance (feeding, drinking), non-intake maintenance (self-grooming, allo-grooming), exploratory (raising up, sniffing), active (standing, stretching), inactive (laying down, sitting), and abnormal (bar biting or gnawing). The frequency of a specific behavior was counted for each group and transformed into a percentage with respect to the overall observations.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software [47] with a generalized linear model. The significance of breed, group, and their interaction were calculated. Differences were considered statistically significant at $p \leq 0.05$. To determine letters for interaction, the Duncan Test [48] along with the MSTAT program were employed. In order to determine the percentage of change in productive and economic results, the formula proposed by [37, 49] was utilized. This formula calculates the percentage of change as percentage of change as= [(New Value - Original Value) / Original Value)] *100.

Results

Growth performance results

The impact of incorporating DOC and GDP in rabbit diets at concentrations of 7.5% or 10%, along with the optional addition of bentonite at a rate of 1% on the growth performance of growing rabbits for both NZB and VB is presented in table 5. The results demonstrate that VB exhibited substantial enhancements in all aspects of growth performance in comparison to NZB, with the exception of relative growth rate. In terms of the results related to body weight, it was found that among all treatment groups, G10% B in VB exhibited the highest marketing weight at 2551.7 gm. This reflected an increase of 11.92% compared to the control group. Additionally, this group also demonstrated the highest recorded BWG of 1885 gm, along with the highest recorded RGR value of 117.09%. On the contrary, in NZB, the lowest marketing body weight was recorded at 2212.2 gm, which indicated a decline of 4.58% when compared to the control group. Moreover, the same treatment group also observed the lowest values for both BWG with just 1560 gm and corresponding RGR value of 108.94%, all within treatment group G7.5%.

Regarding the quantity of feed, in relation to NZB, all experimental groups exhibited reduced feed quantities when compared to the control group. Conversely, for VB, it was observed that groups G10%B and G7.5% had the highest amounts of feed intake at 5725 gm and 5626.1 gm, respectively, whereas treatment G7.5%B consumed the lowest amount at 5248.9 gm. Based on the results from FCR, incorporating DOC and GDP into the diet of growing rabbits resulted in an improved value of FCR compared to control groups. The group with the best value of 3.01 was observed in G10%B in NZB. According to findings conducted by PI, it was observed that all groups subjected to treatment exhibited greater outcomes in comparison to the control groups. Among these groups, G10%B in VB attained the highest level of 84.28, closely followed by a similar treatment implemented in NZB, which yielded an outcome of 80.08.

Economic evaluation results

An economic evaluation was conducted to assess the impact of integrating DOC and GDP in rabbit diets by 7.5% or 10% with or without bentonite addition at 1% on production costs. According to the findings presented in table 6,

TABLE 5. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V- line rabbit breeds on parameters of production performance.

Item	New Zealand B						V- line B						MSE		*P*-Value		
/rabbit	G 0%	G 7.5%	G 10%	G 7.5%B	G 10%B	B Overall mean	G 0%	G 7.5%	G 10%	G 7.5%B	G 10%B	B Overall mean	G	B	G	B*G	B
WBW (g)	650.00^{ab}	652.22^{ab}	650.00^{ab}	646.67^{b}	650.00^{ab}	649.78^{B}	670.00^{a}	**668**.89^{a}	666.67^{ab}	668.89^{a}	666.67^{ab}	668.22^{A}	6.57	2.94	0.027	0.001	0.001
MBW (g)	2318.3^{de}	2212.2^{f}	2367.8^{cd}	2336.7^{cde}	2398.9^{bc}	2326.8^{B}	2280.0^{ef}	2458.9^{b}	2381.7^{cd}	2403.9^{bc}	2551.7^{a}	2415.2^{A}	24.31	10.87	0.001	0.001	0.001
BW%	--	4.58^{g} ↓	2.13^{def} ↑	0.79^{ef} ↑	3.48^{cde} ↑	0.36 ↑	--	7.85^{b} ↑	4.46^{cd} ↑	5.43^{bc} ↑	11.92^{a} ↑	5.93 ↑	1.5	0.67	0.001	0.001	0.001
T BWG (g)	1668.3^{de}	1560.0^{f}	1717.8^{cd}	1690.0^{cd}	1748.9^{bc}	1677.0^{B}	1610.0^{ef}	1790.0^{b}	1715.0^{cd}	1735.0^{bcd}	1885.0^{a}	1747.0^{A}	22.82	10.20	0.001	0.02	0.001
RGR (%)	112.41^{b}	108.94^{c}	113.78^{b}	113.81^{b}	114.71^{b}	112.63	109.14^{c}	114.36^{b}	112.53^{b}	112.92^{b}	117.09^{a}	113.21	0.77	0.34	0.001	0.001	0.24
TFI(g)	5618.9	5053.3	5427.2	5135.0	5265.6	5300.0^{B}	5380.0	5626.1	5358.9	5248.9	5725.0	5467.8^{A}	130.8	58.51	0.04	0.062	0.05
FCR	3.37	3.24	3.16	3.04	3.01	3.16	3.34	3.14	3.12	3.03	3.04	3.13	0.061	0.027	0.001	0.51	0.44
PI	69.11^{c}	68.42^{c}	75.04^{b}	77.04^{b}	80.08^{ab}	73.84^{B}	68.58^{c}	78.37^{b}	76.48^{b}	79.61^{ab}	84.28^{a}	77.46^{A}	1.60	0.72	0.001	0.001	0.01

WBW: weaning body weight, MBW: marketing body weight, TBWG: total body weight gain, TFI: total feed intake, RGR: relative growth rate, FCR: feed conversion ratio and PI: performance index, g: grams, G: group, B: breed, ↑: increase, ↓: decrease.

MSE: mean slandered error, the mean values with different superscript letters within the same row differ significantly at ($P < 0.05$). (Small letters for groups and capital litters for breed) there is a decrease in manufacturing costs per kg of diet for the treated groups. The lowest cost was recorded in G 10% at 11.9 LE, followed by 12.17 LE for G7.5%. On the other hand, non-treated group G0% had the highest cost at 12.98 LE. This reduction in Kg cost resulted in an overall reduction in feed expenses for the treated groups and a decline in the cost per kg of weight gained. Furthermore, significant cost savings were evident when comparing the treated groups with the control group. The highest level of cost savings was observed in groups treated with bentonite with 10% DOC and GDP concentration in NZB (7.86 LE/kg), followed by the same treatment in VB by (7.65 LE/kg). Conversely, the lowest cost saving per kg of weight gained was 4.26LE/kg recorded in G7.5% in NZB. Consequently, these findings clearly demonstrate lower TVC and TC for the treated groups when compared to those of the control group.

Based on the return analysis presented in Table 7, it is evident that all of the experimental groups exhibited higher returns from body weight gain. The group with the highest return was G10%B in VB, which recorded a value of 160.23 LE. At the same time, G7.5% in NZB has the lowest return of 132.60 LE. Furthermore, all other treated groups demonstrated higher total revenue and net profit values. A significant rise in TR and NP was observed among the majority of the treated groups, as compared to the control group. The largest increase in G10%B found in VB led to an 11.67% rise in TR and a substantial 64.76% increase in NP. In contrast, when compared with the control group, the G7.5% treatment applied to NZB resulted in a decrease of 4.49% for TR but an elevation of 4.99% for NP values.

In regard to economic efficiency measures in table 8, the results of all treated groups showed significantly better outcomes compared to the control group. It is evident that both breeds exhibited higher economic efficiency in the bentonite-treated groups, with groups without bentonite coming next. Additionally, groups with a 10% incorporation rate outperformed those with a 7.5%. Furthermore, when considering breeds, VB demonstrated superior results compared to NZB.

Gene expression results

The effects of adding DOC and GDP at concentrations of 7.5% or 10%, as well as the optional addition of bentonite at a rate of 1%, on the gene expression profiles of growth and antioxidant markers are presented in table 9. Adding DOC and GDP at 7.5% revealed significant down-regulation of *IGF-I*, and *PGAM2* markers in comparison to the control group. However, DOC and GDP at 10% elicited non-significant differences in the mRNA levels for the investigated genes compared to the control group. The effects of adding DOC and GDP at concentrations of 7.5% or 10% with added bentonite at a rate of 1% evoked a significant up regulation of *SOD*, *CAT*, and *GPX* genes compared to the other investigated groups.

Histological examination results

Histological sections of the cecum and duodenum were assessed after staining with H&E. Upon examination, the cecum displayed a mucosa with varying lengths of folds, a submucosa, and a thick tunica muscularis Fig.1. Measurements taken revealed that the mucosal folds length (MFL) was considerably increased in the bentonite-treated groups, specifically with the highest observed length in the VB groups. Moreover, similar findings were observed for both mucosal thickness (MT) and tunica muscularis thickness (TMT), as depicted in Fig.4. The duodenum displayed a mucosal lining consisting of villi and crypts Fig.2. Measurements were taken to determine the length of the villi (VL) and depth of the crypts (CD), with their ratios subsequently calculated. Among all groups, it was observed that G10%B in VB exhibited the highest ratio, signifying an augmented surface area dedicated to absorbing nutrients Fig.4.

Histological examination of the H&E-stained liver sections showed hepatic lobules comprising a central vein encircled by hepatocyte cords Fig.3. The analysis focused on evaluating the area of the central vein (CV) and hepatic lobule in different groups. In comparison to the control groups, bentonite-treated groups from both breeds had similar CV areas, while untreated groups exhibited dilated central veins. Additionally, the central vein encircled by hepatocyte cords that showed different degrees of fatty change Fig.3. Furthermore, in the G10%B of VB, there was an observed increase in the size of hepatic lobules, indicative of enhanced blood flow within the liver Fig.4.

Behavioral observation results

Table 10 displays the data on how the inclusion of DOC and GDP (7.5% or 10%), with or without bentonite at a concentration of 1%, in rabbit diets

TABLE 6. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V- line rabbit breeds on costs parameters of production.

Item	New Zealand B						V- line B						MSE		P- Value		
LE/Rabbit	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G	B	G	B*G	B
Price of Kg feed	12.91^{a}	12.17^{b}	11.89^{c}	12.17^{b}	11.9^{c}	12.21	12.91^{a}	12.17^{b}	11.89^{c}	12.17^{b}	11.9^{c}	12.21	0.04	0.06	0.001	1	1
FEC	72.54^{a}	61.49^{c}	64.53^{bc}	62.49^{c}	62.66^{c}	٦٤.74^{B}	69.46^{a}	68.47^{ab}	63.72^{bc}	63.88^{bc}	68.13^{ab}	66.73^{A}	1.61	0.72	0.001	0.101	0.05
↓ of FEC	0^{c}	15.66^{a}	11.51^{a}	14.3 ^{c}0^{a}	14.07^{a}	11.21^{A}	0c	2.65^{bc}	9.40^{ab}	9.17^{ab}	3.13^{bc}	5.12^{B}	2.25	1.01	0.001	0.001	0.001
FEC/ Kg WG	43.46^{a}	39.41^{b}	37.58^{bc}	36.98^{c}	35.81^{c}	٣٨.65	43.11^{a}	38.26^{bc}	37.12^{bc}	36.85^{c}	36.14c	38.29	0.76	0.34	0.001	0.62	0.46
↓ in FEC/ Kg BWG	0^{c}	4.26^{b}	6.10^{ab}	6.70^{a}	7.86^{a}	5.03	0c	5.52^{ab}	6.66^{a}	6.94^{a}	7.65^{a}	5.49	0.76	0.34	0.001	0.52	0.34
TVC	152.54^{a}	141.49^{c}	144.53^{bc}	142.49^{c}	142.66^{c}	144.74^{B}	149.46^{a}	148.47^{ab}	143.72^{bc}	143.88^{bc}	148.13^{ab}	146.73^{A}	1.61	0.72	0.001	0.10	0.05
TFC	8	8	8	8	8	8	8	8	8	8	8	8	-----	----	-----	-----	----
TC	160.54^{a}	149.50^{c}	152.53^{b}c	150.50^{c}	150.66^{c}	152.74^{B}	157.46^{a}	156.47^{ab}	151.72^{bc}	151.88^{bc}	156.13^{ab}	154.73^{A}	1.61	0.72	0.001	0.10	0.05

MSE: mean slandered error, the mean values with different superscript letters within the same row differ significantly at ($P < 0.05$). (Small letters for groups and capital litters for breed) TC: Total costs, TVC: Total variable costs, TFC: Total fixed costs, Price of weaned rabbit: 80 LE, (all prices related to time of experiment). L.E: Egyptian Pound, B: breed, G: group, FEC: feed cost, ↓ of FEC: decrease of feed cost, FEC/Kg WG: feed cost/ kilogram weight gained, ↓in FEC/Kg BWG: Savings in feed costs/kilogram of body weight gained.

TABLE 7. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V- line rabbit breeds on return parameters from production.

Item	New Zealand breed						V- line breed						MSE		P- Value		
LE/Rabbit	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G0%	G7.5%	G10%	G7.5%B	G10 %B	B Overall mean	G	B	G	B*G	B
Return of weight gain	141.81^{de}	132.60^{f}	146.01^{cd}	143.65^{cd}	148.65^{bc}	142.55^{B}	136.85^{ef}	152.15^{b}	145.78^{cd}	147.48^{bcd}	160.23^{a}	148.49^{A}	1.94	0.87	0.001	0.02	0.001
TR	201.06^{de}	192.04^{f}	205.26^{cd}	202.62^{cde}	207.91^{bc}	201.78^{B}	197.80^{ef}	213.01^{b}	206.44^{cd}	208.33^{bc}	220.89^{a}	209.29^{A}	2.07	0.92	0.001	0.001	0.001
Percent of change in TR%	--	- 4.49^{g} ↓	2.09^{def} ↑	0.77^{ef} ↑	3.41^{cde} ↑	0.36^{B}	--	7.68^{b} ↑	4.37^{cd} ↑	5.32^{bc} ↑	11.67^{a} ↑	5.8^{A}	1.04	0.46	0.001	0.001	0.001
NP	40.52^{c}	42.54^{c}	52.73^{b}	52.12^{b}	57.25^{b}	٤٩.03^{B}	40.34^{c}	56.54^{b}	54.72^{b}	56.45^{b}	64.76^{a}	54.56^{A}	1.78	0.80	0.001	0.001	0.001
Percent of change in NP%	--	4.99^{c} ↑	30.14^{b} ↑	28.64^{b} ↑	41.28^{b} ↑	21.01^{B}	--	40.15^{b} ↑	35.66^{b} ↑	39.93^{b} ↑	60.54^{a} ↑	35.26^{A}	4.41	1.97	0.001	0.001	0.001

MSE: mean slandered error, the mean values with different superscript letters within the same row differ significantly at (P < 0.05). (Small letters for groups and capital litters for breed), TR: Total returns, NP: Net profit. Price of kg rabbit at marketing time: 85 LE (all prices related to time of experiment). L.E: Egyptian Pound, G: group, B: breed, ↑: increase, ↓: decrease.

TABLE 8. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V- line rabbit breeds on economic efficiency measures.

Item	New Zealand B						V- line B						MSE		P- Value		
LE/Rabbit	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G0%	G7.5%	G10%	G7.5%B	G10 %B	B Overall mean	G	B	G	B*G	B
TR/TC	1.25^{c}	1.29^{c}	1.35^{b}	1.35^{b}	1.38^{ab}	1.32^{B}	1.26^{c}	1.36^{b}	1.36^{b}	1.37^{b}	1.42^{a}	1.35^{A}	0.01	0.01	0.001	0.02	0.001
NP/TC	0.25^{c}	0.29^{c}	0.35^{b}	0.35^{b}	0.38^{ab}	0.32^{B}	0.26^{c}	0.36^{b}	0.36^{b}	0.37^{b}	0.42^{a}	0.35^{A}	0.01	0.01	0.001	0.02	0.001
EE %	0.96^{d}	1.16^{c}	1.27^{abc}	1.30^{ab}	1.39^{a}	1.22	0.98^{d}	1.23^{bc}	1.30^{ab}	1.31^{ab}	1.36^{a}	1.24	0.04	0.02	0.001	0.61	0.45

MSE: mean slandered error, the mean values with different superscript letters within the same row differ significantly at (P < 0.05). (Small letters for groups and capital litters for breed), TC: Total costs, TR: Total returns, NP: Net profit. L.E: Egyptian Pound, G: group, B: breed, EE: economic efficiency.

TABLE 9. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V- line rabbit breeds on gene expression.

Item	New Zealand B						V-line B						MSE		*P*-value		
Gene	G0%	G7.5%	G10%	G7.5%B	G10 %B	B overall mean	G0%	G7.5%	G10%	G7.5%B	G10%B	B overall mean	G	B	G	B*G	B
IGF-I	1^{a}	0.51^{b}	0.94^{a}	0.42^{c}	1^{a}	0.77^{B}	1^{a}	0.68^{b}	1^{a}	0.61^{c}	1^{a}	0.85^{A}	0.16	0.14	0.001	0.001	0.001
PGAM2	1^{a}	0.36^{c}	1^{a}	0.46^{b}	0.85^{a}	0.73^{B}	1^{a}	0.57^{bc}	1^{a}	0.51^{c}	0.91^{a}	0.79^{A}	0.17	0.13	0.001	0.001	0.001
SOD	1^{b}	0.62^{c}	0.84^{b}	1.51^{a}	1.50^{a}	1.09^{A}	1^{c}	0.71^{d}	0.96^{c}	1.61^{b}	1.74^{a}	1.01^{A}	0.11	0.09	0.001	0.34	0.28
CAT	1^{b}	0.42^{c}	0.92^{b}	1.36^{a}	1.41^{a}	1.02^{A}	1^{ab}	0.63^{c}	0.98^{b}	1.48^{a}	1.48^{a}	1.11^{A}	0.18	0.11	0.001	0.16	0.12
GPX	1^{b}	0.54^{c}	1^{b}	1. 47^{a}	1.51^{a}	1.10^{A}	1^{c}	0.71^{d}	1^{c}	1. 53^{a}	1.43^{b}	1.13^{A}	0.15	0.13	0.001	0.26	0.24

MSE: mean slandered error the mean values with different superscript letters within the same row differ significantly at ($P < 0.05$), (Small letters for groups and capital litters for breed), G: group, B: breed. (Small letters for groups and capital litters for breed), IGF-I= Insulin-like growth factor-1; PGAM2= Phosphoglycerate mutase 2; SOD= Super oxide dismutase; CAT= Catalase; GPX= Glutathione peroxidase; and ß. actin= Beta actin.

affects the percentages of exhibited behaviors in NZB and VB from week 7 to week 12 of age. Concerning the impact of breed, it was found that the frequency of most observed behaviors was higher in VB compared to NZB. However, lying down and sitting behaviors were actually more prevalent in NZB.

Among the various groups of both breeds, it was observed that G10%B in the VB breed exhibited superior results in various behaviors such as feeding (26.75), drinking (13.62), gnawing (20.25), and sniffing (14.37). Similarly, G10% in VB showed higher instances of standing (8.5), stretching (4.75), and gnawing (6.5) behaviors, whereas NZB with a G7.5% showed an increased occurrence of lying down (16.75) and sitting (14.62) behaviors. In terms of feeding and drinking behaviors, the lowest results were observed in NZB for G7.5% with a score of 18.37 and 7.37, respectively. Similarly, NZB for G0% also exhibited the lowest frequencies of standing (5.75), stretching (1.37), grooming (12.5), and rearing up (2.87). Additionally, gnawing behavior was less frequent in G7.5 %B from the NZB, with a score of 4.37. In contrast, VB showed the least instances of sniffing (8) and lying down (7:25) for groups G0% and G10%B.

Discussion

The continuous rise in the global population indicates that growth is unlikely to cease. Ensuring accessible and affordable food resources has become a top priority for nutrition specialists and policymakers around the world. As a result, the need for alternative animal feed options has become increasingly pressing. On the other hand, the cost of commercially available pellet diets for rabbits has risen significantly due to the increasing prices of concentrates. Moreover, after the weaning stage, feeding contributes to approximately 40% of the overall expenses [50].

Therefore, there is a need to find ways to minimize expenses while ensuring continued production efficiency. The rate of inclusion of alternative feed resources is primarily influenced by the levels of crude fiber and crude protein, ensuring that they do not surpass the allowable limits for each stage of growth. However, the utilization of unconventional diets may have an impact on the function of the caecal microflora of rabbit [51]

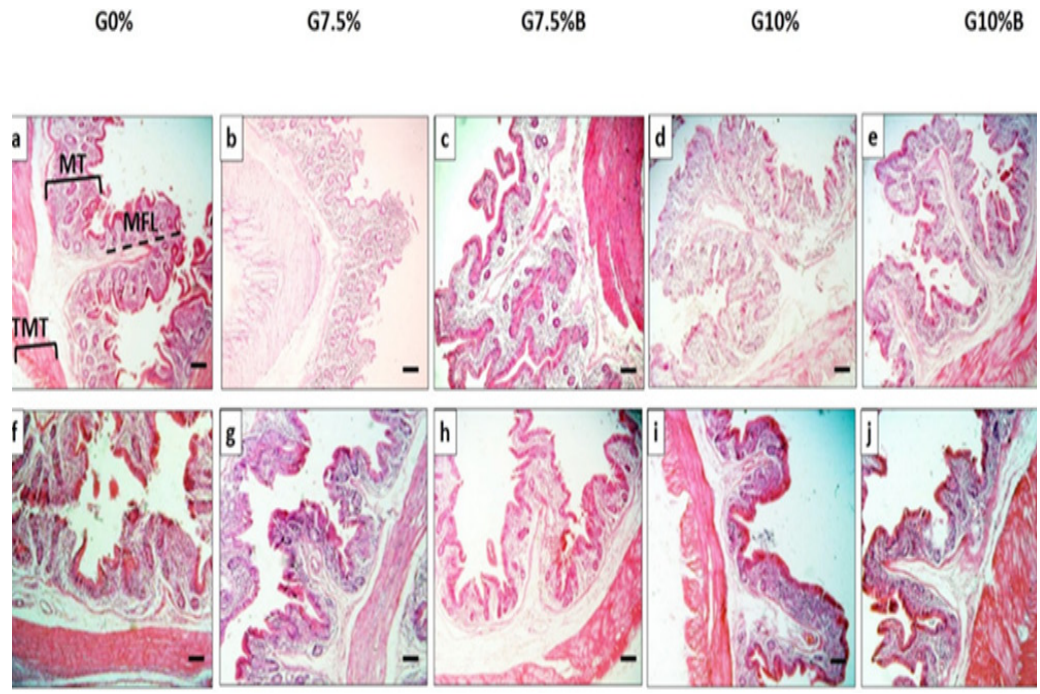

Fig.1. Photomicrograph of H&E-stained slide of the cecum of the two breeds showing NZB (a-e) and VB (f-j), the cecum appeared with mucosal folds of different length. MFL=mucosal fold length, MT=mucosa thickness, TMT= tunica muscularis thickness. Scale bars=100µm.

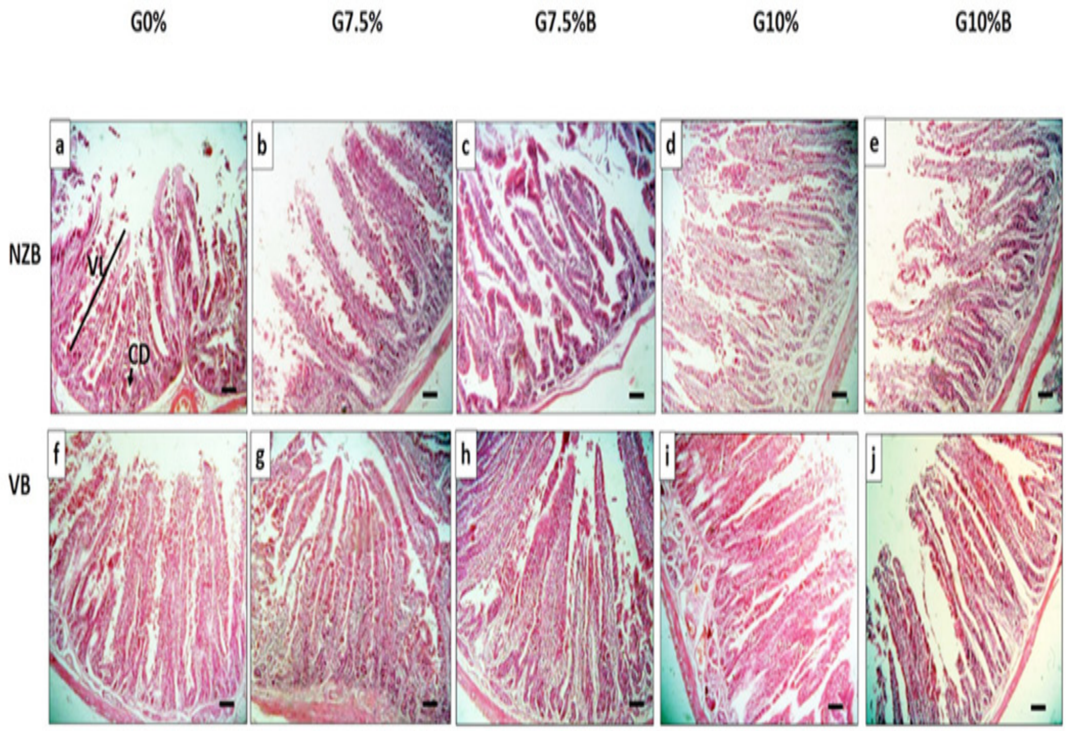

Fig.2. Photomicrograph of H&E-stained slide of duodenum of the two breeds showing NZB (a-e) and VB (f-j), the duodenal mucosa showed various villi and crypts. VL=villi length, CD=crypt depth. Scale bars=100µm.

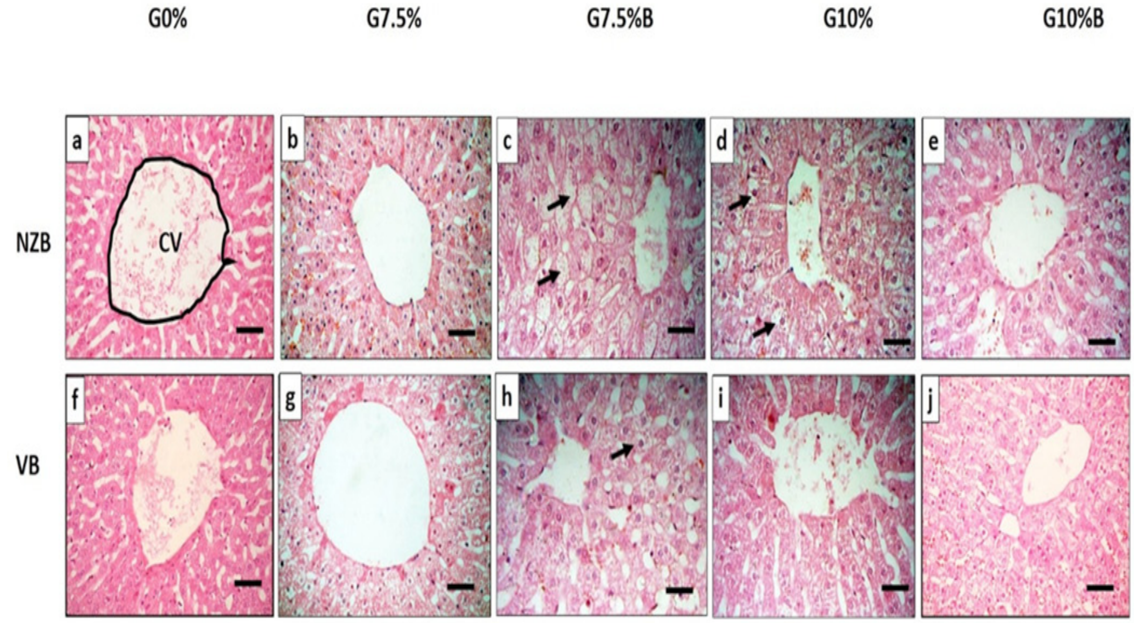

Fig. 3. Photomicrograph of H&E-stained slide of liver of the two breeds showing NZB (a-e) and VB (f-j), liver with cords of hepatocytes surrounding a central vein encircled by hepatocyte cords. Interestingly, hepatocytes of G7.5%, G7.5% B, G10% and G10%B groups showed different degree of fatty change, CV=central vein. Scale bars=50µm.

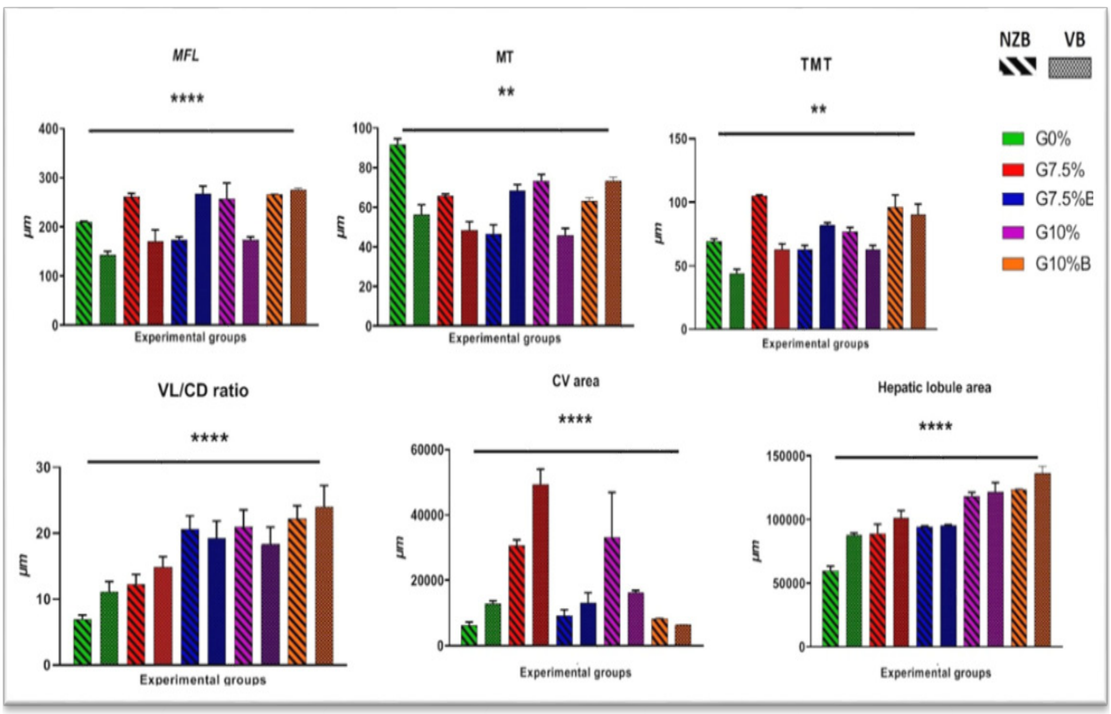

Fig.4. Graphs of different parameters (MFL=mucosal fold length, MT=mucosa thickness, TMT= tunica muscularis thickness, VL/CD=villi length/crypt depth ratio, CV area=central vein area, hepatic lobule area. Significant results are represented by asterisks ($p \leq 0.01$=, $p \leq 0.0001$=****).**

TABLE 10. Effect of including dried olive cake and ground date palm with or without bentonite in diet of New Zealand and V-line rabbit breeds on behavioral expression.

Item	New Zealand B						V- line B						MSE		P- Value		
Behavioral pattern	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G0%	G7.5%	G10%	G7.5%B	G10%B	B Overall mean	G	B	G	B*G	B
Feeding	24.25^{abc}	18.37^{c}	23.50^{abc}	18.87^{c}	20.87^{abc}	21.18	22.50^{abc}	25.62^{ab}	21.00^{abc}	20.06^{bc}	26.75^{a}	23.19	1.98	0.88	0.07	0.18	0.12
Drinking	10.75^{ab}	7.37^{b}	9.87^{ab}	8.12^{ab}	9.37^{ab}	9.10	11.25^{ab}	13.25^{a}	8.87^{ab}	8.50^{ab}	13.62^{a}	11.10	1.65	0.74	0.15	0.19	0.07
Standing	5.75	6.25	6.87	8.75	8.37	7.40	8.25	7.00	8.50	7.87	6.00	7.52	1.27	0.57	0.63	0.05	0.69
Stretching	1.37	2.50	1.62	1.87	3.12	2.10	1.62	2.50	4.75	3.12	3.12	3.02	1.10	0.49	0.57	0.08	0.19
Lying down	16.00^{a}	16.75^{a}	11.62^{bc}	15.50^{a}	9.00^{c}	14.57^{A}	15.00^{ab}	8.25^{c}	9.36^{c}	10.50^{c}	7.25^{c}	10.07^{B}	1.43	0.64	0.0001	0.01	0.0001
Sitting	13.12^{ab}	14.62^{a}	8.62^{cde}	12.13^{abc}	9.00^{cd}	10.55^{A}	9.89^{bcd}	5.89^{de}	6.18^{de}	7.50^{de}	4.63^{e}	6.81^{B}	1.29	0.58	0.0001	0.0001	0.0001
Grooming	12.50^{d}	13.00^{d}	17.37^{abc}	15.75^{bcd}	19.75^{a}	15.67^{B}	13.00^{d}	15.50^{cd}	19.00^{ab}	17.75^{abc}	20.25^{a}	17.10^{A}	1.07	0.48	0.0001	0.0001	0.043
Sniffing	8.37^{d}	10.00^{bcd}	12.87^{abc}	9.50^{cd}	13.75^{ab}	10.90	8.00^{d}	10.50^{abcd}	11.87^{abcd}	11.25^{abcd}	14.37^{a}	11.20	1.27	0.57	0.01	0.03	0.71
Rearing up	2.87	4.37	5.00	5.62	4.37	4.45	4.37	5.37	4.12	6.87	3.12	4.77	1.47	0.66	0.75	0.28	0.73
Gnawing	5.00	6.25	3.12	4.37	2.75	4.30	6.12	6.12	6.50	6.00	3.25	5.60	1.28	0.57	0.27	0.21	0.12

MSE: mean slandered error, the mean values with different superscript letters within the same row differ significantly at ($P < 0.05$). (Small letters for groups and capital litters for breed) G: group, B: breed.

. One highly valuable unconventional feed resource is olive cake meal. It stands out for two significant reasons in particular. Firstly, it contains a notable amount of residual oil (6.8%), which can serve as an additional energy source in animal diets. Secondly, the unique composition of unsaturated fatty acids in olive cake, including high levels of oleic acid (62.4%), linoleic acid (18.2%), linolenic acid (1.1%), and palmitoleic acid (2.7%), may potentially affect how fatty acids are stored in different body compartments over the lifespan of animals and consequently impact meat quality [52]

. Similarly, date palm can be incorporated into animal diets as an energy source for both ruminants and rabbits [53]

. Ground date palm have high palatability and digestibility, making them valuable feed ingredients that can potentially replace a portion of the concentrates in animals' ration to provide energy [54].

To mitigate the negative impacts on cecal microflora activities, certain feed additives, like bentonite, can be utilized. Clay is a natural feed additive commonly incorporated into rabbit and other animal diets. When included in the animals' diet, clay effectively binds to specific compounds that are detrimental to intestinal health, ultimately reducing their absorption through the intestinal mucosa and preventing any toxic effects they may have on the animal. Several studies have demonstrated that the use of clay, such as bentonite, can effectively detoxify poisons [55], aflatoxin [56], enterotoxins, and plant metabolites such as alkaloids and tannins [57] from animal diets.

In relation to the impact of incorporating DOC and GDP, with or without bentonite, on the growth performance of rabbits, our study revealed that both breeds showed significantly better results compared to the control group. These findings align with previous studies by [58, 59], which reported enhanced productive performance (such as final body weight, BWG, and RGR) in NZB rabbits when fed a diet containing 6.25% olive cake or date stone. Similarly, [60] reported similar results when rabbits were fed diets containing 30% olive cake meal along with a 1% bentonite addition. Additionally, our findings were consistent with those of [61] who found that incorporating 10% of date waste meal into the diet led to better final BW compared to higher percentages such as 30% and 40%. Furthermore, [11, 62] also supported these trends by noting enhancement in finishing BW among groups exposed to diets containing olive cake pulp, or DOC. According to [63], the inclusion of dates in animal diets has been reported to have positive effects on BWG and fattening. This may be attributed to the presence of growth-promoting compounds in dates that enhance energy and protein utilization, as suggested by [64, 65] resulting in a better synchrony between energy release and protein degradation, leading to increased microbial protein yield. In contrast, our research contradicts prior studies by [62, 66, 67, 68], who found no significant variations in the live BW and BWG of rabbits fed diets containing olive by-products.

Regarding feed amount, the highest consumption was observed in group G10%B in VB. Interestingly, this group demonstrated the best FCR and had the highest PI. These findings align with previous studies by [69] who found that rabbits fed on a diet containing 25% discarded dates had the highest feed intake and best FCR. Similarly, [61] reported that feeding rabbits with 10% date waste meal resulted in superior FCR performance. Furthermore, [70] as well as [71] noted increased feed intake when using date by-products; this might be attributed to their favorable palatability properties, supporting earlier suggestions by [67]. This is also in accordance with previous findings by [24, 72] that showed improved FCR in rabbits fed diets supplemented with 10% olive pulp or olive cake. Similar results were reported by [62, 73, 74], where higher concentrations of olive cake at 22, 20, and 25%, respectively, led to significant improvements in FCR for rabbits. In addition, our findings on PI aligned with the research conducted by [60], which reported that the highest PI was observed in a group supplemented with 10% date waste meal, as compared to both the control group and those receiving higher concentrations of supplementation.

In relation to the impact of breed type, our research reveals that the VB outperformed the NZB in response to dietary treatment, exhibiting superior BWG, marketing weight, RGR, FCR, and PI. This advantageous performance can likely be attributed to the known genetic superiority of VB. These findings are consistent with those reported by [75, 76], who found similar results regarding FCR, body weights, and BWG.

In reference to feed expenses, our experiment indicates that the cost per kg of treated diets is significantly lower compared to commercially available control diet. This cost reduction can be attributed to the utilization of low-priced non-conventional feed resources such as DOC and GDP. These results support the conclusions made by [77], who found a decline in feed costs through the inclusion of date stone meal due to its low price. Similarly, [27, 39] also reported a decrease in Kg feed cost through the inclusion of date pits. Additionally, [34] demonstrated that incorporating corn cobs reduced the cost/kg of manufactured diet. This reduction in kg cost consequently results in a significant reduction in overall feed expenses, with a high percentage of cost reduction for the treated groups compared to the control group. Several studies have supported these findings, including research on incorporating olive by-products into the diet [11, 67, 78, 79].

Similarly, lower feed costs have been reported when utilizing date by-products and waste materials, as demonstrated by [60, 80, 81] .

The decrease in feed cost led to a reduction in the costs per kg of weight gained, with greater cost savings observed in the treated groups compared to the control group. This finding is consistent with previous studies by [58, 67, 78, 82], who also found that incorporating alternative feed ingredients such as olive cake, olive pulp, DOC, date stone and wasted dates resulted in reduced feeding costs per kg of weight gained. Conversely, [83] reported no significant effect on meat costs when using date pits. The cost savings was evident among the groups that received a 1% bentonite treatment, as compared to those without bentonite. This may be due to the ability of bentonite to enhance nutrient digestibility and utilization in diets, which can impact hormone activities such as growth hormones and thyroid hormones [84], leading to a decrease in feed intake and improved weight gain [85].

The expenditure on animal feed comprises more than 70% of the total production expenses [86, 87].

Consequently, reducing these costs would lead to a decrease in overall production expenses, as in our experiment, decreased feed costs for treated groups resulted in lower overall costs compared to the control groups. This is consistent with the findings of [11, 60] who observed a reduction in TC when alternative feed sources such as discarded dates and DOC were used.

The return from weight gain was higher in treated groups when compared to control groups, resulting in a higher TR for these groups. Higher TR with lower TC result in higher NP in treated groups. This aligns with previous findings by [11, 60, 88], who also observed higher TR and NP when applying similar studies with cull dates and DOC.

In the case of the group that received 7.5% treatment from both DOC and GDP without B in NZB, there was an observed reduction in overall costs compared to the control group as a result of decreased feed costs. However, it is important to note that this treatment resulted in a lower body weight gain return when outcomes compared to the control group. In contrast, the same treatment in VB yields superior outcomes compared to the control group. This could be explained by a variety of genetic [89, 90] and hematological traits [91] that influence an animal's ability to successfully gain weight. Nevertheless, the reduction in its expenditures on feed with improved FCR was significant enough to generate a higher profit percent compared to the control group, as reported by [60], who recorded improved FCR when incorporating olive cake meal in to their diet. In contrast, our analysis when comparing this particular group with another group of the same breed (NZB) receiving the same treatment (7.5%) along with bentonite as an additional supplement revealed a significant enhancement in terms of TR and NP percent change when compared to the control group. This improvement can potentially be attributed to including pelleted feed containing bentonite, which may have contributed to improved growth and increased activity of the naturally occurring microflora within the gastrointestinal tract lining [92].

In terms of economic efficiency, feeding growing rabbits with treated diets containing DOC and GDP, even without bentonite, resulted in improved economic efficiency values compared to the control groups. This can be attributed to reduced feed costs in these groups along with a higher return from weight gain. These findings are consistent with previous studies that have demonstrated the positive impact of incorporating date byproducts, such as cull dates [93, 94], discarded dates [82], and date waste meal [95], in improving economic efficiency when included in animal diets. Similarly, this is consistent with previous studies conducted on olive byproducts such as olive pulp [67] and olive cake [11, 24, 74] that also demonstrated that incorporating these byproducts into animal diets can enhance overall economic efficiency.

Regarding breed type, the results of the current study indicate that NZB rabbits exhibited decreased feed costs, TVC, and TC. Conversely, VB rabbits displayed higher TR and NP. These findings are consistent with previous research conducted by [76]. Similarly, these findings align with studies by [75, 96], which recommend that VB surpasses NZB.

In this context, *IGF-I* and *PGAM2* markers were significantly down-regulated when DOC and GDP were added at a 7.5% ratio to the control group. In contrast to the control group, DOC and GDP at 10% produced non-significant variations in the mRNA levels for the genes under investigation. In comparison to the control group, the addition of DOC and GDP at concentrations of 7.5% or 10% resulted in a notable up-regulation of *SOD*, *CAT*, and *GPX* genes observed in the groups that added bentonite. Interestingly, V-line rabbits exhibited a markedly higher mRNA level of growth markers than NZB ones. To our knowledge, this is the first study that elucidates the effect of adding DOC and GDP on the growth and antioxidant properties of rabbits from a gene expression perspective. According to our results, adding DOC and GDP at 10% evoked better results than 7.5%. The non-significant differences in the gene expression pattern of growth markers may be attributed to the low content of crude protein (CP) and high content of crude fiber (CP) exhibited by DOC and GDP [91, 97]. The improved antioxidant status in rabbits fed DOC and GDP at 7.5% or 10% alongside bentonite is due to the fact that bentonite possesses antioxidant properties that could increase the expression of antioxidant markers [98, 99].

The duodenum is the initial part of the small intestine and is responsible for the majority of digestion. Various enzymes are produced by and into the small intestine to facilitate food breakdown. The height of villi and the depth of crypts are crucial determinants of gut health. Increased length of villi leads to a larger surface area, facilitating improved nutrient absorption, which enhances the overall growth performance and well-being of animals [100]. This enhanced villi length is associated with more efficient expression of brush border enzymes and enhanced nutrient transportation [101]

. Our findings demonstrated a higher VL/CD ratio in the treated groups. This can be attributed to the inclusion of GDP in their diet, which is known for its rich content of essential phytochemicals such as carotenoids, phenolics, and flavonoids [102].

Furthermore, previous studies by [103, 104] have highlighted how phytogenic feed additives can improve gut morphology, enhance digestive functions, and ultimately contribute to improved animal growth. Apart from its antioxidant effect, GDP has been shown to mitigate the impact of peroxides and free radicals, which are present in high concentrations in active organs like the intestinal epithelium. These findings are consistent with the research conducted by [105], who observed longer and wider duodenal villi in birds that were fed diets containing date palm pollen. Likewise, [106] reported that supplementation with bentonite enhanced intestinal morphology in rabbits, indicating improved capacity for nutrient absorption. Furthermore, a study by [107] showed that rabbits fed diets supplemented with DOC exhibited improved intestinal morphology.

The cecum plays a critical role in the rabbit's digestive system. It serves as a habitat for numerous microorganisms and bacteria, which ferment, break down, and metabolize the substances entering the cecum. Based on the histological examination of the cecum, it is proposed that dietary bentonite addition may potentially alter the cecal lining structure, leading to improvements in nutrient absorption and growth performance. This finding supports previous research conducted by [108], which showed positive outcomes related to microbial fermentation, metabolic processes, and animal health when rabbits were fed diets containing bentonite along with multi-enzymes.

Regarding the liver morphology, the development of fatty alterations in hepatocytes can be linked to a high fructose content in GDP [109], and an elevated fat content in DOC [110]. These findings align with [111, 112] conclusion that both fructose and high fat levels contribute to the progression of fatty liver. Additionally, [113] observed time-dependent changes in rabbit livers fed a high-fat diet, displaying characteristics of fatty liver compared to control groups over increasing exposure periods of 3,6,9 weeks. Furthermore, [63], discovered that there was a notable increase in fat infiltration in hepatocytes within groups fed diets containing 10% and 20% DOC. They suggested that the elevated fat intake from olive oil content in these diet mixtures could be accountable for the fat infiltration observed in various tissues. Additionally, [114], highlighted that the degree of histopathological changes in the liver of fish fed diets containing DOC intensified with higher levels of DOC inclusion. On the contrary, [107], observed no morphological alterations in the hepatic parenchyma of broilers across all groups that incorporated dried olive pulp into their diets.

In our research, the G10%B hepatic alterations improved, possibly due to success of the suggested combination percentage in which olive cake is known for its hepato-protective properties. This was noted by [115], who recommended supplementing with olive oil to reduce fat accumulation in the liver by restoring abnormal lipid metabolism. Additionally, high levels of phenolics and flavonoids in GDP offer hepato-protection [116]. Furthermore, [106] found that including bentonite in the diet had a significant protective effect on liver histopathological changes.

Elevated levels of self-care (comfort) and exploration behaviors are often regarded as a sign of enhanced welfare in rabbits. The findings of our study revealed that the rabbits in groups fed treated diets exhibited higher levels of comfort behavior, such as grooming and exploratory activities like sniffing, compared to the control groups. This observation suggests that these rabbits may experience improved welfare and better health status. This can potentially be attributed to the antioxidant capacity of DOC, as suggested by [97] who found that incorporating DOC in the diet provides higher protection against oxidative damage and improves health condition. Additionally, GDP has strong antioxidant activity due to its content of vitamins (B1, B2, B12, A, E, and C), amino acids, minerals (selenium, zinc, copper, and iron), flavonoids, carotenoids, tannins, and volatile unsaturated fatty acids, according to [117, 118]. Moreover, supplementing rabbits' diet with bentonite has shown significant benefits in improving health status, growth performance, and reducing mortality rate [119]. Stereotypic behaviors, as defined by researchers [120], are repetitive actions that lack an apparent purpose. These behaviors can be indicators of poor welfare when their frequency increases. Rabbits have been reported to exhibit various stereotypic behaviors including excessive grooming, sham chewing (chewing without anything in their mouths), bar biting, licking parts of the cage, digging against the cage, biting water nipples, sliding their noses against bars, head pressing, and running repeatedly in a specific pattern [46, 121]. The results of our study revealed a decrease in sedentary behaviors, such as sitting or lying down, and stereotypical actions like gnawing within the treated groups. It could be interpreted as these animals reallocating their time to engage more actively in other advantageous behaviors, such as engaging in regular grooming sessions and further exploring their surroundings.

The results of our investigation suggest that VB rabbits displayed elevated levels of feeding and self-grooming behaviors, while exhibiting reduced amounts of inactivity compared to NZB rabbits. This finding is consistent with prior studies conducted by [122], highlighting the significant impact of genotype on behavioral manifestations. Furthermore, [123] stated how rabbit genotype influences distinct behaviors such as social interactions and maintenance within various rabbit breeds.

Conclusion:

Based on our findings, it can be concluded that the incorporation of both DOC and GDP resulted in improved growth performance for both rabbit breeds. Specifically, concentrations of 7.5% and 10% of both DOC and GDP in the VB breed yielded superior economic efficiency outcomes, better growth performance, as well as genetic, histological, and behavioral results compared to the control group. Markedly, the addition of bentonite at concentration of 1% to the diet further enhanced these positive effects. On contrary, NZB breed showed unfavorable outcomes with a 7.5% concentration that only slightly improved with a 10% concentration. However, adding bentonite at 1% concentration remains essential for achieving optimal economic, productive, genetic, histological, and behavioral results in both concentrations of 7.5% and 10% for DOC and GDP in NZB. After comparing the responsiveness of two breeds in this study, it can be concluded that the VB breed outperformed the NZB breed in terms of net profits, economic efficiency, and growth performance, genetic, histological, and behavioral measures.

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Conflict of interest

The authors assert that they have no conflicts of interest.

Authors' contributions

Noha M. Wahed designed this study, wrote the manuscript and analyzed the economic efficiency measures with Samer S. Ibrahim. Shimaa A. Sakr performed a growth performance analysis. Ahmed I. Ateya performed a genetic analysis of growth-associated and antioxidant genes. Zeinab Shouman performed histological analysis. Asmaa Saad evaluated the behavioral measures. All authors also read, revised, and approved the article for publication.

Availability of data

Data are available upon request from the corresponding author.

Ethics approval statement

This study was approved by the animals' ethical approval committee of Mansoura University, (MU-ACUC), No. (VM.R.23.02.54).

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التقييم الاقتصادى والإنتاجى والسلوكى لاستخدام تفل الزيتون المجفف ونخيل التمر المطحون فى علائق نمو الأرانب: تأثير التعبير النسيجى والجينى

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كان الهدف من هذه الدراسة هو تقييم تأثير دمج نسب مختلفة (٧,٥٪ و ١٠٪) من تفل لزيتون المجفف وتمر النخيل المطحون مع أو بدون إضافة البنتونيت (١٪) على الكفاءة الاقتصادية ، أداء النمو ، التعبير الجيني ، النتائج النسيجية المرضية ، والاستجابات السلوكية في سلالاتي الأرانب النيوزيلندي والفيلاين. تم استخدام مائه أرنب من عمر الفطام ، تتراوح أعمارهم بين ٥ أسابيع ويبلغ متوسط وزن الجسم ٦٥٩±٢٠,٩٣ جم . تم إيواؤهم بشكل فردي وتوزيعهم عشوائيا إلى خمس مجموعات لكل سلالة تحتوي على عشرة أرانب لكل منها. تم تصنيف المجموعات بناء على المعاملات المستخدمة . تشير النتائج التي توصلنا إليها إلى أن إدراج البنتونيت بتركيز ١٪ مع كل من تفل الزيتون المجفف و تمر النخيل المطحون أدى إلى تعزيز أداء النمو لكلا السلالتين. كان هذا واضحا من خلال ارتفاع أوزان الجسم التسويقية ، فضلا عن التحسينات الكبيرة في نسبة تحويل العلف. من منظور اقتصادي ، هناك انخفاض للتكاليف لكل كيلوغرام من العلف ، وتكاليف الأعلاف الإجمالية مما أدى إلي انخفاض إجمالي تكاليف الإنتاج. كشفت النتائج عن زيادة كبيرة في إجمالي العائد وصافي الربح ومقاييس الكفاءة الاقتصادية التي لوحظت على وجه التحديد ضمن مجموعة (B ٪G١٠) لكلا السلالتين. كشفت نتائج التعبير الجيني عن اختلاف غير معنوي في العلامات المرتبطة بالنمو أما فيما يتعلق بعلامات مضادات الأكسدة، فإن تغذية المجموعات المضاف إلي العلف الخاص بها البنتونيت ١٪ زادت من مظهر التعبير عن علامات مضادات الأكسدة. تم التحقق من هذه النتائج أيضا من خلال التغيرات الملحوظة في السمات النسيجية المختلفة بما في ذلك زيادة طول الطيات المخاطية ، الزغابات ، عمق القبو في الأمعاء ومنطقة الفصيص الكبدي الموسع في الكبد. وبالمثل ، أظهرت المجموعات التي استهلكت الوجبات الغذائية المعالجة سلوكا معززا من خلال زيادة أنشطة التغذية والاستمالة ، فضلا عن تقليل فترات عدم النشاط. أبرزت نتائجنا أيضا أن سلالة الفيلاين أظهرت نتائج أفضل مقارنة بسلالة نيوزيلندا عند إعطائها نفس الاضاقات والتعديلات الغذائية المتطابقة. في الختام ، فإن إدراج تفل الزيتون المجفف و تمر النخيل المطحون معا بمعدل ٧,٥٪ أو ١٠٪ لكل منهما جنبا إلى جنب مع ١٪ بنتونيت إضافي في النظام الغذائي للأرانب النامية ، لديه القدرة على تحقيق نتائج فعالة من حيث الإنتاج والتكلفة والعائد.