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Research Article

Impact of Partial Replacement of Clover by Anise, Basil, Mint and Fennel Remnants in Rabbits Diet on Blood Constituents

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Abstract

Background and Objective: The clover plant (alfalfa) is considered the primary fiber source in the rabbit diet in Mediterranean Sea countries like Egypt, so researchers are always trying to find alternatives and aromatic and medicinal plant remnants could be one of them. So, this study was designed to determine the effects of some aromatic and medicinal plant remnants on New Zealand white rabbits' blood hematological and biochemical aspects. **Materials and Methods:** A total of 108 weaned white New Zealand rabbits at five weeks of age were used to consider the effect of using remnants of mint, fennel, basil and anise with or without probiotics to replace 50% from alfalfa hay in rabbits' diets. Four remnants were obtained after etheric oil distillation and were incorporated in rabbit diets at level 17.5% without probiotics and with probiotics (replacement 50% of alfalfa hay). Rabbits were randomly assigned into nine experimental groups; the experimental period lasted eight weeks. Rabbit blood hematological and blood biochemical were analyzed. **Results:** The highest values of RBC 6.03 μ L, HCT 37.13%, WBC 12.70 μ L and lymph percentage were found in the basil+probiotics group. In contrast, the highest value of hemoglobin (HGB 10.50 g/dL), MCV 64.13 fl, MCH 23.27pg, MCHC 36.40 g/dL, PLT 463 μ L, urea 50.33 mg/dL and creatinine 1.30 mg/dL were found in anise+probiotic group. In contrast, RDW-CV 33.17%, Mid 13.17 μ L, granulocytes (Gran 7.13 μ L) and PDW 16.73 in the mint group. Furthermore, RDW-SD (34.40 fl) and procalcitonin (PCT 0.35%) were found in the control group and the highest values ALT 142 IU/L and AST 77.33 IU/L were found in the fennel group. The highest albumin value (3.10 g/dL) was found in the anise group and the highest total protein (TP 5.23 g/dL) was found in the mint+probiotic group. **Conclusion:** The results proved that using these medicinal plant remnants and probiotics as substitutes for half the amount of alfalfa used in the diet of New Zealand white rabbits did not have a negative effect and improved their health condition.

Key words: Rabbits, anise, basil, mint, fennel, remnants, blood, probiotic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been effectively used in healthcare treatment to achieve significant increases in health and animals' productivity and the use of traditional medicinal plants is cheaper, easier and more sustainable compared to synthetic medicines and pharmaceuticals¹. Furthermore, nutrition is one of the most critical factors for maintaining animal health^{2,3} and organic products in organic production systems may lead to organic life⁴⁻⁶. Using hay from some medicinal plants in rations improves digestibility parameters and nutritional values compared to alfalfa hay. Replacing alfalfa straw with any medicinal plant wastes increased nitrogen balance and biological values. Differences were significant ($p < 0.05$)⁷.

Abdelsalam and Fathi⁸ noted that many studies have found that replacing antibiotics in rabbit feed with natural plants or feed additives increases productivity and improves immune capacity, especially under heat-stress conditions. On the other hand, much research has focused on the elucidation of the biochemical compositions and physiological functions of various feed additives such as probiotics, prebiotics, organic acids and plant extracts for the beneficial effects of herbs and spices; they can be added to feed as dried plants or parts of plants and as extracts, maintaining the health of farm animals is also essential to obtain healthy animal products⁹.

Plant extracts and herbal-derived materials are legal and safe alternatives. Research has confirmed the effectiveness of herbal materials as natural growth stimulants and proven that they represent an alternative to banned antibiotics. Improved health is the most apparent result of the application of herbs and herbal feed additives, which is reflected in blood tests and has proven to be a positive factor concerning both animal health and productivity in terms of quality and quantity in livestock (milk and beef), pigs (pork productivity and reproductive performance) and poultry (egg laying yield in laying hens and broiler chickens)¹⁰ when Mirzaei-Aghsaghali¹¹ said that biologically active substances found in herbs such as (alkaloids, glycosides, tannins, essential oils, phenolic compounds and others) affect the bodies of animals, as they have antibacterial properties, improve the functioning of the immune system and regulate feed intake and appetite in animals by enhancing the flavor and regulating the functioning of the digestive system. Konmy *et al.*¹² confirmed that using medicinal plants in feeding rabbits led to their treatment of some diseases, including eliminating animal worms.

The HEMA and responses of rabbits fed the dietary treatments like the PCV, RBC, MCHC and Hb across the dietary treatments were insignificant. Meanwhile, Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV)

were significantly higher at 50% and above-based millet offal diets. The test diets did not significantly influence other blood variables. They concluded that the White Blood Cells (WBC) were considerably higher at 50% and above-based millet offal diets. The test diets did not significantly influence other blood variables. However, eosinophils, lymphocytes, basophils and monocytes across the dietary treatments were not significant¹³. Nonetheless, Duwa *et al.*¹⁴ indicated that the hematological showed significant differences among the treatments in Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH). Still, there was no significant effect on hemoglobin and Mean Corpuscular Hemoglobin Concentration (MCHC). On the other hand, they showed that the result also revealed significant differences in serum globulin, glucose, cholesterol, urea and creatinine. Still, there was no significant effect on serum albumin and total protein. While Oladunjoye *et al.*¹⁵ indicated that no significant impact of diets was observed in Hb, WBC and MCHC and showed that dietary treatments did not affect serum composition as the values obtained for total protein, albumin, glucose, serum creatinine, ALT and AST were not significantly different across the treatments. Therefore, this study aimed to determine the effect of using medicinal and aromatic plant remnants to replace 50% of alfalfa hay on New Zealand white rabbits' blood hematological and biochemical aspects.

MATERIALS AND METHODS

Location: The rabbits used in this study were New Zealand white rabbits at the Experimental Station of the Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt and a private Rabbit's farm at El-Qalyubia Governorate.

Plants and diets: The four remnants (mint, fennel, basil and anise) were obtained after oil extraction and incorporated in rabbit diets at 17.5% without probiotic and with probiotic (replacement 50% of alfalfa hay), as shown in the experimental design in Table 1 and 2.

Chemical analysis of the experimental diets was shown in Table 3; chemical analysis of the different diets was carried out to determine dry matter (DM), ash, crude fiber (CF), crude protein (CP) and ether extract (EE) for tested plants was done according to the methods recommended by Horwitz and AOAC¹⁶, the nitrogen-free extract (NFE) and the organic matter (OM) contents were calculated. At the same time, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to van Soest *et al.*¹⁷. Hemicellulose and cellulose were calculated.

Table 1: Experimental design and diet

Item	Experimental diets				
	Alfalfa (clover) (%)	Mint (%)	Fennel (%)	Basil (%)	Anise (%)
Control	35.0	-	-	-	-
Mint	17.5	17.5	-	-	-
Mint+probiotic	17.5	17.5	-	-	-
Fennel	17.5	-	17.5	-	-
Fennel+probiotic	17.5	-	17.5	-	-
Basil	17.5	-	-	17.5	-
Basil+probiotic	17.5	-	-	17.5	-
Anise	17.5	-	-	-	17.5
Anise+probiotic	17.5	-	-	-	17.5

Table 2: Formulation of the experimental diets

Ingredients (kg)	Control (kg/100 kg)	Treatment group (kg/50 kg)							
		Mint		Fennel		Basil		Anise	
		Mint	Mint+probiotic	Fennel	Fennel+probiotic	Basil	Basil+probiotic	Anise	Anise+probiotic
Yellow corn grains	25	25	25	25	25	25	25	25	25
Soybean meal	15	15	15	15	15	15	15	15	15
Barley grains	10	10	10	10	10	10	10	10	10
Wheat bran	10	10	10	10	10	10	10	10	10
Alfalfa Hay	35	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
Mint straw	-	17.5	17.5	-	-	-	-	-	-
Fennel straw	-	-	-	17.5	17.5	-	-	-	-
Basil straw	-	-	-	-	-	17.5	17.5	-	-
Anise straw	-	-	-	-	-	-	-	17.5	17.5
Molasses	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
Limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Common salt	1	1	1	1	1	1	1	1	1
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Probiotic	-	-	+	-	+	-	+	-	+

-: Without probiotic and +: With probiotic

Table 3: Chemical composition of the experimental rabbit diets

Item	Experimental diets				
	Remnants diets with or without probiotic				
	Control	Mint	Fennel	Basil	Anise
Chemical composition (%)					
DM	90.76	90.44	89.85	89.78	89.54
OM	91.98	91.96	91.56	92.05	91.99
Ash	8.02	8.04	8.44	7.95	8.01
CP	17.36	16.42	16.62	16.99	17.04
EE	2.40	2.38	2.44	2.33	3.17
CF	13.62	13.31	12.06	13.88	12.21
NFE	58.60	59.85	60.44	58.85	59.57
Fiber fractions (%)					
NDF	24.87	26.96	24.05	26.49	23.12
ADF	14.45	15.87	14.13	15.43	13.18
ADL	4.04	4.53	4.22	4.37	3.24
Cellulose	10.41	11.34	9.91	11.06	9.94
Hemi cellulose	10.42	11.09	9.92	11.06	9.94

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen-free extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber and ADL: Acid detergent lignin

Experimental animals: One hundred eight weaned New Zealand white rabbits (5 weeks of age and about 635 ± 5 g average body weight) were used. They were individually weighed to the nearest and randomly assigned into nine experimental groups. Each group was divided into three replicates of four rabbits each. All rabbits were kept under the same managerial and hygienic conditions and were vaccinated against common diseases and they were under veterinary control. Feed and water were available during the experimental period. The experimental period lasted for eight weeks. Body weight gain and feed intake were recorded weekly and the feed conversion ratio was calculated. Rabbits in all treatments were kept under the same management system and environmental conditions. The building was naturally ventilated and provided with sided electric fans.

Blood analysis: Forty five blood samples of white New Zealand rabbits were taken at the end of the experiment during the process of slaughtering in two clean tubes with or without heparin to analyze.

Blood hematological aspects: Hematocrit value (Ht (%)) was read and recorded directly as described by Schalm and Jain¹⁸, Hemoglobin (HGB) (g/dL), was determined by colorimetric method according to Moore *et al.*¹⁹ and the differential white blood cells were done according to Hawkey *et al.*²⁰. Neutrophils/lymphocytes ratio (N/L) was calculated for all taken samples.

Blood biochemical: Total protein was determined using quantitative colorimetric according to Melillo²¹, albumin was determined using quantitative colorimetric according to Doumas *et al.*²², Aspartate Aminotransferase or Glutamic Oxaloacetic Transaminase (AST or GOT) and Alanine Aminotransferase or Glutamic Pyruvic transaminase (ALT or GPT) values (IU/L) were determined as described by Zanardo *et al.*²³. Urea was determined using Berthelot method according to Fawcett and Scott²⁴ and creatinine was determined using colorimetric kinetic method according to Bartels *et al.*²⁵.

Statistical analysis: Statistical analysis was carried out using the F-test for significance at $p \leq 0.05$ and computing of least significant difference (LSD) test values to separate means in different statistical groups according to the described method by Hills and Little²⁶.

RESULTS AND DISCUSSION

Chemical composition analysis of diets: The chemical composition of the experimental diets was presented in Table 3. Four medicinal plant remnants were used in this study (mint, fennel, basil and anise). In remnant diets with or without probiotics, the control diet showed the highest percentage of DM, while anise showed the lowest percentage. Still, in OM analysis, basil showed the highest percentage, whereas fennel showed the lowest. When fennel showed the highest percentage of ash and basil showed the lowest. In CP analysis, control showed the highest percent, mint showed the lowest percent, anise showed the highest percent in EE analysis and basil showed the lowest percent. In contrast, in the CF analysis, basil showed the highest percentage and fennel showed the lowest percentage. When fennel showed the highest percentage in NFE analysis, the control showed the lowest percentage.

The analysis of fiber fraction to the roughage and diets showed that in remnant diets with or without probiotics, the mint diet observed the highest percent of NDF, ADF and ADL, while anise showed the lowest percent. In cellulose and hemicellulose analysis, mint showed the highest percentage, whereas fennel showed the lowest.

Effect of remnant medicinal and aromatic plants on blood hematological aspects: Red blood cells (RBC) (μL) for all dietary treatments ranged between 4.68-6.03 μL in NZW-growing rabbits presented in Table 4. The highest value of RBC was found in basil+probiotics, while the lowest was found in fennel treatments. These results disagreed with Chineke *et al.*²⁷, who indicated that the values of RBC in rabbits' blood ranged between 5-8 μL . There were also insignificant differences between all treatments and control. The dietary treatments did not affect red blood cell parameters. These results disagreed with Abd El-Hady *et al.*²⁸, who found that red blood cell count was significantly higher in Alexandria-growing rabbits of G2 (fed on 300 mg digestion) and G1 (fed on 400 mg digestion) than in the control group. Furthermore, Abd-El-Hady²⁹ found a significant increase in RBC counts in males of New Zealand white rabbits fed diets at the age of eight weeks supplemented with 0.5% peppermint.

Hemoglobin (HGB) (g/dL) for all dietary treatments ranged between 10.13-13.5 g/dL. These results disagreed with Chineke *et al.*²⁷, who showed that the values of HGB in rabbits' blood ranged between 10-17 g/dL. The highest value of HGB was found in anise+probiotics treatment,

Table 4: Red blood cells for growing rabbits fed on diets containing alfalfa, mint, fennel, basil and anise remnants

Treatments	RBC (μL)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RDW-CV (%)	RDW-SD (fl)
Ref. range of rabbit	5-8	10-11	33-50	58-67	17-24	29-37	-	-
Control	4.70±0.26 ^a	10.13±0.58 ^c	28.23±1.59 ^b	60.20±0.78 ^{ab}	21.50±0.17 ^{bc}	35.87±0.22 ^{ab}	15.33±1.69 ^a	34.40±2.40 ^a
Mint	5.1±0.47 ^a	10.40±0.70 ^{bc}	29.77±2.28 ^{ab}	58.63±1.24 ^b	20.43±0.59 ^c	34.93±0.29 ^b	15.6±0.93 ^a	33.17±1.17 ^a
Mint+probiotics	4.85±0.53 ^a	10.53±0.87 ^{bc}	29.57±2.38 ^{ab}	61.43±2.00 ^{ab}	21.80±0.64 ^{abc}	35.57±0.12 ^{ab}	14.7±0.56 ^a	32.63±1.09 ^a
Fennel	4.68±0.29 ^a	10.23±0.54 ^c	28.40±1.65 ^b	60.83±0.43 ^{ab}	21.83±0.23 ^{abc}	36.00±0.36 ^a	14.4±0.66 ^a	32.40±1.32 ^a
Fennel+probiotics	4.94±0.23 ^a	10.77±0.57 ^{abc}	30.33±1.53 ^{ab}	61.40±1.64 ^{ab}	21.73±0.42 ^{abc}	35.47±0.28 ^{ab}	14.2±0.67 ^a	32.67±0.38 ^a
Basil	4.91±0.44 ^a	10.67±0.68 ^{abc}	29.63±2.07 ^{ab}	60.77±1.75 ^{ab}	21.83±0.73 ^{abc}	35.97±0.22 ^a	13.97±0.35 ^a	31.47±0.65 ^a
Basil+probiotics	6.03±0.76 ^a	13.3±1.62 ^{ab}	37.13±4.68 ^a	61.73±0.97 ^{ab}	22.07±0.45 ^{ab}	35.80±0.25 ^{ab}	14.53±0.49 ^a	33.37±0.81 ^a
Anise	4.84±0.28 ^a	10.5±0.23 ^{bc}	29.50±0.90 ^{ab}	61.33±1.82 ^{ab}	21.73±0.77 ^{abc}	35.57±0.62 ^{ab}	14.1±0.23 ^a	32.67±1.09 ^a
Anise+probiotics	5.78±0.65 ^a	13.5±1.59 ^a	37.00±4.04 ^a	64.13±0.29 ^a	23.27±0.15 ^a	36.40±0.31 ^a	14.17±0.45 ^a	33.60±1.27 ^a
LSD _{0.05}	1.4654	2.9074	8.1914	4.2716	1.6203	1.0148	2.3805	3.8624

RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation and RDW-SD: Red cell distribution width-standard deviation. Treatments not significantly different at the 5% level are indicated by the same letters in the same column and error bars are ±SE

while the lowest was found in control treatments. The dietary treatments are affected by hemoglobin parameters. It can be explained by the improvements in all the blood parameters in this study, which may be partially due to increased animal resistance to any physiological or environmental stress. These results suggested that anise enhances immune system activity. Hemoglobin value was increased due to the addition of anise. This indicates the positive effects of this supplementation on hematological parameters. These results were agreed with Attia *et al.*³⁰ and Badr³¹, who revealed that hemoglobin value increased due to adding anise. This shows the positive effects of this supplementation on hematological parameters. And the positive impact on the liver, spleen and other tissues like bone marrow, where red blood cells are synthesized.

There was a significant difference between basil+probiotics and anise+probiotics in all treatments. In contrast, there were also insignificant differences between control, fennel treatments, mint, mint+probiotics treatments and fennel+probiotics and basil treatments. The dietary treatments are affected by hemoglobin parameters. Özkan *et al.*³² said that the statistical comparison between male and female animals revealed a statistical difference for HGB ($p<0.001$) among hematological parameters.

Hematocrit (HCT) (%) concentration values of the dietary treatments ranged between 28.23-37.13%. These results disagreed with Chineke *et al.*²⁷, which concluded that the concentration of HCT in rabbits' blood ranged between 33-50%. The highest HCT in rabbits' blood ranged between 33-50%. The highest concentration of HCT was found in basil+probiotics treatment, while the lowest value was found in control treatments. The HCT concentration was increased due to the addition of basil+probiotics.

This indicates the positive effects of this supplementation on hematological parameters. However, these results disagreed with Attia *et al.*³⁰ and Badr³¹, who showed that hemoglobin value was increased due to adding Anise. There were significant differences between control and fennel with all treatments. In contrast, there were also insignificant differences between control and fennel treatments, between mint, mint+probiotics, fennel+probiotics, basil and anise treatments and between basil+probiotics and anise+probiotics. The dietary treatments are affected by hematocrit parameters. Whereas Özkan *et al.*³² showed that the statistical comparison between male and female animals revealed a statistical difference for HCT ($p<0.001$) among hematological parameters.

Measurement of red blood cells indicators: Mean cell volume (MCV) (FL) for all dietary treatments ranged between 58.63 and 64.13 fl. These results disagreed with Chineke *et al.*²⁷, which showed that the values of MCV in rabbits' blood ranged between 58- 67 fl. The highest value of MCV was found in anise+probiotics treatment, while the lowest was found in mint treatments. The MCV value was increased due to the addition of anise+probiotics. This indicates the positive effects of this supplementation on hematological parameters. This result agreed with Attia *et al.*³⁰ and Badr³¹, who showed the positive impact of anise supplemented on hematological parameters. There were significant differences between mint and anise+probiotic with all treatments, whereas there were also insignificant differences between control, mint+probiotics, fennel, fennel+probiotics, basil, basil+probiotics and anise treatments. The dietary treatments are affected by the mean cell volume parameter.

Mean cell hemoglobin (MCH) (pg) for all dietary treatments ranged between 20.43 and 23.27 pg. These results disagreed with Chineke *et al.*²⁷, which observed that the values of MCH in rabbit blood ranged between 17-24 pg. The highest values of MCH were found in anise+probiotics treatment, while the lowest was found in mint treatments. The MCH value was increased due to the addition of Anise+probiotics. This indicates the positive effects of this supplementation on hematological parameters. This result agreed with Attia *et al.*³⁰ and Badr³¹, who showed the positive impact of anise supplemented on hematological parameters. There were significant differences between control, mint, basil+probiotics and anise+probiotics with all treatments. In contrast, there were also insignificant differences between mint+probiotics, fennel, fennel+probiotics, basil and anise treatments, the dietary treatments affected by mean cell hemoglobin parameter.

Mean cell hemoglobin concentration (MCHC) (g/dL) for all dietary treatments ranged between 34.93 and 36.40 g/dL. These results disagreed with Chineke *et al.*²⁷, which said that the values of MCHC in rabbits' blood ranged between 29-37 g/dL. The highest value of MCHC was found in anise+probiotics treatment, while the lowest was found in mint treatments. The MCHC value was increased due to the addition of Anise+probiotics. This indicates the positive effects of this supplementation on hematological parameters. This result agreed with Attia *et al.*³⁰ and Badr³¹, who showed the positive impact of anise supplemented on hematological parameters. There were significant differences between mints with all treatments, whereas there were also insignificant differences between control, mint+probiotics, fennel+probiotics, basil+probiotics and anise and between fennel, basil and anise+probiotics treatments. The dietary treatments are affected by the mean cell hemoglobin concentration parameter. While

Özkan *et al.*³² said there was no statistically significant difference in MCHC between male and female animals.

Red blood cell distribution with a coefficient of variance (RDW-CV) (%) for all dietary treatments ranged between 13.97 and 15.6%. The highest value of RDW-CV was found in the mint treatment, whereas the lowest was in the basil treatment. There were also insignificant differences between all treatments and control. The dietary treatments did not affect red blood cell distribution with the coefficient of variance parameter.

Red blood cell distribution with standard deviation (RDW-SD) (fl) for all dietary treatments ranged between 31.47-34.40%. The highest value of RDW-SD was found in the control treatment, whereas the lowest was in the basil treatment. There were also insignificant differences between all treatments and control. The dietary treatments did not affect red blood cell distribution with a standard deviation parameter.

Differential white blood cells: White Blood Cells (WBC) (μL) for all dietary treatments ranged between 6.27-12.70 μL in NZW-growing rabbits presented in Table 5. These results disagreed with Chineke *et al.*²⁷, which indicated that the values of WBC in rabbits' blood ranged between 5-12.5 μL. The highest value of WBC was found in mint and basil+probiotics, while the lowest was found in anise treatments. There were significant differences between anise with all treatments. In contrast, there were also insignificant differences between control and fennel, mint and basil+probiotics treatments, mint+probiotics, anise+probiotics, fennel+probiotics and basil+probiotics. Dietary treatments are affected by white blood cell parameters. When Özkan *et al.*³² said, there was no statistically significant difference in WBC between male and female animals.

Table 5: White blood cells for growing rabbits fed on diets containing alfalfa, mint, fennel, basil and anise remnants

Treatments	WBC (μL)	Lymph (μL)	Mid (μL)	Gran (μL)	Lymph (%)	Mid (%)	Gran (%)
Ref. range of rabbit	5-12.50	1.6-10.60	-	-	30-85	-	-
Control	8.13±1.22 ^{bc}	2.63±0.37 ^b	0.97±0.09 ^c	4.53±0.76 ^b	32.57±0.48 ^{cd}	12.43±0.75 ^{ab}	55.0±1.13 ^a
Mint	12.70±2.34 ^a	3.90±1.18 ^b	1.67±0.41 ^a	7.13±1.39 ^a	30.73±6.62 ^d	13.17±0.91 ^a	56.1±6.03 ^a
Mint+probiotics	9.40±1.01 ^{abc}	3.87±0.48 ^b	1.17±0.09 ^{abc}	4.37±1.33 ^b	42.77±8.93 ^{bcd}	12.43±0.59 ^{ab}	44.8±9.01 ^{ab}
Fennel	7.83±0.52 ^{bc}	3.87±0.03 ^b	1.07±0.03 ^{bc}	2.90±0.49	50.07±3.47 ^{abc}	13.33±0.42 ^a	36.6±3.81 ^{bc}
Fennel+probiotics	11.57±1.98 ^{ab}	5.33±1.02 ^{ab}	1.57±0.19 ^{ab}	4.67±0.79 ^{ab}	45.83±1.27 ^{abcd}	13.70±0.75 ^a	40.47±0.78 ^{abc}
Basil	10.83±0.97 ^{ab}	5.73±0.57 ^{ab}	1.57±0.23 ^{ab}	3.77±0.35	51.87±0.93 ^{ab}	14.07±0.98 ^a	34.07±0.29 ^{bc}
Basil+probiotics	12.70±1.85 ^a	8.13±1.82 ^a	1.03±0.18 ^{bc}	3.53±1.50 ^b	63.67±12.05 ^a	8.67±2.49 ^b	27.67±10.39 ^c
Anise	6.27±0.68 ^c	3.27±0.43 ^b	0.77±0.15 ^c	2.23±0.19 ^b	52.33±3.03 ^{ab}	11.90±1.37 ^{ab}	35.77±1.97 ^{bc}
Anise+probiotics	8.83±1.50 ^{abc}	4.83±1.43 ^b	0.90±0.15 ^c	3.10±0.10 ^b	52.40±6.28 ^{ab}	10.50±1.43 ^{ab}	37.1±5.76 ^{bc}
LSD _{0.05}	4.41	3.12	0.57	2.57	18.55	3.78	17.10

WBC: White blood cells, MID: Other white blood cells not classified as lymphocytes or granulocytes and Gran: Granulocyte. Treatments not significantly different at the 5% level are indicated by the same letters in the same column and error bars are ±SE

Lymphocytes (Lymph) (μL) for all dietary treatments ranged between 2.63-8.13 μL . These results disagreed with Chineke *et al.*²⁷, which indicated that the values of lymphocytes in rabbits' blood ranged between 1.6 and 10.6 μL . The highest value of lymph was found in basil+probiotics, while the lowest was found in control treatments. There were significant differences between basil+probiotics with all treatments, whereas there were also insignificant differences between control, mint, mint+probiotics, fennel, anise and anise+probiotics treatment and between fennel+probiotics and basil treatments. The dietary treatments are affected by lymph parameters.

Minimum inhibitory dilution (Mid) (μL) for all dietary treatments ranged between 0.77-1.67 μL . The highest value of Mid was found in mint, while the lowest was found in anise treatments. There were significant differences between mint and mint+probiotics with all treatments. In contrast, there were also insignificant differences between control, anise and anise+probiotics treatment, fennel and basil and fennel+probiotics and basil treatments. The dietary treatments are affected by Mid-parameters.

Granulocytes (Gran) (μL) for all dietary treatments ranged between 2.23-7.13 μL ; the highest value was found in mint, while the lowest was found in anise treatments. There were significant differences between mint and fennel+probiotics with all treatments, whereas there were also insignificant differences between control, mint+probiotics, fennel, basil, basil+probiotics, anise and anise+probiotics treatment. The dietary treatments are affected by the Gran parameter. When Özkan *et al.*³² showed no statistically significant difference in granulocytes between male and female animals.

Lymphocytes (Lymph) (%) for all dietary treatments ranged between 30.73-63.67%. These results disagreed with Chineke *et al.*²⁷, which indicated that the concentration of

lymphocytes in rabbits' blood ranged between 30-85%. The highest concentration of lymph was found in basil+probiotics, while the lowest concentration was found in mint treatments. There were significant differences between all treatments and insignificant differences between basil, anise and anise+probiotics treatment. The dietary treatments are affected by lymph parameters.

Minimum inhibitory dilution (Mid) (%) for all dietary treatments ranged between 8.67 and 14.07%. The highest concentration of Mid was found in basil, while the lowest was found in basil+probiotics treatments. There were significant differences between basil+probiotics with all treatments, whereas there were also insignificant differences between control, mint+probiotics, anise and anise+probiotics treatment and between mint, fennel, fennel+probiotics and basil treatments. The dietary treatments are affected by the parameters.

Granulocytes (Gran) (%) for all dietary treatments ranged between 27.67-56.1%. The highest concentration of Gran was found in mint, while the lowest value was found in basil+probiotics treatments. There were significant differences between mint+probiotics, fennel+probiotics and basil+probiotics with all treatments, whereas there were also insignificant differences between control and mint and between fennel, basil, anise and anise+probiotics treatment. The dietary treatments are affected by the Gran parameter. Whereas Özkan *et al.*³² showed that the statistical comparison between male and female animals revealed a statistical difference for granulocytes ($p < 0.001$) among hematological parameters.

Blood platelets (thrombocytes): The effect of diets containing remnants of medicinal plants on red blood cells in NZW-growing rabbits was presented in Table 6.

Table 6: Blood platelets for growing rabbits fed on diets containing alfalfa, mint, fennel, basil and anise remnants

Treatments	PLT (μL)	MPV (fl)	PDW	PCT (%)
Ref. range of rabbit	250-650	-	-	-
Control	463.67 $\pm$ 71.61 ^a	7.53 $\pm$ 0.41 ^a	15.63 $\pm$ 0.37 ^b	0.35 $\pm$ 0.07 ^a
Mint	331.00 $\pm$ 182.35 ^{ab}	7.90 $\pm$ 0.49 ^a	16.73 $\pm$ 0.28 ^a	0.24 $\pm$ 0.12 ^{ab}
Mint+probiotics	349.00 $\pm$ 99.45 ^{ab}	7.23 $\pm$ 0.33 ^a	15.93 $\pm$ 0.03 ^{ab}	0.24 $\pm$ 0.06 ^{ab}
Fennel	178.33 $\pm$ 58.81 ^b	7.27 $\pm$ 0.09 ^a	15.97 $\pm$ 0.28 ^{ab}	0.13 $\pm$ 0.05 ^b
Fennel+probiotics	251.67 $\pm$ 79.42 ^{ab}	7.33 $\pm$ 0.60 ^a	15.87 $\pm$ 0.42 ^{ab}	0.19 $\pm$ 0.06 ^{ab}
Basil	125.00 $\pm$ 40.07 ^b	8.07 $\pm$ 0.65 ^a	16.37 $\pm$ 0.62 ^{ab}	0.10 $\pm$ 0.03 ^b
Basil+probiotics	169.67 $\pm$ 51.96 ^b	7.57 $\pm$ 0.46 ^a	16.37 $\pm$ 0.61 ^{ab}	0.13 $\pm$ 0.04 ^b
Anise	328.00 $\pm$ 49.37 ^{ab}	7.40 $\pm$ 0.15 ^a	15.87 $\pm$ 0.07 ^{ab}	0.24 $\pm$ 0.03 ^{ab}
Anise+probiotics	463.00 $\pm$ 102.31 ^a	7.23 $\pm$ 0.15 ^a	15.77 $\pm$ 0.09 ^{ab}	0.33 $\pm$ 0.07 ^a
LSD _{0.05}	270.49	1.1535	1.08	0.19

PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width and PCT: Procalcitonin. Treatments not significantly different at the 5% level are indicated by the same letters in the same column and error bars are $\pm$ SE

Table 7: Liver functions for growing rabbits fed on diets containing alfalfa, mint, fennel, basil and anise remnants

Treatments	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	TP (g/dL)
Ref. range of rabbit	48-80	14-113	2.4-4.6	5.4-8.3
Control	87.00±54.03 ^a	51.67±26.69 ^a	2.67±0.17 ^{ab}	4.30±0.15 ^c
Mint	81.67±56.71 ^a	49.33±26.87 ^a	2.67±0.17 ^{ab}	4.63±0.41 ^{bc}
Mint+probiotics	53.00±12.00 ^a	41.00±8.00 ^a	2.80±0.30 ^{ab}	5.23±0.17 ^a
Fennel	142.00±53.00 ^a	77.33±25.17 ^a	2.50±0.00 ^b	4.77±0.13 ^{abc}
Fennel+probiotics	56.33±13.67 ^a	45.00±10.00 ^a	2.50±0.00 ^b	4.97±0.150 ^{ab}
Basil	98.67±49.46 ^a	61.00±22.37 ^a	2.50±0.00 ^b	5.03±0.03 ^{ab}
Basil+probiotics	109.67±42.70 ^a	72.00±15.01 ^a	2.67±0.17 ^{ab}	5.03±0.23 ^{ab}
Anise	64.00±0 ^a	55.00±0 ^a	3.10±0.30 ^a	4.70±0.10 ^{abc}
Anise+probiotics	68.00±2.00 ^a	58.67±0.67 ^a	2.67±0.17 ^{ab}	4.97±0.07 ^{ab}
LSD _{0.05}	117.76	54.92	0.57	0.55

ALT: Alanine transaminase, AST: Aspartate transaminase and TP: Total protein. The same letters in the same column indicate treatments that are not significantly different at the 5% level and error bars are ±SE

Platelets count (PLT) (μL) for all dietary treatments ranged between 125.00-463.67 μL in NZW growing rabbits presented in Table 6. These results disagreed with Chineke *et al.*²⁷, which indicated that the values of PLT in rabbits' blood ranged between 250-650 μL. The highest value of PLT was found in control, while the lowest was found in basil treatments. There were significant differences between all treatments, whereas there were also insignificant differences between control and anise+probiotics, between mint, mint+probiotics, fennel+probiotics and anise treatments and between fennel, basil and basil+probiotics treatments. The dietary treatments are affected by blood platelet parameters. The nutritional treatments are affected by lymph parameters. Whereas Özkan *et al.*³² showed no statistically significant difference in PLT between male and female animals.

Mean Platelet Volume (MPV) (fl) for all dietary treatments ranged between 7.23-8.07 fl. The highest value of MPV was found in basil, while the lowest was in mint+probiotics and anise+probiotics treatments. There were also insignificant differences between all treatments and control. The dietary treatments were not affected by mean platelet volume parameters.

Procalcitonin (PCT) (%) for all dietary treatments ranged between 0.10-0.35. The control had the highest PCT concentration, while the basil treatment found the lowest value. There were significant differences between all treatments, whereas there were also insignificant differences between control and anise+probiotics treatments, between mint, mint+probiotics, fennel+probiotics and anise treatments and between fennel, basil and basil+probiotics. Dietary treatments are affected by procalcitonin parameters.

Blood biochemical

Liver function for growing rabbits experimental: Table 7 presents the effect of diets containing remnants of medicinal plants on liver function in NZW-growing rabbits.

Alanine Transaminase (ALT) (IU/L) for all dietary treatments ranged between 53.00-142.00 IU/L in NZW

growing rabbits presented in Table 7. These results disagreed with Chineke *et al.*²⁷, which indicated that the values of ALT in rabbits' blood ranged between 48- 80 IU/L. The highest value of ALT was found in fennel, while the lowest was found in mint+probiotics treatment. These results disagreed with Badr³¹, who indicated that adding anise to growing rabbits' diets significantly increased ($p \leq 0.05$) the concentrations of serum transaminase enzymes (ALT). There were also insignificant differences between all treatments. The dietary treatments did not affect the alanine transaminase parameter. These results agreed with Abd El-Hady *et al.*²⁸, who indicated that levels of serum ALT were not significantly different in all groups and these results indicated normal liver functions in all experimental rabbits. However, blood components and enzyme activities are intimately related to metabolism. Furthermore, Paika *et al.*³³ concluded that ALT concentration was not significantly affected by adding fennel hay to growing rabbit diets.

Aspartate Transaminase (AST) (IU/L) for all dietary treatments ranged between 41.00-77.33 IU/L. These results disagreed with Chineke *et al.*²⁷, which indicated that the values of AST in rabbits' blood ranged between 14-113 IU/L. The highest value of AST was found in fennel, while the lowest was found in mint+probiotics treatments. These results disagreed with Badr³¹, who indicated that adding anise to growing rabbits' diets increased significantly ($p \leq 0.05$) the concentrations of serum transaminase enzymes (AST). There were also insignificant differences between all treatments. The dietary treatments did not affect the aspartate transaminase parameter. These results agreed with Abd El-Hady *et al.*²⁸, who indicated that levels of serum AST were not significantly different in all groups and these results indicated normal liver functions in all experimental rabbits. However, blood components and enzyme activities are intimately related to metabolism. Furthermore, Sherlock³⁴ reported that AST levels reflect the impairment of liver function when their levels increase.

Albumin (g/dL) for all dietary treatments ranged between 2.50-3.10 g/dL. These results disagreed with Chineke *et al.*²⁷, which observed that the values of albumin in rabbits' blood ranged between 2.4-4.6 g/dL. The highest value of albumin was found in anise, while the lowest value was found in fennel, fennel+probiotics and basil treatments. There were significant differences between anise and all treatments. There were also insignificant differences between control, mint, mint+probiotics, basil+probiotics and anise+probiotics treatments and between fennel, fennel+probiotics and basil treatments. The dietary treatments are affected by albumin parameters. These results agreed with Abd El-Hady *et al.*²⁸, who indicated that serum albumin levels were not significantly different in all groups and these results indicated normal liver functions in all experimental rabbits. However, blood components and enzyme activities are intimately related to metabolism. Furthermore, Pałka *et al.*³³ concluded that adding fennel hay to growing rabbit diets did not significantly affect albumin concentration.

Total protein (TP) (g/dL) for all dietary treatments ranged between 4.30 and 5.23 g/dL. These results disagreed with Chineke *et al.*²⁷, which observed that the values of TP in rabbits' blood ranged between 5.4-8.3 g/dL. The highest total protein value was found in mint+probiotics, while the lowest was in control treatments. Significant differences existed between control, mint and mint+probiotics with all treatments. There were also insignificant differences between fennel, fennel+probiotics, basil, basil+probiotics, anise and anise+probiotics treatments. The dietary treatments are affected by the total protein parameter. These results agreed with Abd El-Hady *et al.*²⁸, who indicated that levels of serum TP were not significantly different in all groups and these results indicated normal liver functions in all experimental rabbits. However, blood components and enzyme activities are intimately related to metabolism. Furthermore, Pałka *et al.*³³

concluded that TP concentration was not significantly affected by adding fennel hay to growing rabbit diets. Abd-El-Hady²⁹ reported that the metabolic changes of blood TP significantly improved in males of New Zealand rabbits aged eight weeks supplemented with 0.5% peppermint.

Kidney function for growing rabbits experimental

Blood urea: Urea (mg/dL) for all dietary treatments ranged between 32.00-50.33 mg/dL in NZW growing rabbits presented in Table 8. These results disagreed with Chineke *et al.*²⁷, which showed that the values of urea in rabbits' blood ranged between 13-29 mg/dL. The highest value of blood urea was found in anise+probiotics, while the lowest was found in mint+probiotics treatments. There were significant differences between anise and anise+probiotics with all treatments. There were also insignificant differences between control, mint and mint+probiotics treatments, fennel and basil+probiotics and fennel+probiotics and basil treatments. The dietary treatments are affected by urea parameters. Whereas, Özkan *et al.*³² showed no statistically significant difference in blood urea between male and female animals.

Creatinine: Creatinine (mg/dL) for all dietary treatments ranged between 0.43 and 1.30 mg/dL. The highest creatinine value was found in anise+probiotics, while the lowest was in mint treatments. There were significant differences between all treatments. These results disagreed with Chineke *et al.*²⁷ which concluded that the creatinine values in rabbits' blood ranged between 0.5-2.5 mg/dL. There were also insignificant differences between fennel and basil treatments and between fennel+probiotics and basil+probiotics treatments. Dietary treatments are affected by carnitine parameters. While Özkan *et al.*³² showed no statistically significant difference in creatinine between male and female animals.

Table 8: Kidney functions for growing rabbits fed on diets containing alfalfa, mint, fennel, basil and anise remnants

Treatments	Urea (mg/dL)	Creatinine (mg/dL)
Ref. range of rabbit	13-29	0.5-2.5
Control	32.33±4.06 ^c	0.60±0.23 ^{cd}
Mint	33.33±3.84 ^c	0.43±0.23 ^d
Mint+probiotics	32.00±3.00 ^c	0.80±0.00 ^{bcd}
Fennel	37.00±1.53 ^{bc}	0.87±0.03 ^{abcd}
Fennel+probiotics	42.00±1.53 ^{abc}	1.03±0.07 ^{abc}
Basil	41.67±1.67 ^{abc}	0.90±0.00 ^{abcd}
Basil+probiotics	39.00±0.58 ^{bc}	1.07±0.23 ^{abc}
Anise	46.00±6.03 ^{ab}	1.17±0.18 ^{ab}
Anise+probiotics	50.33±4.67 ^a	1.30±0.20 ^a
LSD _{0.05}	10.42	0.488

Same letters in the same column indicate treatments that are not significantly different at the 5% level and error bars are ±SE

Table 9: Chemical names of volatile oils of alfalfa

No.	Control (Alfalfa)
1	Scopoletin
2	Ferulic acid
3	4-Hydroxyphenylpyruvic acid
4	Anethole
5	24,25-Dihydroxyvitamin D3
6	Lactose
7	β -Guaiane
8	p-Cresol and 2,6-di-tert-butyl- α -methoxy-
9	3,4-Dimethoxycinnamic acid
10	Cis-10-Heptadecenoic acid
11	Ecgonine methyl ester
12	Myristic acid and methyl ester
13	o-Isoeugenol
14	Palmitic acid and methyl ester
15	Arachidic acid
16	Chromone and 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-
17	Linolenin, 1-mono-
18	2,6-Di-tert-butylhydroquinone
19	Chrysin

Table 10: Chemical names of volatile oils of mint and fennel

No	Mint	Fennel
1	Phenol, 4-(2-aminopropyl)-	Camphenol, 6-
2	β -Asarone	D-Limonene
3	Shikimic acid	β -Pinene
4	1,8-Cineole	D-Fenchone
5	p-Menth-1(7)-en-2-one	Ocimene
6	D-menthone	Lanceol
7	Menthol	Cis-p-mentha-2,8-dienol
8	Anethol	Ethyl linalool
9	Pulegone	Carveol
10	Levo-carvone	Anethole
11	Berbenone	trans-Chrysanthenyl acetate
12	6-Allyl-2-cresol	α -Terpinyl propionate
13	β -Guaiane	Cuminaldehyde
14	Caryophyllene-(I1)	Terpinyl formate
15	Apigenin 7-glucoside	Perillol
16	2,6-Di-tert-butylquinone	24,25-Dihydroxyvitamin D3
17		β -Guaiane
18		Santalol
19		Nerolidyl acetate
20		Apiol
21		α -Elemene
22		Myristic acid
23		Oleic acid and 12-hydroxyl
24		Eugenol acetate
25		Geranyl isovalerate
26		Phenol, 2,4-diisopropyl-
27		Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-
28		Linoleic acid
29		Cis-11-Eicosenoic acid
30		Palmitoleate
31		Estragole
32		2,6-Di-tert-butylquinone

Volatile oil component of the experimental plants: Results given in Table 9-11 show the volatile oils of control and the four remnant plants shown in Table 9-11 cleared all volatile oils found in alfalfa hay and mint, fennel, anise and basil remnants. These results observed that 19 volatile oils were

found in alfalfa hay, whereas the volatile oils that were found in mint remnant had 16 volatile oils; on the other hand, these results indicated that the fennel remnant was contested composed of 32 volatile oils when the anise and basil contained 22 volatile oils.

Table 11: Chemical names of volatile oil of anise and basil plants

No	Anise	Basil
1	5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone	Phenol, 4-(2-aminopropyl)-
2	Flavone, 2 ¹ ,5,6 ¹ -tetramethoxy	Limonene
3	Ocimene	α -Terpineol
4	α -Pinene	Linalool and formate
5	Anethole	β -Camphor
6	4-(2-Methyl-2-propenyl)phenol	Methyl chavicol
7	3-Anisaldehyde	Sinapic acid
8	Cinnamic alcohol	Cuminaldehyde
9	β -Guaiene	α -Himachalene
10	α -Bisabolene	Ocimene
11	Terpinolene	Caryophyllene-(I1)
12	Junipene	Linalyl octanoate
13	Ylangene	Apiole
14	Patchoulene	3,5-Dimethoxycinnamic acid
15	Eugenol	Farnesol
16	p-cresol, 2-tert-butyl-	Eugenol acetate
17	γ -Himachalene	Apigenin 7-glucoside
18	8-Octadecenoic acid, methyl ester	2,6-Di-tert-butylquinone
19	Coniferyl alcohol	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-
20	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	10-Octadecenoic acid and methyl ester
21	Estragole	2,3-Dihydroxybenzoic acid
22	Flavone, 5-hydroxy-7-methoxy	Colchicine

In mint remnant, these results in agreement with many authors who indicated that the most critical compounds in peppermint are menthol, menthone and pulegone^{35,36}. These oils play an important pharmacological role; this role was illustrated in menthol's vital role as an anti-inflammatory, analgesic, antifungal and central nervous system excitation effect. Pulegone, menthone and carvone were tested for insecticidal and genotoxic activities on *Drosophila melanogaster*. In contrast, the most effective insecticide was pulegone, whereas the most effective for genotoxic activity was menthone^{36,37}. In fennel, these results agreed with many authors who indicated that fennel plant constituents include anethol, fenchone, phenolic compounds and estragole³⁸. *Foeniculum vulgare* is essential to exhibit antifungal, antibacterial, antioxidant, antithrombotic and hepato-protective activities, supporting the rationale behind several of its therapeutic uses³⁸. Whereas, in anise, these results were in agreement with many authors who indicated that anise plant constituents include flavone^{39,40}, β -bisabolene, γ -himachalene⁴¹, anethole, γ -himachalene, methyl chavicol (estragole), anisaldehyde^{42,43} and terpenoids⁴⁴. Anise oils exert their growth-promoting activity, are antioxidants and stimulate birds' immune systems. Another possibility is that anise oil may improve nutrient digestion and absorption by increasing the activity of pancreatic enzymes⁴⁵. Anise is used as a preservative material in canned food to increase its half-life and in human medicine, it is used for kidney failure treatment. It has an antimicrobial, antifungal, insecticidal and antioxidative effect of this herb on human health⁴⁶. On the

other hand, in basil, these results agreed with many authors who indicated that anise plant constituents include limonene⁴⁷, linalool^{48,49}, methyl chavicol and eugenol^{50,51}. Basil is used as a medicinal herb in medical treatments for headaches, coughs, diarrhea, worms and kidney malfunctions. It is also considered a source of aroma compounds and possesses a range of biological activities such as insect repellent, nematocidal, antibacterial, antifungal agents and antioxidant activities⁵².

CONCLUSION

The results proved that using these medicinal plant remnants and probiotics as substitutes for half the amount of alfalfa used in the diet of New Zealand white rabbits did not have a negative effect and led to an improvement in their health condition. The results showed that the highest value of red blood cells (RBC (μ L)), Hematocrit (HCT%), White Blood Cell (WBC (μ L)) and lymph (%) were found in basil+probiotics treatment, while the highest value of Hemoglobin (HGB) (g/dL), mean cell volume (MCV (fl)), mean cell hemoglobin (MCH) (pg), mean cell hemoglobin concentration (MCHC) (g/dL), platelet count (PLT) (μ L), urea and creatinine were found in anise+probiotic treatment. Whereas the highest values of Alanine Transaminase (ALT) (IU/L) and Aspartate Transaminase (AST (IU/L)) were found in fennel treatment. When the highest value of albumin (g/dL) was found in anise treatment and the highest value of total protein (TP) (g/dL) was found in mint+probiotic treatment.

SIGNIFICANCE STATEMENT

This research proposes improving the health status of New Zealand white rabbits by using some residues of medicinal and aromatic plants (mint, fennel, basil and anise) with or without adding probiotics and studying their effect on blood components and liver and kidney functions. The results of this research represent a contribution to the field of rabbit nutrition, as it allows the use of some medicinal and aromatic plant residues because of their important benefits that help raise the nutritional value of feeds while reducing the cost of producing feeds to work to improve the health status of New Zealand white rabbits.

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