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Productive and Immunological Performance of Small Ruminants Offered Some Medicinal Plants as Feed Additives

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ABSTRACT

The current study aimed to investigate the effect of some medicinal herbs on ewes and their lambs performance represented in blood parameters, rumen liquor parameters, growth performance, and digestibility. In a factorial experiment with repeated measurements, 11 ewes in the second parturition and eight lambs with three months of age with an average bwt 19.03kg were divided into six groups. The experimental diets were: T1 fed Control, T2 fed T1 plus garlic powder (1gm/kg DM), T3 fed T1 with garlic powder (1.4gm/kg DM), T4 fed T1 plus fennel seed powder 1.2gm/kg DM, T5 fed T1 plus dill (0.8gm/Kg DM) and T6 fed T1 in addition to marjoram leaves powder (1gm/kg DM). We weighed the experimental animals fortnightly. We took blood and rumen liquor monthly for the six months of the experimental period. The use of medicinal herbs as feed additive by lambs showed a significant (P \leq 0.05) increase in DMI and average daily gain, especially with the use of fennel, leading to an increase in average daily gain by 161.11gm/day versus 121.11gm/day for the control group. Ewes fed experimental rations didn't affect rumen pH, but the use of fennel, dill, and marjoram increased significantly (P \leq 0.05) TVFA's produced from rumen fermentation. All treatments improved blood parameters, especially total protein and globulin, and increased total weight gain (P \leq 0.05) during the experimental period against the control group.

Key words: Medicinal Herbs, Sheep, Blood Parameters, Rumen Fermentation, Digestibility, Growth Parameters.

INTRODUCTION

The main objective for nutritionists is to improve animal production by improving rumen fermentation. Patra et al. (2006) illustrated that improving rumen fermentation had a positive effect on fiber digestibility and reduced both of gas emission (NH4) and nitrogen extraction. Use of natural additives like medicinal and aromatic herbs is coming as a main topic in feed additives to improve animal production and health through rumen fermentation. Moreover, antibiotic, ionophores, probiotic used as feed additive in improvement of rumen fermentation (Vahabzadeh et al. 2021). For decades producers success to improve ruminant production by used antibiotics as feed additives, but with the increase of concern about the negative effect of antibiotic on public health, specially residues in ruminant production like milk and meat had led to panned using of antibiotics all over the world. So, they turned to search about alternative components which did not have a negative effect on

animal health or products safety and quality for humans (Makkar et al. 2007).

The using of medicinal herbs, or their extracts, leads to improve nutrients utilization and its digestion through enhancement of ruminal fermentation without undesirable side effects on animal health (Busquet et al. 2005; Patra et al. 2006). Many natural additives and herbs or herbs have been added as powder (Hajalizadeh et al. 2019; Degla et al. 2022) or as essential oils (Simitzis et al. 2008) in ruminants' diet. Because of bioactive component which medicinal herbs or their essential oils have inclusive of carvacrol in marjoram (Bravo et al. 2014) carvone and limonene in dill (Delaquis et al. 2002), fenchone and anethol in fennel (Cabuk et al. 2003). However, many other components in medicinal herbs had an antimicrobial, antifungal and antioxidant effect which had a positive effect on ruminal fermentation and nutrient digestibility. The main objective of present study is to evaluate the effect of some medicinal herbs (garlic, fennel, dill and marjoram) as natural feed additives in rumen fermentation, animal health and performance in sheep.

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MATERIALS AND METHODS

Ethical Statement

All experimental protocols and practices in this study were confirmed with the research ethics guidelines in compliance with the EU Directive for protection of experimental animals (2010/63/EU).

Location and Source of Animals

This study was conducted at the farm and laboratories of Department of Animal Production, Faculty of Agriculture, Ain Shams University during the period from year 2018 to 2021, to evaluate the effect of feeding four types of feed additives at six different levels of medicinal herbs on both of dry matter (DM) and organic matter (OM) disappearance, in vivo digestibility, rumen activity, some blood constituents and performance for Rahmani sheep.

In Vitro Incubation

Four medicinal herbs are used as treatments (T_1 = garlic, T_2 = fennel, T_3 =dill and T_4 =marjoram) with six doses (D_1 =0.2, D_2 =0.4, D_3 = 0.6, D_4 =0.8, D_5 =1 and D_6 = 1.2 gm/Kg DM) were used to evaluate it's effect on DM and OM disappearance for basal diet contain (60% concentrate feed mixture (CFM), 20% Alfalfa hay and 20% rice straw). In vitro incubation was carried out according to in-vitro Batch culture Method and by using McDougall 's Buffer described by McDougall et al. (1948).

Feeding Trials (In vivo)

Growth Trials

A factorial design with repeated measurements was followed in a growth for eight lambs which were divided randomized for six groups, each group was housed in pen, animals were three months of age with average live bwt 19.03kg, animals were weighted biweekly before morning feeding and were fed a basal contain concentration feed mixture (CFM) 60%, Alfalfa hay 20% and rice straw 20%.

Experimental Rations

The six trial rations containing: control (concentrates60%, alfalfa hay20% and rice straw20%). The experimental diets were: $T_{1=}$ Control, $T_2=T_1+$ garlic (1gm/kg DM), $T_3=T_1+$ garlic (1.4gm/kg DM), $T_4=T_1+$ fennel (1.2gm/kg DM), $T_5=T_1+$ dill (0.8gm/Kg DM) and $T_6=T_1+$ marjoram (1gm/kg DM). Rations and

proximate analysis of diet according to AOAC (2005) shown in Table 1.

Rumen Liquor Sampling

At the end of the feeding trial, ruminal fluid samples were collected from ewes using stomach tube. Samples were collected before the morning (zero time) feeding, three and six hours after feeding. Fluid samples collected were filtered immediately through four layers of gauze and determine pH by digital pH meter, total volatile fatty acids according to steam distillation as described by Warner (1964) were analyzed in the samples.

Digestibility Trials

Six adult Rahmani rams (average weight 65 ± 0.5 kg) divided into two groups and tested consecutively for six experimental rations in 3 stages every stage has taken one month (21 days for adaptation and one week to collect samples).

Daily extracted feces collected for every treatment three times daily and treated immediately with H_2SO_4 10% and formaldehyde 10% to stop fermentation and frozen, ration samples and feces were dried and analyzed for whole proximate analysis and silica was to use as natural marker to calculate nutrients digestibility all proximate analysis according to the method described by AOAC (2005). Samples for each animal were prepared by following Kamra et al. (1991) method for protozoa count. The digestibility coefficient of a certain nutrient was calculated according to the method described by Schneider and Flatt (1975).

Blood Sampling

Blood samples were collected via jugular vein in tubes had EDETA from each dietary treatment just before morning feeding,3 and 6 hours post feeding. Blood samples were centrifuged at 4000 rpm for 20 min, separated plasma was used for enzymes determination while the other part was frozen at -20°C till analysis for the ALT and AST according to the method described by Reitman and Frankel (1957), Albumin content according to the method described by Doumas et al. (1971), total protein (TP) content according to the method described by Gornal et al. (1949), globulin content calculated as the difference between total protein and albumin, creatinine content according to the method described by Bartles et al. (1972).

 Table 1: Composition of the experimental rations and analysis of feed ingredients

	Treatments							
Component	Control (T1)	T2	T3	T4	T5	Т6		
Garlic powder (g/kg DM)	-	1	1.4	-	-	-		
Fennel (g/kg DM)	-	-	-	1.2	-	-		
Dill (g/kg DM)	-	-	-	-	0.8	-		
Marjoram (g/kg DM)	-	-	-	-	-	1		
	Che	mical analysis	(DM basis)	%				
Items	DM	Ash	OM	CP	CF	EE	NFE	
Concentrate feed mixture (CFM)	88	10	78	13.2	20.1	5	39.7	
Rice straw	90	16.3	73.7	4	39.8	1	28.9	
Alfalfa hay	91	10	81	10.2	22.4	2.6	45.8	
ration (calculated)	89	11.26	77.74	10.76	24.5	3.72	38.7	

In each treatment, concentrate feed mixture (CFM), alfalfa hay and rice straw were 60, 20 and 20%, respectively.

Statistical Analysis

The obtained data were analyzed using factorial model with repeated measurements procedure of SAS (2002), as the following model:

 $Y_{ijkl}=\mu+Animal (time)_{l} +Treat_{j}+Time_{k}+ (treat*time)_{jk} + error_{ijkl}$. Where:

 μ =overall mean I = 11 (1,2,3,4,5,...11) J=6 (1,2,3,...,6)

RESULTS

In-vitro Incubation (Nutrients Disappearance)

Table 2 presented results of four types of medical herbs (garlic, dill, fennel and marjoram) with doses 0.6, 0.8, 1, 1.2, 1.4 and 1.6gm/kg DM used. and all results compared with control. Garlic powder with both of doses 1 and 1.4gm/kg DM increased the dry matter disappearance (DMD) and organic matter disappearance (OMD) significantly compared with both of control and the other doses with the same additive, in the other types of medical herbs fennel showed the highest value with dose 1.2gm/kg DM with DMD & OMD values were 66.399and 55.33% respectively. The same for other feed additives the significant values for dill and marjoram with doses 0.8and 1gm/kg DM. with a percentage of DMD 46.466, 53.27% and OMD 78.251 and 71.084%.

Digestion Coefficients and Nutritive Value (%) of Experimental Diets of Sheep

Table 3 illustrate a significant different ($P \le 0.05$) between the treatments and control on nutrients digestibility, where all treatments showed superiority on DM and OM digestibility against control the same for both of crude fiber (CF) and ether extract (EE) digestibility, used fennel as feed additive improve digestibility of DM, OM and crude protein CP versus other treatments, control and both of fennel and garlic improve EE digestibility with the same degree compared to other treatments and control. CF digestibility improved by used dill as feed additive. Previous result agrees with research of Hajalizadeh et al. (2020) who reported that when used fennel seed powder as feed additive wasn't affected nutrient digestibility.

Rumen Parameters

Results of rumen fermentation activities are shown in Table 4. Rumen liquor pH didn't significantly (P<0.05) different between treatments by sheep and this result agree with the research of (Wanapat et al. 2008; Kongmun et al. 2011) who reported that used of garlic powder as feed additive in sheep diets had no significant effect on rumen liquor pH, Total volatile fatty acids (TVFA's) affected by using additives as showed in Table 4. where it turns out that generally using fennel increased significantly TVFA's concentration compared with control in the same time using garlic with does 1.4gm/kg DMI had no significant effect compared with control. And illustrate the effect of treatments on count of Entodinium and Holotricha as count for protozoa population where use of marjoram significantly increases both, Garlic 1gm/kg DM increase entodinium but had no significant effect for holoticha other treatment did not effective compared to control.

Blood Biochemical Parameters

Table 5 illustrated the effect of treatments on some blood plasma parameters and explain that there are significant different between treatments and control on some of them like total protein and liver function parameters, but all values were in normal range of pregnant ewes. Use of dill, fennel and garlic (1gm/kg DM) increased blood total protein and plasma globulin whereas marjoram had no significant effect on plasma total protein but increase globulin and had no significant effect on liver function blood parameters, all additives decreased creatinine.

Table 2: Effect of supplementing diet with different levels of garlic, fennel, dill and marjoram on ruminal nutrient degradability

Items		Control	D1	D2	D3	D4	D5	D6	Overall mean
	DMD	43.066 ^{cd}	-	-	-	-	-	-	-
	OMD	45.667 ^h	-	-	-	-	-	-	-
T1	DMD	-	47.999°	36.199 ^{hi}	65.666ª	30.733 ^j	66.533ª	52.733 ^b	49.977ª
	OMD	-	45.583 ^h	44.167 ^h	54.500^{fg}	43.000 ^h	53.250^{fg}	50.250gh	48.458 ^b
T2	DMD	-	38.933 ^{fghi}	50.333 ^{bc}	36.820 ^{hi}	66.399ª	49.133 ^{bc}	35.733 ⁱ	46.225 ^b
	OMD	-	45.083 ^h	49.417 ^{gh}	44.233 ^h	55.333^{fg}	43.083 ^h	43.667 ^h	46.803 ^b
Т3	DMD	-	37.666 ^{ghi}	46.466 ^{cde}	40.799^{fgh}	38.933 ^{fghi}	38.933 ^{fghi}	39.333 ^{fghi}	40.355 ^d
	OMD	-	63.668 ^{cde}	78.251ª	63.418 ^{cde}	58.001 ^{ef}	59.334 ^{def}	59.501 ^{def}	63.596ª
T4	DMD	-	37.199 ^{hi}	42.266 ^{efg}	53.266 ^b	50.866 ^{bc}	42.133 ^{efg}	46.733 ^{cde}	45.410 ^{bc}
	OMD	-	64.334 ^{bcde}	46.251 ^{bcde}	71.084 ^b	69.251 ^{bc}	65.668 ^{bcd}	49.251 ^{gh}	63.973ª
Overall mean	DMD		42.04 ^c	43.82°	49.14 ^a	46.73 ^b	49.18 ^a	43.63°	45.47
	OMD		52.31 ^{cd}	59.02ª	58.31 ^{ab}	56.4 ^{ab}	55.33 ^{bc}	50.67 ^d	55.11

Means in the same raw having different superscript letters are significantly different (P<0.05).

Table 3: Dry matter intake and digestibility of nutrient in lambs' rations contain experimental feed additives

	÷							
Items	T1 (Control)	T2	T3	T4	T5	T6	Overall mean	P Value
DM intake (kg)	0.928^{f}	1.195 ^b	1.101 ^d	1.141°	1.328 ^a	0.968°	1.11	0.0001
			Dige	estibility %				
Dry matter	59.05 ^{bc}	63.91ª	59.17 ^{bc}	60.44 ^b	57.55°	58.43°	59.76	0.0001
Organic matter	58.11 ^d	66.32ª	60.95 ^b	60.34 ^{bc}	57.51 ^d	58.66 ^{cd}	60.32	0.0001
Crude protein	57.54 ^{ab}	50.8 ^{cd}	56.86 ^{bc}	47.71 ^d	50.65 ^{cd}	63.4ª	54.48	0.0014
TDN	55.88ª	46.84 ^{cd}	44.15 ^d	55.8ª	48.90 ^{bc}	52.56 ^{ab}	50.69	0.0002

Means in the same raw having different superscript letters are significantly different (P<0.05).

Table 4: Ruminal pH, TVFAs and protozoa populations of sheep fed experimental rations

Parameters		Mean	P value							
	T1(control)	T2	T3	T4	T5	T6				
pН	6.53	6.43	6.46	6.46	6.5	6.48	6.478	0.0001		
TVFAs (mg/ dl)	18.6 ^c	19.1 ^{bc}	18.7°	20.7ª	20.2^{ab}	20^{ab}	19.5	0.023		
Protozoal populations (*	* 10 ⁵ cells/mL)									
Entodinium	32.56 ^{ab}	22.3°	27.92 ^{bc}	27.49 ^{bc}	35.52ª	24.33°	28.3	0.0002		
Holotricha	2.12 ^{ab}	1.61°	2.10 ^{ab}	2.10 ^{ab}	2.24 ^a	1.73°	1.99	0.016		
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Means in the same raw having different superscript letters are significantly different (P<0.05).

Table 5: Blood parameters of sheep fed the experimental rations

Items	Rations								
	T1 (Control)	T2	T3	T4	T5	T6			
Total Proteins (g/dL)	7.081°	7.548 ^b	6.9°	8.136 ^a	7.99ª	6.77°	7.403		
Albumin (g/dL)	4.351 ^a	4.205 ^a	4.395ª	4.568ª	3.475 ^b	3.314°	4.047		
Globulin (g/dL)	2.730°	3.343 ^b	2.506°	3.568 ^b	4.517 ^a	3.459 ^b	3.356		
ALT (units/L)	17.660 ^a	17.39 ^{ab}	15.453°	16.421 ^{bc}	15.712°	18.155ª	16.796		
AST (units/L)	36.354 ^{cd}	34.910 ^d	39.723ª	37.514 ^{bc}	32.114 ^e	39.306 ^{ab}	36.651		
Creatinine (mg/dL)	0.876ª	0.844 ^{ab}	0.822 ^{ab}	0.813 ^b	0.815 ^b	0.733°	0.817		

Means in the same raw having different superscript letters are significantly different (P<0.05).

Table 6: Growth rate and feed utilization efficiency for lambs fed experimental diet

	T1	T2	T3	T4	T5	T6	Mean
DM intake (kg)	0.93 ^f	1.19 ^b	1.1 ^d	1.14°	1.33ª	0.96 ^e	1.11
IBW (kg)	15.6	22.2	19.5	18.1	23.6	15.2	19.03
FBW(g)	37.4 ^f	46.1°	43.4 ^d	47.1 ^b	52.3ª	40.1 ^e	44.4
ADG (gm)	121.11 ^e	132.78 ^d	132.78 ^d	161.11ª	159.44 ^b	138.33°	140.93
Average weight (kg)	26.5 ^f	34.15 ^b	31.45 ^d	32.6°	37.95ª	27.65 ^e	31.72
	Fee	d utilization ef	ficiency (g/g)				
DM/Gain	7.66	9	8.29	7.08	8.33	6.99	7.89
TDN/Gain	5.21	4.53	4.33	4.78	4.91	4.54	4.72
DCP/gain	0.52	0.67	0.49	0.48	0.49	0.39	3.04

Means in the same raw having different superscript letters are significantly different (P<0.05).

Dry Matter Intake and Growth Performance

Data of feed intake and feed utilization efficiency are presented in Table 6 Which showed that generally used of additives increased dry matter intake (DMI) compared to control group, especially the use of dill increased feed intake approximately 43% more than control group and that may be due to the dill content of essential oils which improved appetite and that reflected positively on final bwt and average daily gain. Total bwt gain (kg) and daily bwt gain (gm) were significantly affected with treatments (T2, T3, T4, T5 and T6) compared with control T1 specially T5 (Dill treatment).

DISCUSSION

According to the result for our recent study use of medicinal herbs as feed additives increased feed intake in treatment groups compared with control group, and that agree with study reported that use of fennel and marjoram as feed additives increased DMI and average daily gain (Cabuk et al. 2003) who return the increase in DMI to fennel content of anethol and estragol which have positive effect on appetite. Yang et al. (2010) and Geraci et al. (2012) explained the main point according to type of ration ingredient, animal growth stage and a rumen microbial adaptation or interaction between both of rumen population, ration ingredient and feed additives essential oils. Generally, Ruíz García et al. (2011) noted that use of the herbal extract with lambs' diet was increased the dry matter intake the increase in DMI for treatments may return to the effect of medicinal herbs essential oils content which

had positive effect on appetite. Hart et al. (2008) explained selectivity of protein degradation when using essential oils and their component to the effect of this compounds on certain species of bacteria which explain differentiation on CP digestibility in our study. Other nutrient digestibility may be affected with medical herbs content of essential oils like carvacrol which Zamiri et al. (2015) reported that in its study had no positive effect on digestibility in rams. The same for Khalesizadeh et al. (2011) who reported that use of garlic oil or turmeric powder had no effect on apparent digestibility in sheep. While had effect on CP digestibility on in-vitro study (Kongmun et al. 2010) this difference to the recent study may be due to the use of various herbs used in the study and the essential oils content.in current study, the use of medical herbs as feed additives improves both of final bwt and daily gain for lambs significantly compared with control group. It may be reverse to the ability of additives to improve rumen fermentation, use of diet contain feed additives had no significant effect on pH and that agree with results of Vahabzadeh et al. (2021). Whereas rumen TVFA's concentration increased the same result found for (Chaves et al. 2008) which played the main role as metabolizable energy for ruminants, in recent study we noticed decrease in protozoa in both garlic 1gm/Kg DM and marjoram treatment which indicates an improvement in protein digestibility and ammonia nitrogen decreased and reflected on average daily gain and final weight (Williams and Coleman 1997).

Immune response and immunity statues are affected with using Garlic, Fennel, Dill and marjoram as feed additives because its antioxidant, antimicrobial response from essential oils compounds which it's had all blood parameters in normal range due to Piccione et al. (2009).

Conclusion

It is concluded that supplementation of medicinal plants as feed additives to diets of Rahmani lambs and ewes at different levels has positive of beneficial effects of digestion, feeding values and feeding efficiency.

Authors Contributions

HSA: conducted the study, collected, and analyzed the data, wrote the first manuscript draft, ASM: Revised manuscript; KMM: Revised manuscript; SFA: Designed and supervised study; Revised manuscript. All authors read and approved the final manuscript.

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