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PRODUCTIVE PERFORMANCE OF WEANED MALE SHEEP FED DIETS SUPPLEMENTED WITH GARLIC ADDITIVES

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ABSTRACT

Twenty one male Barki lambs, which were apparently healthy, of nearly the same body weight (20.43 ± 0.21) were used in this study to investigate the effect of garlic powder and garlic extract supplementation on rumen feremntation, cellulolytic bacteria acount and growth performance of growing lambs. Lambs were allotted into three equal groups, first group fed on the basal diet without any supplement while second group fed on the basal diet and garlic powder (2% of DM) , the 3rd group was weekly dosage with 25 ml of garlic extract for each lamb for continuous 12 weeks. Statistical analysis of the obtained data indicated that the supplementation of garlic powder and garlic extract in rations did not affect the daily intakes of concentrate, roughage and total feed. However, both of garlic powder and garlic extract treatments significantly ($P \leq 0.01$) decreased ammonia nitrogen concentrations values at 4h post feeding as compared with untreated group. While, the TVFAs concentration showed an opposite trend to that of NH3-N where both treated groups (garlic powder and garlic extract) were significantly ($P \leq 0.01$) higher than that of control group at 4h post feeding samples. Beside, a significant increase ($P<0.05$) in total cellulolytic bacteria counts by garlic powder and garlic extract treatments compared to control diet after 6 and 12 weeks of experiment. Despite of that the average daily gain for lambs fed garlic powder rations (T1) and their mates which doesed garlic extarct (T2) increased by 11.3% and 22.7%, respectively compared with control ration. It was found that, the total feed intake cost was higher by 11.2% and 19.5% for T1 and T2 respectively, compared with control ration. Generally, diets supplemented with garlic powder or its extarct garlic had significant improving on growth performance of growing lambs and feed efficiency. While, it is not economically recommended for fattening sheep under desert conditions.

Key words: Garlic extract, Garlic powder, rumen fermentation, cellulolytic bacteria, daily gain, weaned sheep

INTRODUCTION

Over the last decades, antibiotic as growth promoters have been included successfully in ruminant animal diet additives to improve animal performance besides reducing diseases probabilities (El-Katcha et al. 2016). Nevertheless, the World Health Organization (WHO/OIE/WHO, 2004) and the Food and Agriculture Organization (FAO) have drawn attention to the threat of the transfer of antibiotic resistant pathogens to humans beside the hazard of their residues in meat and milk on human health (FAO/OIE/WHO, 2004). Therefore, the European Union has prohibited their use (EC, 2003).

In recent years, aromatic plants and their extracts have received increased attention as potential alternatives to growth promoters. Garlic (*Allium sativum*) has been used as spice and folk medicine since antiquity (Rivlin, 2001). Bioactive components of garlic, including several sulfur-containing compounds such as alliin, diallylsulfides and allicin, may partly account for some effects of garlic (Chouhan et al. 2023). In this regard, effects of garlic and its bioactive components have been partly demonstrated on rumen manipulation (e.g. defaunation, decreased methane production, decreased ruminal degradation of dietary proteins, reducing the proportion of acetate and increasing that of pro-pionate) and consequently on animal production and performances (Kholif et al., 2012). This experiment was conducted to investigate the effect of garlic powder and garlic extract supplementation on feed intake, rumen fermentation pattern, celluletic bacteria and growth performance of fattening lambs under desert conditions.

2. MATERIAL AND METHODS

2.1. Experimental location:

This study was conducted at Maryout Research Station belongs to Desert Research Center (DRC), 35 km south of Alexanderia, Egypt during November 2022 to February 2023. The experimental procedures were approved by the Animal and Poultry Production Division of DRC committee and as followed by the Veterinary and Animal Care Department.

2.2. Experimental animals and diets

Twenty one male Barki lambs (20.43 ± 0.21) kg initial body weight and 4-5 months of age) were selected for 12 weeks experiment and divided into three groups (n=7). Animals received the same basal diet that consisted of 70% concentrates feed mixture (CFM) and 30% Alfalfa hay (*Medicago sativa*) that was formulated to meet the growing lambs' feeding requirements. The control group (C) received the basal diet without additives; the second group (T1) received the basal diet supplemented with garlic powder as 2% of DM intake while the third group (T2) was weekly dosage with 25 ml of garlic extract for each lamb. The compositions of animal diets are presented in Table 1. The weights of the lambs were recorded at the beginning of the experiment then biweekly to calculate offered feed depending on the changes of live body weight. Lambs were housed in shaded pens and drinking water was offered twice a day.

Table 1. Chemical composition and cell wall constitutes of Alfalfa hay and concentrate.

Item	Alfa alfa hay	Concentrate
Dry matter	87.98	85.91
Chemical analysis (%) on DM basis		
Organic matter	89.73	93.82
Crud protien	15.93	14.89
Ether extract	0.98	3.36
Crud fiber	34.15	6.3
Nitrogen free extract	38.67	69.54
Ash	10.27	6.18
Cell wall constitutes (%)		
NDF	44.84	42.94
ADF	38.04	20.66
ADL	6.80	1.41
Hemicell.	13.06	22.28
Cellulose	24.98	19.25
Minerals content		
Ca ,% DM	4.21	0.64
Mg,% DM	0.64	0.19
P ,% DM	0.37	0.62
K,% DM	3.46	3.21
Na,% DM	0.63	0.46
Mn, (mg/kg DM)	67.00	23.52
Cu ,(mg/kg DM)	7.00	2.53
Zn ,(mg/kg DM)	11.00	18.53
Fe, (mg/kg DM)	3138	180.66
Co, (mg/kg DM)	0.26	-

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.3. Preparation of the garlic extract

Fresh garlic was obtained at local supermarkets. The outer skin of the garlic cloves was peeled off. The garlic cloves were cut into small cubes (approximately 0.3 cm on all side) using a kitchen knife then were air dried for a week and milled. The grinding garlic was soaked overnight in Methanol (1:4 w/v). The methanol was evaporated at a rotary evaporator. The extraction was repeated once more to produce a crude extract. The residue was then dried and stored at -4 °C (Safithria et al., 2011). Allicin concentration in garlic aqueous extract was 26.8 ml/L determined by UPLC (Haiping Wang et al. 2010). The extract was diluted by adding 1 liter of distiller water with 250 gm of dried extraction to get 20% concentration

2.4. Chemical analysis of feed ingredients

Ration ingredients were analyzed for DM and ash, Crude fiber (CF), Crude protein

(CP = Nitrogen% x 6.25) and ether extract (EE) contents according to AOAC (1997). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were analyzed sequentially (Van Soest *et al.,* 1991) using the Ankom200 Fibre Analyzer for NDF and ADF and thereafter soaking the residual with 72% sulfuric acid for 3 hours.. The NDF content was analyzed with 2 additions of heat-stable a-amylase and 1:1 g sodium sulfite per gm sample in the neutral detergent solution (Hansen et al., 2016). NDF and ADF are expressed inclusive of residual ash and hemicellulose and cellulose calculated from NDF, ADF and ADL values. The nitrogen free extract (NFE) was obtained by the difference.

2.5. Sampling of rumen liquor

Rumen liquor samples were taken by stomach tube from all animals at 45 and 90 day of the experiment. The samples were taken before morning feeding (zero-time) and at 4hours post-feeding. The rumen samples were filtered through two layers of cheese-cloth and used quickly as possible for measurement of pH by using Beckman pH meter. Rumen liquor was stored in plastic bottles with a few drops of 0.1 N HCl and stored at a deep freeze (-20˚C) till analysis. Rumen NH3-N and total VFA were determined by steam distillation in Kjeldahl distillation equipment according to the methods of Warner (1964) and AOAC (2005), respectively.

2.6. Preparation of bacterial cultures

Five strains of cellulolytic bacteria were isolated from rumen fluid of three animals in each group and were grown as pure cultural. The separated strains were *Cellulomonas cellulasea, Acetobacter xylinum , Thermonospora fusca , Bacillus sp. and Ruminococcus albus.* The isolation of species used the pour-plate technique for pure preparation of cultures according to A.T.C.C. (1992). The rumen samples were immediately gassed with CO2 and viable counts of rumen cellulolytic bacteria were determined according to the method described by Moir (1951) and Gall *et al*.(1945) and their classification were done according to Pounden and Hibs (1948) and Sleat *et al.* (1984).

2.7. Statistical analysis

Data of the digestibility trial were subjected to one way analysis of variance according to SAS (1998).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Y_{ij} : the observation of j^{th} animal treated with i^{th} treatment

μ : overall mean

T : type of ration

e : experimental error

3. Results and Discussion

3.1. Voluntary Feed intake

Data of concentrate feed mixture, roughage (alfalfa hay) and total feed intakes of the three tested rations are shown in Table 2. At the first month of the experiment, all animals were given the nutrient requirements for maintenance and growth (restricted feeding) according to body weights, therefore total dry matter intakes was similar for all animal groups. At the second and third month of the experiment, the supplementation of garlic powder and garlic extract in T2 and T3 did not affect the daily intakes of concentrate, roughage and total feed. These results were in agreement with those obtained by Rongzhen Zhong *et al.* (2019) for

lambs fed rations included 50g /kg DMI of garlic powder and Kewan *et al.* (2021) for lambs fed rations included 40 g /h/d of garlic powder and Ruchita Khurana *et al.* (2023) for dairy cows fed rations consisted of garlic and citrus extract.

Table 2. Feed intake of weaned male sheep during the fattening period

Items	Experimental groups			
	C	T1	T2	SEM
Lambs No.	7	7	7	
First month				
CFM as DM, kg/h/d	0.500	0.500	0.500	--
Roughage as DM, kg/h/d	0.250	0.250	0.250	--
TDMI, kg/h/d	0.750	0.750	0.750	--
Second month				
CFM as DM, kg/h/d	0.814	0.771	0.786	0.13
Roughage as DM, kg/h/d	0.357	0.306	0.343	0.07
TDMI, kg/h/d	1.171	1.078	1.129	2.04
Third month				
CFM as DM, kg/h/d	1.071	1.050	1.099	0.01
Roughage as DM, kg/h/d	0.471	0.460	0.470	0.14
TDMI, kg/h/d	1.543	1.510	1.569	0.08
overall means of DMI (kg/h/d)	1.155	1.113	1.149	0.02
Total Protein intake (kg/h/d)	0.176	0.170	0.176	1.48
Total TDN intake (kg//h/d)	0.797	0.768	0.793	178

**C: control ration, T1: control ration plus 2% of GP, T2: control ration plus 25 ml of GE.*

3.2. Rumen activity

3.2.1. Rumen fermentation parameters

Ruminal pH values, concentrations of NH3-N and TVFAs are shown in Table (3). The pH value was higher at zero than that at 4 hours post feeding for different periods of experiment (after 6 and 12 days). Gradual decreasing of rumen pH with progressing time could be due to fermentation caused by feeding. It can be observed that, the pH values were not affected by garlic treatment. The same results were obtained by (Nassar et al. 2017 for lactating ewes, Abu El-Kassim et al., 2018 for Ossimi ewes and Ruchita Khurana et al., 2023 for dairy cows). The overall means of pH values after 45 days and 90 days of the experiment were within the normal range (4.96-7.92) reported for sheep, which is suitable for maximal cellulytic activity and microbial

protein synthesis (Kewan et al., 2017).

Otherwise, after 6 and 12 weeks of the experiment, data showed that both of garlic powder (T1) and garlic extract (T2) treatments significantly ($P \leq 0.01$) decreased ammonia nitrogen concentrations values at 4h post feeding as compared with untreated group, while, the TVFAs concentration showed an opposite trend to that of NH3-N where both treated groups (garlic powder and garlic extract) were significantly ($P \leq 0.01$) higher than that of control group at 4h post feeding samples. Previous studies showed that concentration of ammonia-N decreased significantly ($P < 0.05$) by sheep fed garlic powder diets (Abu EL-Kassim et al., 2018) or by dairy cows fed garlic extract diets (Ruchita Khurana et al., 2023) as compared to that fed control diet. This decline may be attributed to the increased incorporation of ammonia in microbial protein, and the stimulation of microbial activity, or it can be a direct effect of garlic additives on the reduction of CP degradation and amino acids deamination resulting improvement in protein digestion and metabolism. (Rongzhen et al. 2019). These findings are in agreement with that obtained by El shereef (2020) when they stated that the apparent digestibility of DM and CP increased by garlic powder supplementation. While the increasing of TVFAs as affected by garlic powder and their extract was supported by Yang et al. (2007) who reported that the supplementation of garlic powder at 5 g/d/head was able to increase the DM and OM digestibility from 49.4% to 55.2% and 49.6% to 55.4%, respectively, which enhance the VFA concentration.

Table 3. Rumen fermentation parameters of weaned male sheep during the fattening period

Items	Experimental groups				SEM
	Time	C	T1	T2	
After 6 weeks					
pH	0hr	7.14	7.4	7.11	0.04
	4hr	6.71	6.51	6.5	0.02
	Mean	6.9	7.0	6.8	0.18
NH3-N (mg/dL)	0hr	18.6	16.25	19.2	1.23
	4hr	26.4[a]	21.8[b]	22.7[ab]	6.04
	Mean	22.5	19.0	21.0	2.70
TVFA(meq/dL)	0hr	6.5	8.1	7.8	0.02
	4hr	9.54[c]	11.69[a]	10.28[b]	2.89
	Mean	8.0	9.9	9.0	0.72
After 12 weeks					
pH	0hr	7.75	7.45	7.54	0.31
	4hr	6.69	6.73	6.67	0.27
	Mean	7.2	7.1	7.1	0.54
NH3-N (mg/dL)	0hr	21.1	20.4	19.6	3.05
	4hr	26.4[a]	23.5[b]	22[b]	4.16
	Mean	23.8	22.0	20.8	1.56
TVFA(meq/dL)	0hr	6.27[c]	8.38[b]	9.25[a]	0.02
	4hr	10.3	11.8	11.5	0.56
	Mean	8.3	10.1	10.4	1.02

[a,b,c] *means at the same row with different superscript are significantly (P<0.05) different.. C: control ration, T1: control ration plus 2% of Garlic Powder, T2: control ration plus 25 ml of Garlic Extract.*

3.2.2. Cellulolytic bacteria

The data showed a significant increase (P<0.05) in total cellulolytic bacteria counts by garlic powder addition and garlic extract treatment compared to control diet after 6 and 12 weeks of experiment (Table 4). The same trend was observed with *Cellulomonas cellulasea; Acetobacter xylinum; Thermonospora fusca; Bacillus sp and Ruminococcus albus*. These results mainly due to that diets supplemented with garlic powder and their extract enhance and stimulate activity of cellulolytic bacteria which capable of hydrolyzing fiber in rumen that clarify the higher VFAs concentration in T1 and T2 as shown in Table 3. Similarly, Ma et al. (2016) reported that dietary supplementation with allicin, which is a secondary plant metabolite in garlic bulbs, decreased the number of protozoa and increased the number of total bacteria and cellulolytic bacteria (F. succinogenes, R. flavefaciens, and B. fibrisolvens) in 12-mo-old ewes for 42. The current study suggests that garlic powder and their extract could modify the microbial population profile, improving of cellulolytic bacteria activity, and enhance VFAs concentration. These findings are in agreement with those obtained by Rongzhen et al. (2019) and El-Naggar and Ibrahim (2018) for growing lambs. On an opposite trend, Yan et al. (2020) and Yang et al. (2021) found that the supplementation of garlic products not affect all nutrients digestibility and rumen VFA concentration

Table 4. Count of cellulolytic bacteria in rumen fluid (No. of bacteria x 10^7 /ml rumen liquor) of weaned male sheep during the fattening period .

Item	Time	Experimental groups			
		C	T1	T2	SEM
After 6 weeks					
Cellulomonas cellulasea	0hr	26.1b	41.4a	21.1b	2.10
	4hr	40.1b	67.2a	42.1b	0.09
Acetobacter xylinum	0hr	30.4 b	40.3a	42.7a	1.02
	4hr	42.9c	62.0a	58.1b	0.08
Thermonospora fusca	0hr	36.6 a	32.2b	27.4c	1.07
	4hr	47.2b	55.2a	44.7b	0.06
Bacillus sp	0hr	34.1a	30.4b	30.1b	0.04
	4hr	45.9b	58.6a	45.8b	0.16
Ruminococcus albus	0hr	33.8c	40.0b	44.6a	1.18
	4hr	48.7c	60.4b	68.6a	0.18
Total count		38.58c	48.77a	42.52b	0.07
After 12 weeks					
Cellulomonas cellulasea	0hr	36.3b	41.7a	37.7b	0.35
	4hr	52b	66.7a	44.7c	0.54
Acetobacter xylinum	0hr	37.3c	47.3b	49.3a	1.02
	4hr	50c	65.3a	62.0b	0.33
Thermonospora fusca	0hr	39.3a	31.7c	38.0b	0.41
	4hr	54.3c	58.7b	59.7a	0.02
Bacillus sp	0hr	40.0a	38.7c	39.3b	0.63
	4hr	52.3b	58.7a	51.7b	0.96
Ruminococcus albus	0hr	28c	36.0b	39.7a	0.91
	4hr	50.3c	64.3b	66.7a	0.09
Total count		43.9c	50.9a	48.8b	1.02

[a,b,c] means at the same row with different superscript are significantly (P<0.05) different.. C: control ration, T1: control ration plus 2% of Garlic Powder, T2: control ration plus 25 ml of Garlic Extract.

3.3. Average daily gain and feed conversion:

Growth performance data are summarized in Table (6). It was observed that total dry matter intake (TDMI) was not affected by different additives. Chouhan et al. (2023) found that feeding garlic did not result in any significant change in voluntary dry matter intake for goat kids. The total digestible nutrients (TDN) and total crude protein intakes followed the same trend of TDMI. However, values of average daily gain for lambs fed T1 or T2 rations were significantly surpassed those fed control ration. The ADG for T1 and T2 group increased by 11.3% and 22.7% compared with control ration, respectively. Increasing ADG reflected on total gain during all period. Increasing ADG for garlic additives groups (T1 and T2) may be due to improving of cellulolytic bacteria activity and enhance VFAs concentration as shown in previous results

which probably enhanced the utilization and the availability of essential nutrients (Kewan et al. 2019).

The feed conversion efficiency showed that the use of garlic powder or garlic extract for feeding weaned rams resulted in better gain and also better feed conversion ratio. In agreement of present result, Duvvu et al. (2018) observed a significant ($P<0.01$) improvement in the body weight gain, average daily gain, body condition score and feed conversion efficiency in garlic supplemented buffalo calves. Similarly, Zivkovic et al. (2019) showed that the use of fermented garlic powder, instead of antibiotics, resulted in better gain and also better feed conversion ratio, in piglet. Strickland et al. (2009) also reported that inclusion of raw garlic in diet of Merino lambs aged 6 months decreased the rate of feed conversion.

Economic efficiency indicators in the present study indicated that daily cost of CFM and roughage was nearly similar. While, the total feed intake cost was higher by 11.2% and 19.5% for T1 and T2 respectively, compared with control ration. The higher cost of total feed intake was due to the additional cost of feed additives compared with control ration. The higher price of total feed cost led to increase in cost of 1 kilo gram gain. Economic efficiency is affected by many factors such as the price of feed ingredients, DM intake, total weight gain and feed conversion. In this study, the best economic efficiency recorded by control ration followed by garlic powder and the worst was observed by garlic extract additive.

Table 5. Lambs performance, and economic efficiency indicators for the experimental rations.

Items	Experimental groups			
	C	T1	T2	SEM
IBW, Kg	20.79	20.29	20.21	---
ADG, Kg	0.203[b]	0.226[a]	0.249[a]	1.78
Total gain, Kg	20.31[b]	22.61[a]	24.89[a]	1.08
TDMI (kg/h/d)	1.155	1.113	1.149	0.02
TCPI (kg/h/d)	0.176	0.170	0.176	1.48
TDNI (kg//h/d)	0.797	0.768	0.793	178
Feed conversion				
DM intake/ Gain, kg/ kg	7.60a	6.68b	6.30b	0.15
DCP intake/ Gain, kg/ kg	0.870a	0.753b	0.707c	0.08
TDN intake/ Gain, kg/ kg	3.923a	3.395b	3.185c	0.03
	Economic efficiency indicators			
Cost of daily concentrate, LE	9.17	8.92	9.17	
Cost of daily Roughage, LE	1.58	1.49	1.56	
Cost of daily feed additives ,LE	0	1.54	2.12	
Cost of total feed intake, LE	10.75	11.95	12.85	
Cost of 1 kg gain,LE	52.96	58.87	63.30	

[a,b,c] *means at the same row with different superscript are significantly (P<0.05) different.. C: control ration, T1: control ration plus 2% of Garlic Powder, T2: control ration plus 25 ml of Garlic Extract.*
CFM= 11530 LE/ ton, garlic powder = 70 LE/ kg, Hay= 4400 LE/ ton, garlic extract = 530 LE/ litre,

4. Conclusion

In conclusion, garlic extract or garlic powder supplementation to ration of weaned Barki lambs had beneficial effects on rumen fermentation, growth rate and improves feed efficiency. While, it is not economically recommended for fattening sheep under desert conditions.

Disclosure of conflict of interest

This work was collaboratively carried out between all authors as follows: Author El Shereef AA. designed the study, managed the literature, chemical analysis and writes the manuscript. Author Nassar MS and Abo Bakr S performed the field work and data collecting.

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