IMPACT OF ADDING AJWAIN SEEDS TO THE MALE LAMBS' RATIONS AS GROWTH PROMOTERS

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ABSTRACT

This study was conducted to investigate the impact of adding ajwain seeds to the rations of the growing Ossimi ram lambs on growth performance, nutrient digestibility, rumen liquor fermentation, and blood biochemical parameters. A total of 15 ram lambs, 5-6 months of age, were randomly allocated to the 3 treatments (5 lambs each). Lambs were fed a basal diet and regarded as a control group (T1), while the second (T2) and third (T3) treatments were fed on a basal diet plus 0.2 and 0.4 % of ajwain seeds respectively. At the end of the trial (150 days), three sheep from each treatment were used for a fourteen-day digestibility trial. Feed intake was similar for all groups. The treated groups exhibited higher (P<0.05) average daily gain and lesser (P<0.05) feed conversion ratio than the T1 group. All nutrients digestibility and nutritive value (TDN and DCP) were significantly higher (P<0.05) in the T2 and T3 groups than in the T1 group. The blood attributes were within the reference values of healthy lambs. Serum cholesterol, triglyceride, LDL, and MDA levels were decreased (P<0.05), whereas, albumin, HDL and TAC concentrations increased in the treated than the control groups. I<n conclusion, adding ajwain seed (0.2 and 0.4%) to the ram lambs' ration can enhance the animal health and promote the growth performance.

Keywords: nutrients digestibility, growth performance, blood parameters, lambs.

تأثير إضافة بذور النخوة الى علائق ذكور الحملان كمحفزات للنمو

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المستخلص

أجريت هذه الدراسة لبيان تأثير إضافة بذور النخوة إلى علائق حملان الأوسيمي الذكرية النامية في أداء النمو وهضم العناصر الغذائية وتخمرات سائل الكرش ومعايير الدم الكيمياحيوية. تم استخدام 15 حملاً ذكرياً من سلالة الأوسيمي بعمر 5– 6 أشهر قسمت عشوائيًا إلى ثلاثة معاملات متساوية (5 حملان لكل معاملة). غذيت الحملان على العليقة الأساسية وعدت كمجموعة سيطرة (T1)، في الوقت الذي غذيت فيه المعاملتين الثانية (T2) والثالثة (T3) على العليقة الأساسية مضافاً لها 0.2 و0.4 % من بذور النخوة على التوالي. في نهاية التجربة (150 يومًا)، تم استخدام ثلاثة أغنام من كل معاملة في تجارب الهضم لمدة 14 يومًا. كان مقدار العلف المتناول متماثل لدى المجاميع كلها. أظهرت المجاميع المغذاة على بذور النخوة أعلى (P<0.05) معدل زيادة وزنية يومية وأقل (P<0.05) معدل تحويل غذائي مقارنةً بمجموعة السيطرة. كانت جميع العناصر الغذائية القابلة للهضم والقيمة الغذائية متمثلة في المركبات الكلية المهضومة والبروتين الحقيقي المهضوم أعلى بشكل ملحوظ في المجموعتين المغذاة على بذور النخوة مقارنةً بمجموعة السيطرة. كانت معايير الدم ضمن المعدل الطبيعي لها للحملان الصحية. انخفض (P<0.05) مستوى الكولسترول والدهون الثلاثية و LDL وMDA ، في حين ازدادت تراكيز الألبومين، وHDL وTAC في المجاميع المعاملة مقارنة بمجموعة السيطرة. يمكن الاستنتاج بأن إضافة بذور النخوة (0.2 و0.4 %) الى علائق الحملان يمكن ان يحسن من صحة الحيوان ويحفز من اداء النمو.

الكلمات المفتاحية: هضم العناصر الغذائية، أداء النمو، مؤشرات الدم، الحملان.

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INTRODUCTION

Since the year 2006, the use of antibiotics as growth promoters has been banned by the European Commission due to its possible accumulation in animal products and could badly hurt consumers' health. This situation has forced nutritionists to find effective, safe, and low-cost economic alternative bioactive substances to replace antibiotics in the feeding of commercial livestock. In animal feeding, many medicinal and herbal plants are used as alternatives to antibiotics and have been shown as beneficial effects on pathogenic bacteria, health, and growth performance of different animals (11, 15, 17, 21, 24, 25). Ajwain (*Trachyspermum ammi L.*) is a medicinal plant, planted in Egypt in arid and semi-arid regions (3), its seeds have enormous health benefits which are attributed to their pharmacological, phytochemical and therapeutic properties like immunomodulatory, antifungal, antibacterial, antiprotozoal, antioxidant activities, growth promoters (8, 16, 35) and also decrease methane production (19). Ajwain seeds contain 2.5–5% essential oil (EO), and its composition varied with geographical location and season production, but in general, it consisted of 87.75 % thymol and 11.17 % caracole and 60.78 % p-cymene as non-phenolic components and small proportions of α-terpinene, γ-terpinene, β-pinene, and α-pinene (22). Also contains calcium, iron, cobalt, copper, phosphorous, iodine, magnesium, riboflavin, thiamine, and nicotinic acid (29). Zarshenas, et al. (40) reported that the chemical compositions of Ajwain seeds were 17.1 % crude protein, 11.9 % crude fiber, 24.6 % carbohydrates, glycosides, and phenolic compounds such as saponins, flavones, and tannins. Therefore, this study was conducted to assess the impact of different levels of ajwain seeds on the nutrient digestibility, growth performance, rumen microbial population, and blood parameters of growing Ossimi lambs.

MATERIALS AND METHODS

Experimental animals and design

This study was conducted at the Research and Production Station of the National Research Centre located in Noubaria, Egypt and the chemical analysis was in laboratories of the Animal Production Department of the National Research Centre. Basal total mixed ration (TMR), in a mash form, contained almost 14% CP and consisted of 30 % wheat straw, 27 % corn, 15 % soya bean meal, 24.5 % wheat bran, 1.3 % minerals and vitamins mixture, 0.8 % salts and 1.4 % limestone. Three experimental rations were fed to animals, basal ration (T1), the same basal ration with the addition of ajwain seeds by 0.2% (T2) and 0.4% (T3) from TMR. Ajwain seeds were purchased from a private herbs shop in Egypt. Animals were injected against internal and external parasites and rottenly vaccinated against seasonally infectious diseases. All animals were housed in an open shaded yard surrounded by steel fences and supplied with internal steel barriers to separate between animal groups.

Growth trial

The growth trial lasted 150 days, 15 healthy heads of Ossimi lambs (36.4 kg average live body weight and 6-7 months old) were randomly divided by weight into three equal groups (5 each) for 150 days. Animal groups were randomly assigned to one of the experimental rations to cover its requirements according to NRC (26), and amounts of TMR were adjusted every 15 days according to changes in live body weights. Clean drinking water was freely available at all times. Animals were fed rations twice daily at 8.0 a.m. and 4 p.m. Feed intakes were recorded daily; meanwhile, daily body weight gains and feed conversions (kg feed/kg gain) were calculated biweekly.

Digestibility trials

At the end of the growth trial, three animals from each experimental group were subjected for digestibility trials lasting 14 days; 7 days were for adaptation, and the 7 days were for the quantitative collection of feces. Animals were individually dwelled in metabolic cages, where feces were separately collected. Daily feed intake and feces output amounts were determined and recorded during the collection period. Feces samples were weighted and dried at 60°C /24 hrs. in a hot oven. Dried samples of feces and feeds were ground to pass through a 1-mm siev was stored in emeried bottles for chemical analysis. Meanwhile, nutrient digestibility and nutritive values of the experimental rations were

calculated. Rumen liquor was collected at the end of the digestibility trial via stomach tube before feeding and then after 3 hrs. after feeding. Samples of rumen liquor, for each animal, were filtered through 4 layers of cheesecloth, and then ruminal pH was immediately recorded using a digital pH meter then stored at -20 C for later ammonia and volatile fatty acids analyses.

Blood parameters

At the end of the growth trial, blood samples (5 ml) were individually collected from the jugular vein randomly from three animals in each group and kept in sterilized glass tubes, centrifuged at 3500 rpm for 15 min. to separate serum from the other blood contents then stored in clean sterilized tubes at − 18 °C until further analysis. Collected blood erythrocytes were homogenized and kept under − 40 °C to determine the total antioxidant capacity (TAC). plasma total protein, albumin, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and serum malonaldehyde (MDA) were determined calorimetrically (Biodiagnostic, Egypt), while globulin was calculated as the difference between total protein and albumin.

Chemical analysis

The chemical composition of Ajwain seeds, feeds, and feces was determined for dry matter (DM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE) and ash according to the standard methods of AOAC (2). Nitrogen-free extract (NFE) was calculated by difference. Collected rumen liquor was directly tested for pH using an Orian 680 digital pH meter. Rumen total ammonia-N (NH_3-N) was determined calorimetrically according to Searle (34). Total volatile fatty acids (VFA' s) of rumen liquor were determined using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a Flame Ionization Detector and a capillary column (HP-INNOWAX, 1909N-133, Agilent Technologies, Santa Clara, CA, USA).

Statistical analysis

Collected data concerning body weight gains, nutrients digestibility, rumen microbial population, and blood parameters were subjected to one-way analysis of variance according to Steel and Torrie (36) applying the general linear model procedure of SAS (36), while, data of the rumen liquor parameters were subjected to two-way analysis by the same previous procedure. Significant differences between means were calculated using Duncan's Multiple Range Test (9).

RESULTS AND DISCUSSION

Data from chemical analysis of the experimental rations are presented in Table 1, mentioning that feed rations were suitable for growing lambs according to NRC (26).

Table 1. Chemical composition as dry matter % of ajwain seeds and total mixed ration fed to Ossimi lambs

Nutrients, %	Ajwain seeds	TMR
Dry matter, DM	90.01	88.35
Crude protein, CP	16.10	13.68
Crude fiber, CF	17.60	15.93
Nutrient detergent fiber, NDF	31.50	37.60
Acid detergent fiber, ADF	27.30	19.45
Ether extract, EE	13.26	1.86
Ash	8.20	4.56
Nitrogen-free extract, NFE	44.84	63.97

Data of digestibility trials (Table 2) observed that the addition of ajwain seeds at a high level (0.4 %, T3) to the TMR significantly increased ($P<0.05$) all nutrients digestibility compared to the control group, the same results were observed with the low-level addition (0.2 %, T2) compared to the control group (T1), while there was no significant difference in organic matter digestibility (OMD), crude fiber digestibility (CFD), ether extract digestibility (EED) and neutral detergent fiber digestibility (NDFD) between T1 and T2. For DMD and OMD were higher ($P<0.05$) in T3 compared to the T2, whereas for other nutrients digestibility, there were no significant differences between them. The same results

were found when buffalo calves fed TMR with 0.05 ajwain oil that all nutrients digestibility improved compared to the control group, except that of CP which was comparable between the two groups (38). Nutritive values as total digestible nutrients (TDN) were significantly (P<0.05) higher for T2 and T3 by 8.28 % and 12.63 % and digestible crude protein (DCP) by 17.77 and 23.70 % respectively compared to the T1. The essential oil in ajwain seeds acted as a modulator for protein metabolism which resulted in enhancing the efficiency of protein utilization (28).

Table 2. Nutrient digestibility and nutritive values of experimental rations

Nutrient digestibility, %	Experimental rations			±SEM
	T1	T2	T3	
Dry matter	60.67[c]	66.67[b]	72.33[a]	1.80
Organic matter	64.67[b]	67.67[b]	77.00[a]	1.98
Crude protein	61.67[b]	72.67[a]	76.33[a]	2.34
Crude fiber	63.67[bc]	67.67[ab]	72.67[a]	1.55
Ether extract	72.67[bc]	74.67 [ab]	78.67[a]	1.09
Nitrogen free extract	72.67[b]	78.00[a]	80.33[a]	1.31
Neutral detergent fiber	65.67[bc]	70.00[ab]	73.00[a]	1.40
Acid detergent fiber	50.00	49.00	52.00	1.27
Nutritive values, %				
TDN	68.10[b]	73.74[a]	76.70[a]	1.35
DCP	8.44[b]	9.94[a]	10.44[a]	0.32

[a,b,c] Means with different superscripts within similar row differ significantly (P ≤ 0.05).

Data (Table 3) recorded that the ajwain seeds supplementation increased rumen pH but in the normal range for better nutrient digestibility (27). The overall mean of NH_3-N concentration in all groups was well above the minimum concentration required for optimal microbial growth (32).

Table 3. Rumen liquor fermentation of lambs fed ajwain seeds-supplemented rations

Items	Sampling time, hr.	Experimental rations			±SEM
		T1	T2	T3	
pH	0	6.20	6.20	6.2	
	3	5.00	5.40	5.53	
	Mean	5.60[b]	5.80[a]	5.87[a]	0.07
NH_3-N, 100 ml RL	0	17.27	16.77	15.90	
	3	20.47	19.77	18.23	
	Mean	18.87[a]	18.27[b]	17.07[c]	0.14
TVFA's meq/dl RL	0	7.43	8.10	8.90	
	3	10.63	10.73	12.53	
	Mean	9.03[a]	9.41[a]	10.72[b]	0.14

[a,b] Means with different superscripts within similar row differ significantly (P ≤0.05).

Whatever its concentration significantly decreased (P<0.05) with increasing the addition of ajwain seeds, this decrease may be due to that some essential oils included in the seeds have activity impact antihyper-NH_3-producing ruminal bacteria such as Peptostreptococcus anaerobic and as *Clostridium astyklandy* (6, 7, 20), also may be regarded to protein binding substances existing in ajwain seeds like tannins which might be reduced dietary protein degradability (11). The same results for the decreasing of ammonia nitrogen concentration were recorded by fistulated buffalo fed various parts of the ajwain plant (30) and with lambs fed diets supplemented with thyme essential oil (14). The overall mean of rumen total volatile fatty acids significantly (P<0.05) increased linearly with T2 and T3 by 4.21 and 18.72 %, respectively compared with T1. This increase in VFA 's concentration might be due to that rumen bacteria assimilate some of the released

amino acids and peptides into fermented amino acids or microbial protein to produce volatile fatty acids (4). These results were in agreement with the findings of Klevenhusen et al. (18), El-Naggar et al. (14 and 13), and Wadhwa and Bakshi (38), that dietary essential oils supplementation increased ruminal VFAs concentration. In general, the improvement in nutrient digestibility might be due to that herbs plants stimulate saliva secretion, which leads to the synthesis of bile in the liver, and pancreatic enzymes (23) and also enhances rumen microflora activity which increases the production of VFA's, and DM, OM and CF digestibility (1, 12). Data in Table 4 showed that there were no significant differences among groups in the initial and final body weight of lambs fed experimental rations, however adding ajwain seeds significantly (P<0.05) increased average daily gain by 12.76 and 17.70 %, respectively compared with those fed T1. These increases might be attributed to the high TDN and nutrient digestibility with T2 and T3, regarding to the presence content of essential oils in ajwain seed which increases the energy availability for animals (14, 39). Adding ajwain seed in the basal ration did not show any impact on feed intake.

Table 4. Effect of ajwain seeds supplementation on growth performance, feed intake, and feed conversion ratio of Ossimi lambs

Item	Experimental rations			±SEM
	T1	T2	T3	
Initial weight, IW, kg	36.40	36.40	36.20	1.89
Final weight, FW, kg	61.00	62.94	64.12	2.44
Average daily gain, ADG, g	162.00[b]	182.67[a]	190.67[a]	4.07
Feed intake, kg/d	1.28	1.30	1.35	-
Feed conversion ratio (kg feed/kg gain)	7.39	7.12	7.10	-

[a,b] Means with different superscripts within the same row differ significantly (P ≤ 0.05).

The daily DM intake was comparable among groups. Similar results on daily DM intake have been reported when the diet of lambs was supplemented with essential oils (13). Wadhwa and Bakshi (38) also reported that there was a non-significant effect on DM intake of buffalo calves fed diets supplemented with or without 0.05 % ajwain oil of TMR. Feed conversion ratio (kg feed/kg gain) enhanced for the rations with ajwain seed by 3.65 and 3.92 % for T2 and T3 respectively compared to T1. The same results were found when researchers used essential oils from thyme oil or *Nigella sativa* oil by two levels 0.1 and 0.2 % from DM intake, as feed additives in the diets of growing lambs (14). Table (5) demonstrated that adding a based diet with ajwain seeds did not have any adverse effect on the blood profile of Ossimi lambs and all the blood parameters were within the reference physiological values.

Table 5. Effect of ajwain seeds on blood biochemical parameters of Ossimi lambs

Item	Experimental rations			±SEM
	T1	T2	T3	
Total protein, g/dl	6.40[c]	6.70[b]	7.03[a]	0.10
Albumin, g/dl	2.90[b]	3.07[b]	3.30[a]	0.06
Globulin, g/dl	3.50	3.63	3.73	0.05
A/G Ratio	0.83	0.85	0.88	0.01
Cholesterol, mg/ dl	78.67[a]	74.00[b]	73.00[b]	1.02
Triglyceride, mg/ dl	32.00[a]	28.00[b]	24.00[c]	1.19
HDL, IU/l	31.00[c]	33.50[b]	35.30[a]	0.66
LDL, IU/l	44.67[a]	42.67[a]	38.00[b]	1.06
MDA, nmol/l	5.20[a]	3.93[b]	3.33[c]	0.28
TAC, mmol/l	0.90[c]	1.43[b]	1.63[c]	0.11

[a,b,c] Means with different superscripts within similar row differ significantly (P ≤ 0.05).

These results for blood profile were the same with found by Ebrahimi et al. (10) when dairy calves fed diets supplemented with ajwain seeds (2ml/day), and recorded by Wadhwa and Bakshi (38) when buffalo calves fed diets supplemented with 0.05 % ajwain oil. Meanwhile, Albumin, HDL, and TAC concentration increased (P<0.05) linearly, and cholesterol, triglyceride, LDL, and MDA decreased linearly with increasing the level of ajwain seeds addition compared to the control group, these results may be due to the inclusion of thymol and saponins in ajwain seeds (37), which is known to reduce cholesterol and triglyceride levels (33), also enhancing the TAC was by declined the MDA which known as non-enzymatic antioxidant activity improved the immunity and the health of animals. The same results agreed with Barwary et al (5) who reported that supplementing medicinal plants to lactating ewes' diet positively affected blood cholesterol levels. In conclusion, the phenolic compound content of ajwain seeds might be responsible for improving antioxidant activities and immunity. We can conclude that ajwain seeds could be considered as a beneficial feed additive used as growth promoters, that ajwain seeds at the two levels 0.2 or 0.4 % addition of TMR resulted in improving nutrients digestibility, rumen fermentation as NH_3-N and VFAs concentration, without any adverse effects on blood profile, in addition to its effect on enhancing blood TAC and MDA concentration which refer to high immunity and the good health of animals, and improved growth performance of lambs.

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