Journal homepage
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Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

GROWTH PERFORMANCE, RUMEN FERMENTATION, AND NUTRIENT DIGESTIBILITY OF AWASSI LAMBS FED DIFFERENT LEVELS OF ORGANIC COPPER IN A BASAL DIET

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Article info

Received: 2024-08-07
Accepted: 2024-09-12
Published: 2024-12-31

DOI-Crossref:
10.32649/ajas.2024.184733

Cite as:
Abdan, A. H., and Saeed, O. A. (2024). Growth performance, rumen fermentation, and nutrient digestibility of awassi lambs fed different levels of organic copper in a basal diet. Anbar Journal of Agricultural Sciences, 22(2): 1542-1556.

Abstract

This study investigated feed intake, growth performance, and rumen fermentation in Awassi lambs on diets supplemented with various levels of organic copper. A total of 24 eight-month-old male lambs weighing 25.5 ± 3.69 kg on average were studied over 90 days. The animals were divided randomly into four treatment groups of 6 lambs each, namely OCC (basal diet), OCF (basal diet + free of organic copper), OCC5 (basal diet + 0.5 g organic copper/kg DM), and OCC1 (basal diet + 1 g organic copper/kg DM). Samples of rumen fluid, feed intake, weight gain, and nutrient digestibility were collected during the trial and examined. The results showed that nutrient digestibility of organic matter and ether extract were higher ($P < 0.01$) in the OCC1 and OCC5 groups compared to the OCC. Similarly, there was a significant increase in concentration of acetic acid or/and NH_3 of OCC1 compared to OCC. CH_4 concentration was significantly lower in both OCC1 and OCC5 than the control group. The OCF treatment had a significant increase ($P < 0.01$) in pH compared to other treatments. No significant differences were found in daily weight gain, growth specific ratios, crude protein digestibility, crude fiber, and both isopropionic and propionic acids. The findings suggest that an Awassi lamb diet containing more than 0.5 g/kg copper feed over 90 days did not cause copper toxicity, improved rumen fermentation, and reduced methane emissions from the animals.

Keywords: Awassi sheep, Copper toxicity, Digestibility, Volatile fatty acid, Methane.

اداء النمو وتخمرات الكرش وقابلية هضم العناصر الغذائية لحملان العواسي المغذاة على مستويات مختلفة من النحاس العضوي في العليقة

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الخلاصة

هدفت هذه الدراسة الى تحديد تأثير اضافة مستويات مختلفة من النحاس العضوي في عليقة حملان العواسي في كمية العلف المستهلك وأداء النمو وتخمرات الكرش. أستخدم 24 حملاً ذكراً بعمر ثمانية أشهر وبمعدل وزن 5 .25 ± 3.69 كغم ولمدة 90 يومًا. وزعت الحيوانات بشكل عشوائي على أربع معاملات علفية شملت المعاملة الاولى (عليقة اساسية) والمعاملة الثانية (عليقة اساسية + بدون اضافة خليط المعادن) والمعاملة الثالثة (عليقة اساسية + 0.5 غم نحاس عضوي/ كغم علف) والعليقة الرابعة (عليقة اساسية + 1 غم نحاس عضوي/ كغم علف). جمعت العينات خلال التجربة لدراسة التغيرات الفسيولوجية في تخمرات سائل الكرش والزيادة الوزنية وقابلية الهضم للحملان. أظهرت نتائج الدراسة وجود اختلاف معنوي بمستوى $(P < 0.01)$ في معاملات النحاس (المعاملة الثالثة والرابعة) على باقي معاملات التجربة في قابلية هضم المادة العضوية ومستخلص الايثر. وكان هناك ارتفاع معنوي بمستوى $(P < 0.01)$ في تركيز امونيا الكرش وحمض الخليك في المعاملة الرابعة. وكان تركيز الميثان CH_4 منخفضا معنوياً في جميع المعاملات مقارنةً بالسيطرة. لوحظ هناك زيادة معنوية في الاس الهيدروجيني للمعاملة الثانية $(P < 0.01)$ مقارنة بباقي المعاملات. لم يكن هناك أي فروق معنوية في الزيادة الوزنية اليومية ونسبة النمو وقابلية هضم المادة الجافة والبروتين الخام والالياف الخام وكل من Iso propionic وكذلك propionic. يمكن ان نستنتج من هذه الدراسة ان تغذية الحملان العواسي على عليقة تحتوي على أكثر من 0.5 غم/ كغم علف ولمدة 90 يوما لم تسبب التسمم بالنحاس وقد أدت إضافة النحاس العضوي في العليقة الى تحسن بيئة الكرش للحملان كما وقللت من كمية الميثان المنتج في الحيوانات.

كلمات مفتاحية: الأغنام العواسي، التسمم النحاس، قابلية الهضم، الاحماض الدهنية الطيارة، الميثان.

Introduction

Several factors influence the efficiency and physiological performance of sheep among which diet is the most critical. Environmental factors also play a role in sheep farming, with particular emphasis on the significance of nutrition. Sheep require certain amounts of readily-accessible essential nutrients in order to meet their nutritional requirements. In assessing the nutritional needs of animals, it is necessary to prioritize calorie and protein intakes in their diets, as well as mineral components, which are often ignored in calculations. The mineral element requirements of sheep depend on their age and the specific stages of their reproductive and productive cycles (2). These elements play vital roles in maintaining overall health, stimulating growth and reproduction, and strengthening the body's organs and tissues (9).

Copper (Cu), an essential element in animal feed additives, is not retained in the body and must be added to diets. In addition, feedstuffs generally contain low concentrations of Cu and need to be included in the diet (34). Copper absorption in ruminants is restricted, usually reaching levels of 25% or less. The main mechanism by which bacteria in the rumen affect this process is their interaction with Cu, which depletes its level in the body. In addition, sheep eat plants that contain moderate amounts of Cu. These plants have a high abundance of molybdenum (4-12 μg/ml) in the rumen. The synthesis of thiomolybdate occurs as a result, which inhibits Cu absorption in the body of sheep (16 and 17). The enhancement of bacterial metabolism and proliferation in the rumen was not sufficient to offset this.

Studies show that adding copper sulphate ($CuSO_4$) at a concentration of 0.11-1.3 mg/kg dry matter enhances carbohydrate digestion (4 and 27). One study demonstrated that introducing Cu in different quantities into the rumen fluid of sheep has a positive effect on the decomposition rate of dry matter, volatile fatty acids, and acetic acid concentration (21).

Given its importance and the limited research on the effects of introducing organic copper on lamb growth and ruminal fermentation in arid and semi-arid regions, this study was conducted in multiple geographical locations. It investigated the effect of different amounts of organic copper in the diets of Awassi lambs on their growth performance, feed intake, nutrients digestibility, rumen fermentation, and methane production.

Materials and Methods

The study was conducted in accordance with the guidelines set by the Scientific Research Ethics Committee at Anbar University on the use of animals (Reference 18/2024). The farm trials were conducted at the sheep farm in the College of Agriculture, University of Anbar (L 33.427117N, 43.332602E) from November 21, 2023 to February 19, 2024. A total of 24 eight-month-old male Awassi lambs weighing an average 25.5 ± 3.69 kg was studied over a period of 90 days. They were housed in individual pens, and allocated randomly into four groups of 6 lambs per treatment.

The four treatments comprised a control (OCC), control diet without mineral premix and organic copper supplementation (OCF), control diet supplemented with 0.5 gm of organic copper per kg/DM of feed (OCC5), and control diet with 1 gm of organic copper per kg/DM of feed (OCC1) (Table 1). Copper proteinate buffermin (15%) (JH Biotech Inc., USA) was used as source of organic copper. It contains chelated copper that is coupled to amino acids and hydrolyzed protein, and has a minimum crude protein ratio of 25% and an excess of 15% copper.

Feed was offered twice daily at 9 am and 4 pm. The diet consisted of a mixture of roughage and concentrate at a ratio of 20:80. The diet components were manually mixed and offered by the animal feeder. At the begin of each week prior to feeding the lambs were weighed with a vertical electronic scale (Asani, China) that could hold up to 200 kg and had a precision of up to 10 grams. Rumen fluid was collected on days 45 and 90 of the experiment at 8 am before feeding using a 60 ml medical syringe connected to a 10 mm in diameter and 1.5 m long plastic tube. The collected rumen fluid was then placed in 50 ml plastic tubes. Four animals were randomly chosen from each treatment group and housed in metabolic cages for 7 days. This period included 2 days for adaptation and 5 days for sample collection of both feed and feces.

Table 1: Ingredients and chemical composition of organic copper concentrations in the lamb diets (DM basis).

Ingredients	**Levels of organic copper in concentration (g/kg)**			
	OCC	**OCF**	**OCC5**	**OCC1**
Straw	200	200	200	200
Barley	600	600	600	600
Wheat bran	120	120	120	120
Soyabean meal	66	68	65.5	65
Copper proteinate	0.00	0.00	0.5	1
Mineral and vitamin premix	4	0.0	4	4
Vitamin premix	0.0	2	0.0	0.0
CaCo3	5	5	5	5
NaCl	5	5	5	5
Total	1000	1000	1000	1000
Chemical composition (%)				
Dry matter (DM)	89.59	91.14	90.23	91.83
Ash	13.40	11.86	12.77	11.17
Crude protein (CP)	5.25	8.31	7.43	6.12
Ether Extract (EE)	6.32	6.04	6.04	6.00
Crude fiber (CF)	9.9	10.7	10.3	11.70
Natural detergent fiber (NDF)	52.38	60.29	55.16	43.91
Acid detergent fiber (ADF)	40.25	38.32	39.82	37.26
Acid detergent lignin (ADL)	1.34	1.27	1.18	1.06
Copper (Cu)(mg/kg)	7.24	6.47	82.23	157.22
Iron (Fe)(mg/kg)	298.96	279.28	298.88	298.8
Nitrogen free extract (NFE)	22.65	13.50	18.60	32.80

The premix provided in treatment 4 consisted entirely of vitamin content. Vitamin premix; Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU. Mineral and Vitamin Premix: Iron (Fe) 5000 mg; Copper (Cu) 1000 mg; Zinc (Zn) 5000 mg; Manganese (Mn) 5000 mg; Magnesium (Mg) 15000; Lodine (L) 80 mg; Cobalt (Co)10 mg; Selenium (Se) 10 mg; Phosphate (p) 2700 mg; Calcium (Ca) 5400 mg; Sodium (Na) 35000 mg; Vitamin A 48; Vitamin D_3 11; Vitamin E 200g;

Vitamin $B_1$10.00 g; Vitamin B_2 30,00 g; Vitamin B_6 14.00 g; B_{12} 0.08 g; Vitamin K_3 6.400 g; Vitamin C 52 g; NICOTINAMIDE 104 g; Calcium Pantothenate 44.00 g; Folic 4.80 g; Biotin 00.40 g.

The food and feces samples were then dried in an oven at 60 °C and then ground using a grinder (BEEM, Germany). This was for the purpose of conducting a preliminary analysis of various components including dry matter, ash, ether extract, crude protein, crude fiber (3), neutral detergent fiber, acidic detergent fiber, and lignin following method as described by (36). Nonfibrous carbohydrates (NFE) was calculated using the equation:

NFE = 100 - (NDF%+CP%+EE%+Ash%)

While the specific growth rate was calculated using:

Specific growth rate = In (Mean Final Weight) - In (Mean Initial Weight) g x 100/time (days)

Six rumen fluid samples were collected for each treatment and their pH measured using a portable pH meter (Hanna Instruments, USA). The samples were poured into a 50-ml plastic container. Then, 2-3 drops of H_2SO_4 were deposited into the rumen fluid to stop microbial activity and the samples stored in a freezer at -20° C until further analyses. The concentration of volatile fatty acids (VFA) in rumen fluid was measured using gas chromatography (Shimadzu, Japan) following the method by (6). The concentration of ammonia (NH_3) in rumen fluid was determined using a spectrophotometer (Hanna Instruments, USA) following (29). The amount of methane (CH_4) produced during rumen fermentation was calculated using a mathematical equation derived from the results of (25), as follows:

$$CH_4 = 0.45 \times (A) - 0.275 \times (P) + 0.4 \times (B)$$

where CH_4 is measured in mmol/L, and A, B, and P represent the concentrations of acetic acid (mmol/L), butyric acid (mmol/L), and propionic acid (mmol/L), respectively.

Data were collected by examining the properties of coefficients, and the general linear model (GLM) was used to analyze the data using statistical analysis software (version 9.4). The statistical techniques of analysis of variance and Duncan's test were employed to determine significant differences between treatments at the probability levels of $P < 0.05$ and $P < 0.01$.

Results and Discussion

The results of this study showed no significant differences in the initial body weight of the lambs across all experimental treatments (Table 2). The fourth treatment (OCC1) recorded the lowest average initial weight at 24.86 kg among all treatments, while OCF had the lowest average final weight (33.00 kg). Also, no significant differences were observed in total weight gain or specific growth rate for treatments. Feed intake for the OCC1, OCC5, and OCF treatments were significantly lower at ($P < 0.05$) compared to OCC which registered the highest consumption per animal at 795.6 g/kg while OCF had the lowest (723.0 g/kg). The results also indicated statistically significant variations ($P < 0.05$) in feed conversion ratios among lambs fed different levels of organic copper. The OCC5 treatment of 0.5 g/kg of organic copper had the highest ratio at 6.59 g/g followed by the OCC1, OCF, and OCC groups at 5.43, 5.07, and 4.98 g/g, respectively.

Table 2: Body weights and feed conversion ratios of different diet treatments.

Parameters	OCC	OCF	OCC5	OCC1	SEM	P value
Initial live weight (kg)	26.91	25.22	26.49	24.86	0.758	0.763
Final live weight (kg)	35.01	33.00	33.83	34.63	1.175	0.942
Average weight gain (g/d)	0.202	0.240	0.125	0.216	0.029	0.581
Feed intake (g/d)	795.6[a]	723.0[b]	775.3[b]	773.2[b]	9.366	0.038
Feed conversation ratio (g:g)	4.98[b]	5.07[b]	6.59[a]	5.43[b]	0.220	0.033

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM. [a, b] Different superscripts in the same rows indicate significant differences in the Means.

Figure 1 shows the quantity of feed consumed by the lambs for each treatment over the 14-week duration of the experiment. The results indicate no significant differences among the experimental treatments.

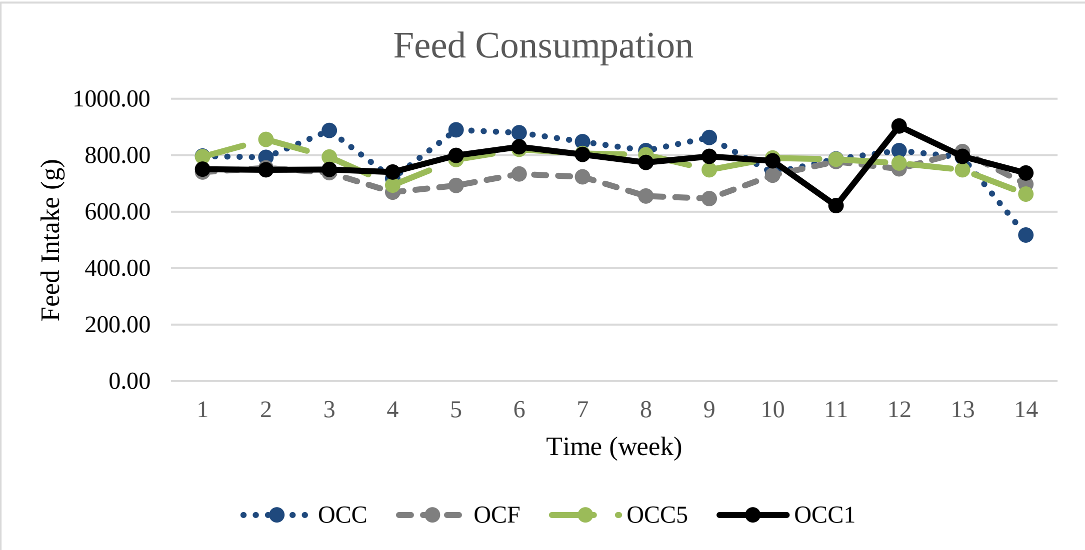

Figure 1: Feed intake of Awassi lambs fed different levels of organic copper.

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM.

In terms of growth performance (Figure 2), all the lambs showed weight gains over the study period regardless of treatment received. The animals were weighed weekly and it was observed that there was a decline in the treatments at the end of the trial in comparison to the control treatment. The remaining treatments were subsequently provided in the order of the OCC1 and OCC5 treatments, followed by OCF treatment. However, these variations did not lead to statistically significant results.

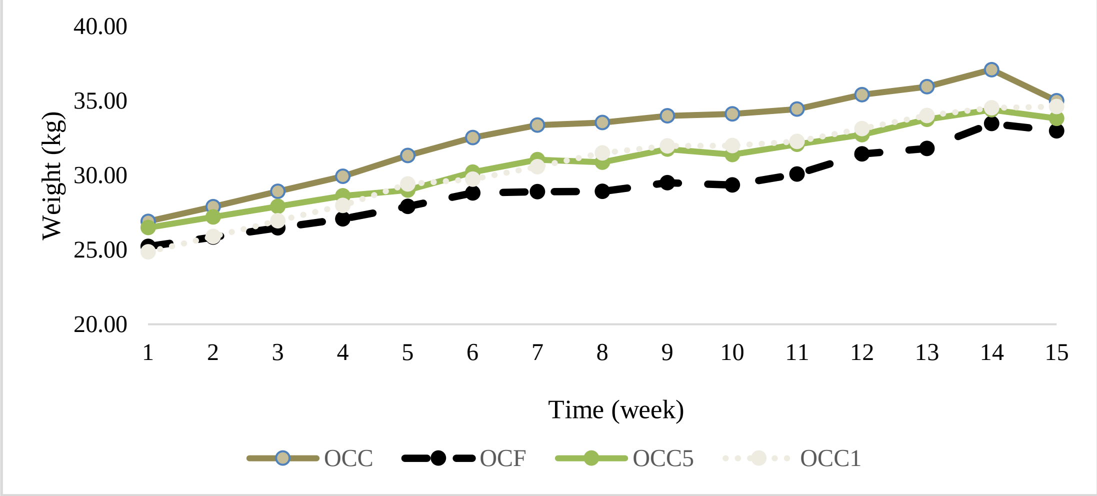

Figure 2: Lamb growth performance based on levels of organic copper in diets.

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM.

The results indicate that introducing organic copper into the diets of the lambs did not significantly affect their growth performance. These results are consistent with (7) who reported that Cu supplementation at doses of 10 or 30 mg per kg dry weight for 88 days did not affect the rate of weight gain in goats. Any differences in the results of this study and other studies may be due to the availability of adequate amounts of zinc or other chelated elements in the diet. These minerals bind to Cu and form complexes that are expelled from the body rather than absorbed. This interaction reduces the expected benefits on lamb growth performance from including organic copper in their diets. However, an animal's rate of weight gain and final weight are also affected by the quality of its feed. The results of this experiment indicate that the feed used in this research was of good quality. Supplementation of organic copper at a concentration of 10 mg/kg feed resulted in an improvement in feed conversion efficiency (19). This improvement in efficiency was observed at different feeding stages and physiological states.

Environmental conditions may also have an impact on the development of the lambs at some point. The sources and concentrations of Cu utilized in field tests may lead to the utilization of Cu supplementation levels below 50 mg/kg of feed. Nevertheless, this study showed that Cu was offered at elevated concentrations into the diets of indigenous lambs under Iraqi climate conditions. Hence, there exists a distinct difference from the results of prior investigations (10, 12 and 35). Copper has a prominent role in promoting growth, mostly because of its bactericidal inhibitory effects. Thus, it is thought to alter the bacterial composition of rumen, leading to a decrease in inflammation caused by infections (11).

The progressive increases observed during the weeks of the experiment may be attributed to weight. The ultimate body weight is variable upon both the animal species and the specific additive utilized (8). This result was confirmed by a recent study that included supplementing the diets of Dorper lambs with feed sources containing a high percentage of Cu. The lambs had greatest final body weight in the diet containing the greatest Cu concentration of 4.84 mg/kg, while the Awassi breed is distinguished from others by its exceptional rate of weight gain and feed consumption (32). Perhaps the significant decrease in feed intake can be attributed to Cu deficiency, which resulted in lower feed intake in the second treatment and may be the real reason for their weakened condition.

In a study by (26), it was noted that the introduction of organic copper in the third and fourth treatments led to a noticeable decrease in the rate of feed intake, which affected the growth performance of lambs. This result agrees (1) who demonstrated that feeding West African dwarf breed sheep with diets containing 0, 5, 10, and 15 mg Cu/kg feed resulted in lower feed intake for the experimental compared to the control groups.

This study results demonstrated a significant ($P < 0.01$) enhancement in nutrient digestibility of the feed component in the OCC1 compared to the other treatments. This improvement was observed in both the digestibility of the organic matter and the ether extract at 3.52% and 71.56%, respectively, while OCC recoded lower digestibility in the organic matter and the ether extract among others. The results also indicate that the OCF showed the lowest value in terms of digestibility of the ether extract at 24.76%. Table 3 shows a significant reduction in the digestibility of crude fiber for the organic copper treatments (OCC5 and OCC1) compared to the OCC treatment. However, there were no significant differences in the digestibility of dry matter and crude protein among all the experimental treatments.

Table 3: Nutrient digestibility of the lamb treatment diets.

Variables (%)	OCC	OCF	OCC5	OCC1	SEM	P value
Organic matter	0.746^{b}	3.270^{ab}	1.773^{b}	3.520^{a}	0.343	0.001
Dry matter	45.22	49.20	48.69	49.58	3.254	0.974
Crude fiber	16.74^{a}	3.876^{b}	0.960^{b}	6.320^{b}	1.881	0.002
Ether extract	49.62^{b}	24.76^{c}	55.57^{b}	71.56^{a}	5.082	0.001
Crude protein	17.75	58.17	44.26	16.74	7.559	0.120

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM. [a, b] Different superscripts in the same rows indicate significant differences in the Means.

This study showed that the inclusion of Cu did not affect the digestibility of all feed dry matter and protein in the ingested diet. It indicates that there was no negative effect on rumen biosynthesis or protein-secreting microbial communities because organic copper supplementation had no adverse effects (5 and 20). Although all experimental treatments experienced a significant reduction in crude fiber digestibility compared to the control, the feed additive involved in enhancing ration quality did not lead to significant improvements in NDF and ADF, as also reported by (32). However, the Cu source available in feeds may influence the ecological diversity of microbiota in rumen. Therefore, this confirms that raising levels of dietary Cu leads to the elimination of bacteria in the rumen, which subsequently affects the digestibility of the animal’s feed (13).

Copper concentrations in the rumen of less than 0.5 mg/kg feed significantly impact bacterial growth, but amounts above this may lead to microbial proliferation (37). Also, improvement in the digestibility of organic matter and ether extract in OCC1 could be due to the addition of organic copper to the feed. Copper promotes microorganism growth in the rumen by acting as an intermediary between negatively charged bacteria and the walls of plant cells thus enhancing fermentation (22). Copper feeds on particular in chemical and physiological conditions thereby affecting the reproduction of microorganisms that produce it. Therefore, it can influence the fermentation process and improve feed digestibility (28).

This study showed significant reduction in CH_4 concentrations for all treatments compared to the control (Table 4). The highest value recorded was 119.5 mmol/L for OCC while the lowest was 13.00 mmol/L for OCC5. Butyric acid and iso butyric acids had statistically significant reductions ($P < 0.05$) in the lamb diets for treatments containing 0.5 (OCC5) and 1 g/kg (OCC1) organic copper over the control treatment. The concentration of total volatile fatty acids (Total VFA) decreased significantly ($P < 0.05$) from 19.89 mmol/L in the OCC treatment to 10.78 and 14.18 mmol/L in the OCC5 and OCC1 treatments. However, there were no significant differences in propionic and isopropionic acid levels among the experimental treatments.

Table 4: Concentrations of methane production (mmol\L) and volatile fatty acids (mmol\ml) in the diet treatments.

Variables	OCC	OCF	OCC5	OCC1	SEM	P value
CH_4	119.5^{a}	40.28^{b}	13.00^{c}	44.99^{b}	14.13	0.015
Total VFA	19.89^{a}	13.73^{b}	10.78^{b}	14.18^{b}	1.169	0.012
Acetic	4.566^{ab}	3.826^{b}	3.730^{b}	4.586^{a}	0.145	0.019
Propionic	9.020	5.800	5.676	6.576	0.572	0.117
Butyric	6.306^{a}	4.106^{b}	1.376^{b}	3.020^{b}	0.564	0.001
Isopropionic	22.64	23.91	24.65	19.37	1.072	0.349
Iso butyric	28.45^{a}	9.796^{b}	9.893^{b}	12.66^{b}	2.417	0.001

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM. $^{a, b}$ Different superscripts in the same rows indicate significant differences in the Means.

Therefore, CH_4 production must be taken into consideration in all experimental treatments in comparison to the control treatment. These results are in line with (31) who suggested that the amount of methane produced could be complemented by the presence of fiber and aerobics in the diet. In addition, it has been found that high levels of diverse fibers lead to increased CH_4 production. This is responsible for the generation of H_2 ions in the drinks of ruminants. Methane production is thought to be related to decreased H_2 levels in the presence of high Cu concentrations. Our findings are supported by (30 and 39) which showed CH_4 concentration of 40 to 100 mg of Cu/kg DM. According to the results of the study, Cu ions can interact with other volatiles, and these compounds are then quantified as feces, reducing Cu in the body at the concentration levels (14 and 15).

The level of acetic acid in the OCC5 group and its superiority over OCC1 indicates that a good combination of dietary fiber produces CH_4, which leads to increased production of acetic acid and produces propionic acid. The onset of concentrations of butyric acid and isobutyric acid in the rumen of both the OCC5 and OCC1 may be attributed to the collection of a number of protozoa in the rumen, leading to the synthesis of the two acids (23). It could be that rumen protozoa, where isobutyric and butyric acids become present during fermentation, is the cause of this phenomenon (32 and 38). However, there were no significant differences in probiotic and isoprobiotic levels between all experimental feed. The propionic and isopropionic ratio is stable under all experimental conditions, thus supporting the results (40).

This study revealed significant increases in pH during the middle phase of the experiment, with the OCF predominant at ($P < 0.05$) over the other treatments. The pH level reached 7.1 in the OCF, while the OCC5 recorded the lowest at 6.4. There were no significant differences in pH levels at the end of the trial or across the different experimental treatments (Table 5). However, significant increases in NH_3 levels were recorded during the 45th day of the trial. This increase was statistically significant ($P < 0.05$) in the OCC1 group of lambs fed organic copper (11.67 mg/dL) compared to the control (4.94 mg/dL). At the end of the experiment, NH_3 concentrations in OCC5 showed a significant increase ($P < 0.05$) compared to the control treatment, with an average of 2.983 mg/dL. OCC had the lowest amount, averaging 3.366 mg/dL. Moreover, there was a significant increase ($P < 0.05$) in total NH_3 concentration in the rumen, with the highest levels observed in OCC1, followed by OCC5, OCC, and OCF.

Table 5: Rumen fermentation of liquid pH and NH3 for the lamb treatment diets.

Variables (%)	OCC	OCF	OCC5	OCC1	SEM	P value
pH						
pH day 45	6.7^{b}	7.1^{a}	6.4^{b}	6.7^{b}	0.096	0.051
pH day 90	6.8	6.8	6.9	6.8	0.058	0.959
Total pH	6.8	6.9	6.7	6.8	0.048	0.306
NH_3						
NH_3 (mg\dL) day 45	4.94^{b}	2.98^{c}	7.31^{b}	11.67^{a}	1.100	0.004
NH_3 (mg\dL) day 90	4.36^{ab}	3.36^{b}	5.03^{a}	3.52^{b}	0.230	0.005
Total NH_3	4.65^{b}	3.17^{b}	6.17^{a}	7.59^{a}	0.570	0.006

OCC: Basal diet; OCF: Basal diet + free of organic copper; OCC5: Basal diet + 0.5 g organic copper/kg DM; OCC1: Basal diet + 1 g organic copper/kg DM. $^{a,\,b}$ Different superscripts in the same rows indicate significant differences in the Means.

This study noted lower pH levels in rumen fluid during the middle of the experiment, possibly due to the concentration of organic copper in the feed. This finding is consistent with (18) who demonstrated that supplementing the diet with 60 mg/kg Cu could directly affect the presence of free Cu ions in the rumen and bacterial proliferation. Including organic copper in the diet of the Awassi lambs did not lead to any noticeable changes in pH levels at the end of the experiment. This lack of effect may be due to the lambs adapting to the forage diet. Thus, there was no significant adverse effect on rumen fermentation traits or microbial populations. This could be attributed to the inability of rumen bacteria to degrade bound nutrients in a way that leads to an increase in pH, in line with the findings of (5 and 31). NH_3 concentrations in the rumen of lambs varied in both the third and fourth treatments over 45 and 90 days. The feed with organic copper resulted in rumen NH_3 concentrations of between 5 - 8 mg, which is the ideal amount for microbial protein synthesis, as noted by (5, 24 and 31).

Conclusions

This study found that organic copper supplementation of between 0.5 and 1 g/kg in the diets of the Awassi sheep effectively enhanced rumen fermentations. It also increased NH_3 production, which assists in microbial protein synthesis. The lower CH_4 levels in their rumen was a positive outcome in the efforts to reduce greenhouse gas emissions. Organic copper in lamb diets influence the absorption of volatile fatty acids, hence promoting pH stabilization in the rumen through the elimination of harmful compounds.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Study concept and design: Osama A. Saeed; data collection: Ahmad H. Abdan; analysis and interpretation of results: Osama A. Saeed; draft manuscript preparation: Ahmad H. Abdan. Both authors reviewed the results and approved the final version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted according to the research plan approved by the department, college, and university.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

The study was carried out following the guidelines approved by the ethics committee at the University of Anbar.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors would like to thank the Dean of the College Agriculture for providing the materials used in this study. We would also grateful to the University of Anbar for the encouragement and support extended to us during the entire duration of the study.

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