Effect of using different ratios of lysophospholipids on in vitro digestibility gas production and some rumen fluid characteristics of local lamb diets

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Abstract:

This experiment investigated the effect of adding different levels of lysophospholipids to the diet on total gas and methane production, some rumen fluid properties, dry matter and organic matter digestibility coefficients, and in vitro protozoa preparation. The results indicated no significant differences (P>0.05) between treatments in total gas volume, reaction rate constant, retardation time, and gas production during different incubation periods. However, the addition of lysophospholipids significantly improved (P<0.05) some nutritional value indicators. The 1.5% LPL treatment recorded the highest gas production over 24 hours, in vitro organic matter digestion, organic matter digestibility, and metabolizable energy. The addition also led to a significant increase (P<0.05) in the concentration of total volatile fatty acids in rumen fluid, particularly at levels of 1.3%, 1.5%, and 1.7% LPL. No significant differences (P>0.05) were observed in pH or ammonia nitrogen concentration. The total number of protozoa and some protozoan genera decreased significantly (P≤0.05) at several addition levels compared to the control treatment. It can be concluded that the addition of lysophospholipids, especially at 1.5%, is the optimal concentration for improving fermentation efficiency and the nutritional value of the diet without negatively impacting rumen stability.

Key words: Lysophospholipids; Lecithin; Fat digestion; gas production.

Introduction:

The increasing demand for animal products due to population growth has created a global need for more animal products. To meet these needs, more food resources are required. This has led to an increased use of concentrates in animal feed [12]. Adding concentrates to ruminant diets increases the intake of unesterified fatty acids, particularly triglycerides. One problem with high concentrate consumption in ruminants is gastrointestinal disturbances such as rumen acidosis, which causes significant economic losses due to increased morbidity and mortality rates [19]. Rumen acidosis results from a diet high in carbohydrates and low in fiber, poor management, or a combination of these factors. Fat supplementation can help improve and reduce negative energy balance in high-producing cows. The beneficial effects of fat supplementation depend on the type and amount of fat. Rumen fat reduces the acetate-to-propionate ratio, decreases fiber digestion, and reduces methane production. [1] found that fat, by

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influencing gastrointestinal hormones such as insulin and leptin, reduces hepatic lipid oxidation and dry matter utilization in cattle. It can increase rumen distension and stimulate rumen stretch receptors, leading to reduced dry matter intake [11]. Simultaneously, hormones such as cholecystokinin and other peptides, as well as proglucagon processing products (glucagon, clegentin, auxin, tomodulin, and glucagon-like peptide), by reducing the transit rate, cause a decrease in dry matter intake in animals fed fat supplements. Naturally, the amount of fat hydrolysis is influenced by rumen pH. Dietary fats are in the form of free fatty acids in the rumen, and fat emulsification in the rumen involves the hydrogenation of 18-carbon linoleic acids, even if the fatty acid uptake is limited in the rumen epithelium. The composition of long-chain fatty acids in the small intestine depends on the saturated and unsaturated fatty acids in the diet. The use of animal fats in the animal diet is more appropriate because unsaturated fatty acids have fewer negative effects on rumen microbiota than saturated fatty acids [10]. Although saturated fatty acids are less digestible than unsaturated fatty acids in the small intestine [6] phospholipids are produced by the enzymatic hydrolysis of phospholipids with the removal of a fatty acid chain. They mainly contain lysophosphatidylcholine,lysophosphatie and lysophosphatidylethanolamine [21]. Lysophospholipids promote the growth of Gram-positive bacteria. Lecithin plays an important role in animal nutrition [16]The emulsification of dietary fats contributes to increased fat absorption in the intestine [17] and [10] The aim of this study is to determine the optimal level of lysophospholipid addition and its effect on the growth and performance of microorganisms.

Materials and Methods:

The experiments and chemical analyses were conducted on the feed samples in the Nutrition Laboratory of the Animal Production Department, Faculty of Agricultural Sciences, and the Faculty of Veterinary Medicine, Al-Qasim Green University. Additionally, laboratory digestion tests were performed in the Nutrition Laboratories of Razi University, Kermanshah, to study the effects of using different proportions of [unclear - possibly "leaf" or "feed"]. The lysophospholipid produced by Simorgh Behin Darou Gostar Arya was tested in the incubation period of dry and organic matter for 96 hours. Total gas and methane gas were produced in vitro, and the metabolizable energy (ME) was calculated in mJ/kg of dry matter, along with the in vitro organic matter digestibility coefficient (IVOMD) and short-chain fatty acids (SCFA) in mmol/100 ml of total gas production. Different proportions of the lysophospholipid were used and mixed with the concentrated feed. The methods of[9] and[18] were used to estimate total gas production, in vitro fermentation and decomposition factors, and in vitro digestibility of fully mixed lamb (TMR) rationsThirty-two5-month-old lambs with an average weight of 23-24 kg were used, with 4 lambs per treatment, from among the eight treatments included in the study. These rations, containing increasing levels of phospholipids in the concentrate, were ground in a laboratory mill with a 1 mm

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sieve. Two hundred milligrams of the experimental TMR rations were collected from three rams with rumen ports and transported to the laboratory in a flask. Anaerobic conditions were then created by introducing CO□ gas into the flask, after which the rumen fluid was filtered using four-layer gauze. After filtering, the sample was placed in a 100 ml glass syringe, and 20 ml of artificial saliva was added. The components of the artificial saliva were collected and prepared before the incubation of the feed samples. The saliva consists of the following chemical components: KCl 0.77 g, Na□HPO□ 2.77 g, NaHCO□ 9.80 g, CaCl□·2H□O 16 g, NaCl 0.47 g, and MgCl□·7H□O 2.16 g. These chemicals were mixed with distilled water while stirring continuously until the components were dissolved, bringing the total volume to one liter. To calculate the total gas production and measure the pH of the rumen fluid, 4 ml of 4% sodium hydroxide was added to 2 injections only to calculate the methane gas production according to the method). The in vitro digestibility coefficient of organic matter % (IVOMD) and short-chain fatty acids (SCFA) mmol/100 ml of the total gas production after a 96-hour incubation period was also calculated using the following equations according to the method of [9]. The McDonald-Orskov equation [14] was used to determine the gas components.

Results and Discussion:

methane production kinetics of local lamb rations.

The results of the statistical analysis in Table (1) showed no significant differences (P>0.05) when adding different levels of lysophospholipids between the treatments in terms of total gas volume, decomposition rate balance, and delay time. Treatment seven recorded the highest total gas production volume (B) at 163.96 at the 1.5% lysophospholipid level compared to the rest of the treatments, while it showed a slight increase in the gas production rate constant, as treatment five recorded 0.055 at the 1.1% lysophospholipid level compared to the control treatment. That is, there are no significant differences (P>0.05) between the treatments, meaning that the fermentation rate was not affected by the addition of lysophospholipids. However, we note a decrease in the delay time (L) in most treatments, as it reached the lowest level in treatment four (2.01 hours) at the 0.9% lysophospholipid level, which indicates an acceleration of the start of microbial activity at these levels. The results indicate that isophospholipids primarily enhance fermentation in rumen fluid at different times of day, while their direct effect on microbial activity and gas production under laboratory conditions may be limited. Furthermore, gas production mainly reflects carbohydrate fermentation, whereas the effect of isophospholipids is more closely linked to improved utilization of fats and other nutrients. These findings are consistent with those of [20] who demonstrated that the addition of isophospholipids did not lead to significant changes in some in vitro rumen fermentation parameters despite improved feed utilization within the animal. They also align with the results of [2] who found that the main effect of isophospholipids is to improve digestion and feed utilization rather than directly impact fermentation products. Finally,

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these results are consistent with those of [15] who indicated that some feed additives may improve nutrient utilization efficiency without necessarily leading to significant changes in gas production or in vitro fermentation kinetics, due to the complexity of microbial interactions within the rumen.

These findings are also consistent with [15] study, which suggested that some feed additives may improve nutrient utilization efficiency without necessarily leading to significant changes in gas production or in vitro fermentation kinetics, due to the complexity of microbial interactions within the rumen.

Table 1. The effect of different level of Lysophosphatidic acid on kinetics of gas production

Treatment	Parameter		
	B	C	L
Control	159.71	0.045	6.02
Treat 1 (L*=0.5%)	162.22	0.041	3.20
Treat 2 (L=0.7%)	154.64	0.050	6.11
Treat 3 (L=0.9%)	156.10	0.052	2.01
Treat 4 (L=1.1%)	141.09	0.055	3.68
Treat 5 (L=1.3%)	146.90	0.052	4.53
Treat 6 (L=1.5%)	163.96	0.046	2.96
Treat 7 (L=1.7%)	157.11	0.048	2.12
SEM	4.05	0.002	0.45
P value	0.90	0.89	0.11

*L=lysophosphatidic acid

B: the asymptotic gas volume (ml); c: the rate constant (/h); L: lag time. Means within rows with different superscript are significantly different at $P<0.05$.

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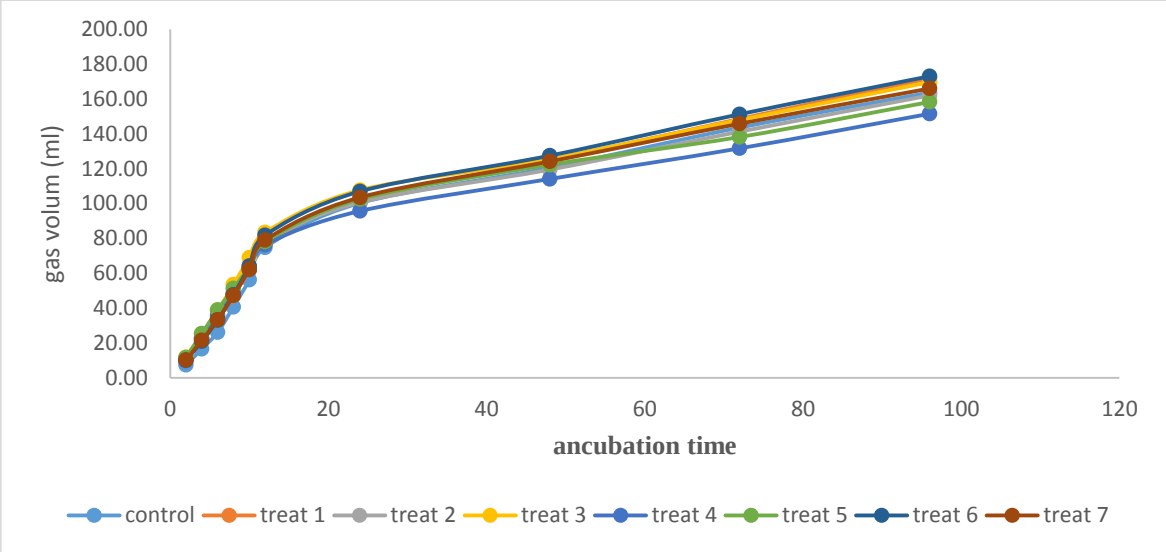
Chart 1. Gas production kinetics of different treatments during 96 hours of incubation



***in vitro* gas production during 96 hours of incubation (ml).**

The results of the statistical analysis in Table (2) regarding the effect of adding different levels of lysophospholipids on methane gas production (2-96 hours) indicate no significant differences (P>0.05) between the different treatments in gas production during the aforementioned time periods. We observe that all treatments gave higher gas results than the control treatment. The gas balance at (2 hours) for the fourth treatment was 11.98 with a concentration of 0.9% lysophospholipids, which is higher than the other treatments, while the fifth treatment recorded the highest gas balance at (96 hours) with a concentration of 1.1% added lysophospholipids. Some results were lower than the treatments compared to the control treatment, which means that increasing the concentration of lysophospholipids may not improve fermentation and may have a negative effect, as it partially activates microorganisms. However, lysophospholipids do stimulate bacterial activity in the rumen and the fermentation of organic matter, as shown in the figure. These results indicate that the addition of lysophospholipids did not significantly affect the overall fermentation activity of rumen microorganisms under laboratory conditions, although there was a numerical trend towards increased gas production at certain levels of addition. This may be because lysophospholipids primarily improve fat emulsification and increase the efficiency of carbohydrate utilization from corn and barley feed within the animal, rather than directly affecting the amount of gas produced from microbial fermentation. Lysophospholipids reduce the surface tension of protein-encapsulated fats and starches in corn, which are difficult for digestive enzymes to penetrate. They break down fat droplets and starch

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molecules into microscopic sizes due to increased contact between microbial enzymes and the feed material, thus increasing the efficiency of hydrolysis before the material reaches the end of the intestine [8] These results are consistent with the study by[20] who demonstrated that the addition of isophospholipids did not lead to significant changes in in vitro rumen fermentation indices, despite improved nutrient utilization and animal productivity. They also align with the findings of[2] who observed no significant effects of isophospholipids on microbial fermentation activity, while noting improvements in carbohydrate digestion and utilization. Furthermore, these results are consistent with the findings of[3], who indicated that some feed additives can improve nutrient utilization efficiency without significantly altering in vitro gas production, and that gas production alone does not always reflect feed efficiency or animal performance.

Table 2. The effect of treatment on *in vitro* gas production during 96 hours of incubation (ml).

Treatment	Time of incubation									
	2h	4h	6h	8h	10h	12h	24h	48h	72h	96h
Control	7.45	16.71	26.23	40.81	56.28	74.83	100.34	120.58	143.61	163.69
Treat 1 (L=0.5 %)	10.74	23.08	34.60	47.24	62.05	77.12	102.04	124.79	148.60	171.84
Treat 2 (L=0.7 %)	10.87	22.32	33.74	47.48	62.16	77.93	100.69	119.50	141.29	162.02
Treat 3 (L=0.9 %)	11.98	25.07	38.49	53.50	68.94	83.44	107.70	125.58	148.04	169.63
Treat 4 (L=1.1 %)	9.88	22.75	35.17	48.58	61.92	76.28	95.75	114.13	131.70	151.52
Treat 5 (L=1.3 %)	11.79	25.40	39.05	51.36	63.86	78.09	102.60	122.28	138.21	158.29
Treat 6 (L=1.5 %)	10.25	21.00	33.20	47.69	64.13	81.85	107.00	127.52	121.27	173.06
Treat 7 (L=1.7	10.25	21.65	33.33	47.26	62.30	79.09	103.57	124.20	145.85	166.01

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%)

SEM	0.41	0.79	1.16	1.56	2.03	2.46	2.84	3.06	3.74	4.00
P value	0.170	0.149	0.153	0.742	0.949	0.995	0.988	0.985	0.951	0.930

*L=lysophosphatidic acid
OMD: *in vitro* organic matter digestibility; PF: partitioning factor; MB: microbial biomass; EMB: efficiency of microbial biomass; ME: metabolizable energy.
Means within rows with different superscript are significantly different at P<0.05.

The means within rows bearing different letters are significantly different at the P < 0.05 level:

T1: Control treatment, T2: 0.5% treatment, T3: 0.7% treatment, T4: 0.9% treatment, T5: 1.1% treatment, T6: 1.3% treatment, T7: 1.5% treatment, T8: 1.7% treatment.

***in vitro* rumen gas production parameters.**

The results of the statistical analysis of Table (3) for gas production at (24 hours) in the incubation indicated significant differences (P<0.05) between the different treatments, where the seventh treatment (122.33 ml) was superior at the level of addition of lysophospholipids (1.5%) compared to the other treatments compared to the control, where the first treatment recorded (94.04 ml). As for the digestible organic matter, the seventh treatment was superior (296.49) at the level of addition of lysophospholipids (1.5%). The seventh treatment was also superior in the digestibility of the organic matter, where it recorded (59.29). As for the fractionation coefficient and biomass efficiency, we note that there are no significant differences between the treatments (P<0.05). Treatment seven also showed superior microbial biomass (182.96) and metabolizable energy (18.42) compared to the control treatment. This indicates that the addition of lysophospholipids significantly improved gas production, organic matter digestion, and the digestibility ratio. Treatment seven, with a concentration of 1.5% lysophospholipids, yielded the highest gas production, organic matter digestion, and metabolizable energy, while no significant effect was observed on the fractionation coefficient or biomass efficiency. These results suggest that lysophospholipids enhance fat emulsification and increase nutrient availability to rumen microorganisms, thereby increasing the rate of organic matter breakdown and boosting the production of volatile fatty acids and energy from fermentation. Furthermore, improved organic matter digestion leads to increased energy availability for the animal, consistent with the improved productivity and digestibility coefficients observed in the current study. These results are consistent with the study by [5] who observed that the addition of lysophospholipids improved organic matter digestibility and increased carbohydrate utilization efficiency by enhancing rumen microbial activity.

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They also align with the study by[20], which indicated that lysophospholipids improved organic matter digestibility and energy utilization from the feed, leading to increased feed efficiency. Furthermore, these findings are consistent with the work of[3] who demonstrated that increased laboratory gas production and organic matter digestibility are associated with increased feed nutritional value and improved microbial fermentation efficiency, which positively impacts metabolizable energy and animal performance.

Table 3. The effect of treatment on *in vitro* rumen gas production parameters.

Treatment	Parameter						
	Gas 24h (ml)	OMD (/500mg)	OMD (%)	PF	MB (mg)	EMB (%)	ME (MJ/kgDM)
Control	94.04	246.21	49.24	2.62	158.93	64.41	15.06
Treat 1 (L=0.5%)	104.62	264.79	52.95	2.53	167.69	64.58	16.51
Treat 2 (L=0.7%)	102.35	260.92	52.18	2.55	165.93	63.33	16.19
Treat 3 (L=0.9%)	93.77	245.38	49.07	2.62	158.35	64.58	15.03
Treat 4 (L=1.1%)	103.33	262.73	52.54	2.54	166.84	63.50	16.33
Treat 5 (L=1.3%)	99.71	256.31	51.26 [b]	2.57	163.77	63.90	15.81
Treat 6 (L=1.5%)	122.33	296.49	59.29	2.44	182.96	61.94	18.42
Treat 7 (L=1.7%)	96.28	250.25	50.05	2.60	160.90	64.33	15.31
SEM	2.56	4.55	0.91	0.017	2.17	0.25	0.34
P value	0.09	0.09	0.09	0.15	0.136	0.13	0.10

*L=lysophosphatidic acid

OMD: *in vitro* organic matter digestibility; PF: partitioning factor; MB: microbial biomass; EMB: efficiency of microbial biomass; ME: metabolizable energy.

Means within rows with different superscript are significantly different at P<0.05.

rumen fluid fermentation *in vitro*.

Table (4) shows the effect of adding different levels of lysophospholipids on rumen fluid fermentation in vitro. Statistical analysis revealed no significant differences (P>0.05) between treatments in pH value. The first

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treatment, the control, had the highest pH (6.50) compared to the fifth treatment (6.32). These results indicate that the fermentation medium remained within the normal range for rumen fermentation (6.2-6.8), meaning that the addition of lysophospholipids did not disrupt the acid-base balance of the medium. The stability of the pH value is an indicator of a healthy microbial environment and the absence of inhibition of fibrotic bacterial activity. Similarly, no significant differences ($P>0.05$) were observed in ammonia nitrogen concentration between the different treatments. The sixth treatment had the highest ammonia nitrogen concentration (117.24) at a 1.3% lysophospholipid addition level compared to the second treatment (107.80). (0.5%) of lysophospholipids, ammonia nitrogen, is an important indicator of the balance of microbial protein degradation used in protein synthesis. The results of the current study indicated that the addition of lysophospholipids had a significant effect ($P<0.05$) on the concentration of total volatile fatty acids. Treatment 6 (1.3%) of lysophospholipids recorded 96.17 mmol/L, treatment 7 (1.5%) 83.33 mmol/L, and treatment 8 (1.7%) 75.83 mmol/L, compared to the control treatment (treatment 1) (31.67 mmol/L). These results indicate that the addition of lysophospholipids, especially at levels of 1.3–1.7% LPL, led to the activation of microbial fermentation processes within the rumen, which was reflected in an increase in the production of volatile fatty acids, the main source of energy in ruminants. This is attributed to the ability of lysophospholipids to improve fat emulsification and increase carbohydrate availability to microorganisms, thereby promoting their growth and fermentation activity. Furthermore, the lack of effect on pH and ammonia nitrogen with the increased concentration of volatile fatty acids indicates that the increase in fermentation activity did not reach a level that would disrupt the rumen environment, but rather occurred within the limits of normal physiological equilibrium. These findings are consistent with the study by[5] who found that the addition of lysophospholipids led to a significant increase in total volatile fatty acid production and improved rumen fermentation properties, without causing significant changes in pH or ammonia concentration. They are also consistent with the findings of [20], who indicated that lysophospholipids improved nutrient utilization and increased rumen fermentation products, particularly volatile fatty acids. The results are also consistent with what [7] stated, who explained that improving fat emulsification in ruminant diets leads to increased microbial fermentation efficiency and increased production of volatile fatty acids without negatively affecting the acid balance of the rumen.

ISSN 2072-3857

Table 4. The effect of treatment on *in vitro* rumen fermentation parameters.

Treatment	Parameter		
	pH	NH_3-N (mmol/L)	TVFA (mmol/L)
Control	6.50	113.70	31.67 [b]
Treat 1 (L=0.5%)	6.38	107.80	33.33 [b]
Treat 2 (L=0.7%)	6.38	114.56	33.33 [b]
Treat 3 (L=0.9%)	6.39	110.25	44.17 [b]
Treat 4 (L=1.1%)	6.32	110.44	42.50 [b]
Treat 5 (L=1.3%)	6.37	117.24	69.17 [a]
Treat 6 (L=1.5%)	6.39	113.83	83.33 [a]
Treat 7 (L=1.7%)	6.46	108.82	75.83 [a]
SEM	0.02	1.30	4.76
P value	0.35	0.66	0.0001

*L=lysophosphatidic acid

NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids;

Means within rows with different superscript are significantly different at P<0.05.

The means within rows bearing different letters are significantly different at the P < 0.05 level:

T1: Control treatment, T2: 0.5% treatment, T3: 0.7% treatment, T4: 0.9% treatment, T5: 1.1% treatment, T6: 1.3% treatment, T7: 1.5% treatment, T8: 1.7% treatment.

the total number of protozoa in the rumen.

Table (5) shows the effect of adding different levels of lysophospholipids on the total number of protozoa in the rumen. The results of the statistical analysis indicated significant differences (P<0.005) between the treatments when adding lysophospholipids in the total number of protozoa, where the third treatment (30.53×10^5 /ml) was superior at a concentration of 0.7% of lysophospholipids, while the sixth treatment (15.01×10^5 /ml) recorded a higher number at a concentration of 1.3% of lysophospholipids. There were also significant differences (P<0.005)

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between the treatments in the numbers of Entodinium (30.05×10^5 /ml) at a concentration of 0.7% for the third treatment, and Isotricha spp. and Diplodiniina. We note that there were no significant differences ($P>0.005$), and the fifth treatment recorded the highest number of protozoa at a concentration of (0.383×10^5 /ml). (1.1%) of lysophospholipids showed significant differences ($P<0.05$) in the total number of Ophryoscolecinae protozoa between treatments when lysophospholipids were added. Treatment 8 (0.100×10^5 /ml) recorded the highest number of protozoa at a concentration of (1.7%) of lysophospholipids. This is mainly related to changes in the Entodinium ssp. numbers, which tend to reduce protozoa populations, potentially indicating an inhibitory effect at high concentrations. These results suggest that lysophospholipids affect the rumen microbial balance, especially at high levels, leading to a reduction in the numbers of some protozoa species. This may be due to changes in cell membrane properties or carbohydrate availability within the rumen environment. Furthermore, a decrease in protozoa numbers may be nutritionally beneficial, as protozoa consume large quantities of rumen bacteria. Therefore, reducing their numbers may allow for increased microbial protein flow into the intestine and improved nitrogen utilization. These results are consistent with the study by[4] who demonstrated that changes in the rumen environment or the use of certain feed additives can lead to a reduction in protozoan populations and improved nitrogen utilization and microbial protein production. They also align with the study by [15] which indicated that a reduction in rumen protozoan populations is generally associated with improved protein utilization and reduced nitrogen loss within the rumen. Furthermore, these findings are consistent with the work of [13] who showed that alterations in the rumen microbial composition can lead to improved fermentation efficiency and increased microbial protein production, particularly when the populations of the dominant protozoa of the genus Entodinium are reduced.

Table 5. The effect of different level of Lysophosphatidic acid on rumen protozoa population ($\times10^5$/ml).

Treatment		Parameter				
		Total protozoa	*Entodinium* spp.	*Isotricha* spp.	*Diplodiiniae*	*Ophryoscolecinae*
Control		27.33 [ab]	26.98 [ab]	0.233	0.100	0.016 [bc]
Treat (L=0.5%)	1	18.18 [c]	17.86 [c]	0.266	0.033	0.016 [bc]
Treat (L=0.7%)	2	30.53 [a]	30.05 [a]	0.350	0.050	0.083 [ab]

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Treat (L=0.9%)	3	18.91 ^{c}	18.83 ^{c}	0.066	0.016	0.000 ^{c}
Treat (L=1.1%)	4	19.31 ^{c}	18.85 ^{c}	0.383	0.000	0.066 ^{abc}
Treat (L=1.3%)	5	15.01 ^{c}	14.81 ^{c}	0.200	0.016	0.000 ^{c}
Treat (L=1.5%)	6	15.93 ^{c}	15.70 ^{c}	0.183	0.016	0.033 ^{abc}
Treat (L=1.7%)	7	22.63 ^{bc}	22.16 ^{bc}	0.300	0.066	0.100 ^{a}
SEM		1.28	1.27	0.03	0.01	0.01
P value		0.003	0.004	0.09	0.21	0.05

*L=lysophosphatidic acid

Means within rows with different superscript are significantly different at P<0.05.

The means within rows bearing different letters are significantly different at the P < 0.05 level:

T1: Control treatment, T2: 0.5% treatment, T3: 0.7% treatment, T4: 0.9% treatment, T5: 1.1% treatment, T6: 1.3% treatment, T7: 1.5% treatment, T8: 1.7% treatment

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