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Effect of Feeding Probiotic Megasphaera Elsdenii and Sodium Bicarbonate on Growth Performance Blood Metabolites and Rumen Fermentation in Awassi Lambs

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Abstract This study evaluated the effects of Megasphaera elsdenii (ME) probiotic and sodium bicarbonate (SB) supplementation on growth performance ,feed efficiency,and blood metabolites in Awassi lambs fed concentrate diet. Eighteen lambs (15 ± 4 kg) were assigned to three treatments (6 lambs/treatment) for a 100 days period : The first group Control (no additives), The second group receiving the diet supplemented with sodium bicarbonate (SB) (10 g kg DM^{-1}), and The third group (ME) where each lamb was dosed with a capsule containing of Megasphera elsedinii (1×10^{10} CFU). Dry matter intake (DMI), daily weight gain (DWG), feed conversion ratio (FCR), and blood metabolites (glucose, triglycerides, lactate, and blood urea nitrogen) were measured. Within the results neither SB nor ME significantly impacted Dry matter intake (DMI) or daily weight gain (DWG) versus the control ($P > 0.05$). However, Lambs consuming SB exhibited a significant reduction in feed conversion ratio (FCR) (7.20) as to control (5.58) and ME (5.52) ($P = 0.02$). All groups showed increases in blood lactate and urea nitrogen ($P < 0.01$) at week 12. Group of ME showed more numerical DMI at the late weeks of study, while the group of SB showed a less numerical DWG between week 9 and 12. no effects of treatments were observed on blood glucose and triglycerides ($P > 0.05$). It could be concluded that SB supplementation may cause adverse effects on feed efficiency in growing Awassi. ME administration results were comparable to that of control and warranting further optimization of probiotic strategies.

Keywords: Megasphaera elsdenii, sodium bicarbonate, Awassi lambs, feed conversion ratio, blood metabolites.

Introduction Sheep production is vital for global food security, particularly in arid and semi-arid regions where environmental conditions restrict other agricultural activities (30). The Awassi sheep, a hardy fat-tailed breed native to the Middle East, is renowned for its adaptability to harsh climates, disease resistance, and high milk yield (14). Despite its economic importance, the productivity of Awassi sheep often falls below its genetic potential due to suboptimal feeding practices and metabolic challenges associated with intensive production systems (14). In modern sheep production, high-concentrate diets are commonly used to enhance growth rates and meet rising demands for animal protein(2). However, these energy-dense rations can disrupt rumen fermentation, leading to digestive disorders such as subacute ruminal acidosis (SARA) (18). SARA occurs when rapid starch fermentation in cereal-based diets lowers rumen pH below 5.6, causing excessive accumulation of volatile fatty acids and lactate (38). This condition impairs nutrient digestibility, reduces

feed intake, and can lead to health issues like liver abscesses and laminitis (23). Awassi lambs, being particularly sensitive to dietary changes, are at risk of reduced growth performance and carcass quality when fed high-grain diets (32).
To address these challenges, dietary interventions such as buffers and probiotics have been explored. Sodium bicarbonate is a widely used rumen buffer that helps neutralize excess acids, maintaining optimal pH for microbial activity (26&22). Studies have shown that sodium bicarbonate supplementation improves fiber digestion, microbial protein synthesis, and feed efficiency in lambs (41). However, its effectiveness is limited in severe acidosis cases, as it does not directly reduce lactate accumulation (34).
An alternative approach is available involves probiotic supplementation, particularly with Megasphaera elsdenii, a lactate-utilizing bacterium that converts harmful lactate into beneficial volatile fatty acids like propionate and butyrate by Meta-analyses indicate that ME administration enhances average daily gain (ADG) by 5.6% in ruminants (40&7). Research indicates that ME can stabilize rumen pH, prevent lactate buildup, and enhance feed efficiency in ruminants on high-concentrate diets (28).
Beyond rumen health, dietary interventions influence blood metabolites, which serve as key indicators of metabolic status. Parameters such as glucose, urea nitrogen, and lactate reflect energy metabolism, protein utilization, and acid-base balance (16). Additionally, nutrient digestibility—especially of fiber—plays a crucial role in determining the economic feasibility of feeding strategies (6). This study aimed to evaluate the effects of Megasphaera elsdenii and sodium bicarbonate supplementation on growth performance and blood metabolites in Awassi lambs fed a high-concentrate diet.

Material and Methods

Ethical approval

The present study was approved by the Committee for Research Ethics, College of Veterinary Medicine, University of Duhok (Approval No.CVM2023/0212UoD, dated 2/12/2023).

Animals and Housing:

Eighteen awassi male lambs with average weight 15 ± 4 kg were used in a completely randomized design according to live body weight with 3 treatments (divided into 3 groups) each of 6 replicates and housed in individual pens (1.5 m × 1.5 m). The experiment duration was 100 days (14 days for adaptation and 86 days was the main period of the experiment.

Diet and treatments:

Lambs received a diet at level of 80% concentrate and wheat straw at 20% in separated feeders that formulated according to NRC (31) – (Table 1). The animals were fed with the concentrate diet, twice daily (at 08:00 and 16:00) to ensure remains residual during 24 hours. Each group of lambs was randomly assigned to one of the three treatments: First one control (no additive), second one Sodium bicarbonate (on the level of 10 g/kg DM) and the third one Megasphaera elsdenii NCIMB 41125 in capsulated (1×10^{10} CFU) was administered orally using a balling gun to deposit the capsule deep in the lambs rumen.

Dry matter intake and Growth Performance:

During the experiment period for estimating the voluntary feed intake, feed offered and residue were recorded daily before morning feeding, and feed samples were stored for subsequent chemical analysis DM, ASH, CP, CF, EE by use procedures from AOAC, (4). In order to determine the average daily gain (DWG), and dry matter intake the lambs were weighed once every 7 days before morning feeding. The average gain on a daily basis was calculated for each lamb by analysis of body weight vs. feed efficiency.

Blood sampling and analysis:

In order to investigate blood biochemical parameters, blood samples (10 mL) was collected from jugular veins periodically at the weeks 0, 4, 8, 10 and 12 of the experimental period just 4 hours after the morning feeding. The blood samples were centrifuged (3,000 × g for 15 min at 4°C) and the serum was separated and frozen at −20°C until measuring biochemical parameters. Glucose, triglycerides, lactate, blood

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urea nitrogen (BUN), were determined by automated analyzer (MEDICA easy ra, Netherlands).

Statistical analysis:

The gathered data submitted to SPSS (Statistical Package for the Social Sciences) software (39) in order to analyze it statistically. Descriptive statistics; Repeated measures ANOVA; GLM (General Linear Model) and correlation analysis between the studied variables were applied. Duncan's multiple range test (12) was used to separate the means within GLM, while Bonferroni test was used for separating means of repeated measures variables. The same statistical program used for drawing the charts of the interaction between studied discrete variables and time.

[i]**Table 1:** Ingredients and Chemical Composition of the Diet

Concentrate		
Ingredients	**Amount (g kg^{-1})**	
Barley	400	
Wheat bran	350	
Soybean meal	200	
Corn	30	
Salt	10	
Vitamin and mineral	10	
Content (g kg DM^{-1})	**Concentrate**	**Wheat straw**
DM (Dry Matter)	885.02	916.21
CF (Crude Fiber)	104.07	363.12
CP (Crude Protein)	121.29	21.03
EE (Ether Extract)	42.28	15.20
Ash	105.71	86.10
NFE (Nitrogen-free Extract)	726.89	491.76

Results and Discussion

Dry matter (DM) Intake:

The means of total DM from both concentrate and roughage are exhibited in Table 2. During the early growth phase (Weeks 0-4), the SB group showed the highest DMI (378.94 g/day), followed by control (349.49 g/day) and ME (326.29 g/day) groups, though these differences were not statistically significant (p = 0.35). aligns with findings by (33), who reported that sodium bicarbonate can transiently increase DMI by stabilizing rumen pH and improving palatability in high-concentrate diets. However, the lack of statistical significance (P=0.35) in our study contrasts with results from Erdman (13), who found significant early-phase DMI increases with SB supplementation in dairy cattle. The numerically higher intake in SB-supplemented lambs may reflect improved palatability or initial buffering effects on rumen pH. In the mid-growth phase (Weeks 5-8), DMI values converged among treatments (control: 300.92 g/day; ME: 300.66 g/day; SB: 307.83 g/day; p = 0.28). This stabilization suggests that any initial effects of the supplements on feed intake diminished as lambs adapted to the high-concentrate diet (19). Similar patterns have been observed in previous studies where dietary buffers showed transient effects on intake (17). At The late growth phase (Weeks 9-12), a different pattern revealed, with ME-administered lambs exhibiting the highest DMI (530.23 g/day), followed by control (514.82 g/day) and SB (488.56 g/day) groups (p = 0.18). The improved intake with ME supplementation in this phase is consistent with recent work by (7), who demonstrated that Megasphaera elsdenii supplementation enhanced DMI in finishing

Table 2. Effect of Megasphaera elsdenii and Sodium bicarbonate on Dry Matter Intake in Growing Lambs.

Daily Weight Gain (DWG):

During the early growth period (W 0-4), The lambs of the control group showed slightly higher DWG (116.7 g/day) as compared to that dosed with ME (101.4 g/day) and that supplemented with SB (92.9 g/day), though this difference was not significant (p = 0.72) These findings are supported by (28), this may suggest either an adaptation period to the treatments or that the basal diet was sufficient for early growth requirements (24) as shown in Table 3.

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In the mid-phase (W 5-8), the lambs from both ME (137.9 g/day) and SB groups (141.7 g/day) numerically outperformed the control (110.1 g/day), but again without statistical significance ($p = 0.22$), These findings are supported by studies in sheep (28) found that both ME (2×10^{12} cells/dose) and SB (2-4% diet) improved lamb DWG by 25-29% (137.9-141.7 vs 110.1 g/day) during weeks 5-8, While are in contrast with studies in cattle (21). this elevation in DWG could indicate a potential benefit of these additives during intermediate growth stages, possibly through improved nutrient utilization (10).
The last weeks of experiment (W 9-12) showed an interesting pattern, with the control group maintaining the highest DWG (183.3 g/day), that of lambs from ME group (173.6 g/day), while the lambs consumed SB exhibited lower gains (132.1 g/day; $p = 0.42$), These findings align with (19), who reported similar growth patterns in Arabi lambs fed high-concentrate diets, where ME supplementation (3 mL/day at 4.5×10^8 CFU/mL + 2 g/day Saccharomyces cerevisiae at 7×10^9 CFU/g) improved propionate production compared to SB (1% of diet) but did not significantly enhance DWG over the control group, Our findings are in contrast with (3) where 0.8% SB increased DWG by 12% in dairy cows ($p<0.05$), while ME (3×10^8 CFU/day) had no effect. These differences may stem from species variation (cows vs lambs), higher SB dose (0.8% vs 0.5%), or longer duration (16 vs 12 weeks), underscoring the need for species-specific dose optimization. The decline in DWG of lambs fed on SB may reflect reduced efficacy of SB as ruminants mature. In early growth phases, SB enhances rumen buffering, supporting microbial fermentation and propionate production. However, In grown animals, the developed rumen becomes less dependent on exogenous buffering, potentially explaining the lack of SB impact (22).

Table 3. Effect of Megasphaera elsdenii and Sodium Bicarbonate on Daily Weight Gain of Lambs.

	Time interval	treatment	Mean(g day^{-1})		p value
DWG	W 0 T0 4	control	116.66	17.93	
		ME	101.42	19.64	
		SB	92.85	25.36	0.72
	W 5 TO 8	control	110.11	11.96	
		ME	137.85	13.10	
		SB	141.66	16.92	0.22
	W 9 TO 12	control	183.33	21.99	
		ME	173.57	24.09	
		SB	132.14	31.10	0.42
	W 0 TO 12	control	158.33	7.04	
		ME	159.04	7.71	
		SB	139.68	9.95	0.28

Feed Conversion Ratio (FCR):
The experimental results demonstrate significant differences (P=0.02) in feed conversion ratio (FCR) among treatment groups, revealing important insights about rumen manipulation strategies (Table 4).The Lambs receiving (SB) showed markedly poorer feed efficiency (FCR=7.20) compared to both control (5.57) and (ME)- received groups (5.51) ,This is supported by (24) who observed no FCR improvement with SB in lambs, However, these results are in contrast with trials reporting SB-enhanced FCR in high-starch diets where it prevents subacute acidosis (15) and subsequent studies confirm that ME supplementation shows greater FCR improvements specifically in high-grain diets (>70% concentrate)(8). The probiotic's lactate-utilizing ability proves most effective during grain intake, where its pH-stabilizing action

directly enhances feed efficiency in both cattle (100 mL of 2×10^8 CFU/mL) and sheep (oral drench) (34).

The impaired FCR performance with SB supplementation is likely to be resulted from multiple factors: One of them is that excessive rumen buffering may have created pH conditions suboptimal for fiber digestion (9), secondly altered electrolyte balance potentially disrupted nutrient absorption (29). In contrast, ME administration maintained feed efficiency equivalent that of control group through its metabolic role in converting lactate to propionate, thereby stabilizing rumen conditions without compromising digestive efficiency (40)

Table 4. Effect of Megasphaera elsdenii and Sodium Bicarbonate on Feed Conversion Ratio (FCR) in Growing Lambs.

FCR			
treatment	Control	ME	SB
Mean (kg kg^{-1})	5.57 b	5.51 b	7.20 a
Std error	0.58	0.62	1.36
P value	0.02		

BLOOD:

There was a significant increase in glucose levels over time ($P < 0.01$) as shown in Table 5 and this might be attributed to enhanced ruminal propionate production from grain fermentation, as starch-rich diets provide more gluconeogenic precursors (20). While ME administration showed a trend for higher glucose levels ($P = 0.07$), the findings are confirmed by(1) dosed with 100 mL (2×10^8 CFU/mL) for cattle. these findings may reflect individual variation in microbial colonization or the time required for ME to establish changes in the rumen ecosystem, furthermore, (21) observed no significant changes in blood glucose in cattle fed on high high-grain diet using ~10^8 CFU/mL ME). Sodium bicarbonate (SB) supplementation increased glucose levels from 80.66 to 100.50 mg dL^{-1} over 12 weeks (P=0.006 for time effect), These findings are confirmed by (36), who reported that SB supplementation (1.5% DMI) significantly increased blood glucose levels in cattle by 15.2 mg/dL through enhanced propionate production and reduced lactate accumulation, also (26) observed no blood glucose change with SB consumption. Here, the glucose level elevation over time may be attributed to the rumen buffering capacity of sodium bicarbonate, which optimizes pH for microbial fermentation and propionate production (36).

Triglyceride of serum remained Indifferent across treatments ($P > 0.05$), These findings are supported by (37) the study compared sodium bicarbonate (1% of diet) and Megasphaera elsdenii (4.5 × 10^8 CFU/mL) + Saccharomyces cerevisiae (7 × 10^9 CFU/g) in lambs on high-concentrate diets and no significant differences ($P > 0.05$) were observed in blood triglyceride levels. This is likely due to homeostatic mechanisms that maintain circulating lipid levels in ruminants (3) and while the results are in contrast with that of (27) supplemented dairy-beef crossbred calves with Megasphaera elsdenii NCIMB 41125 (single and double oral dose at days 15 and 39). The non-significant differences suggest that neither ME nor SB supplementation substantially altered lipid mobilization.

There was a significant effect of time on the mean BUN levels (P=0.004) with the highest level observed at the 12th week (32.74 mg dL-1), aligns with (27) demonstrated that ME improves rumen fermentation and significant effect of time on BUN levels in calves ,Similarly, research

on buffering agents in cattle showed SB administration helps regulate rumen pH , this may support the finding here at the 12th week of experiment BUN(4),and these results in contrast with (19) Reported no BUN elevation in SB-supplemented lambs, possibly due to efficient urea recycling, also results in contrast with (11) ME combined with Saccharomyces cerevisiae increased microbial protein synthesis and reduced BUN in lambs.

Lactate accumulation showed a significant temporal rise (P < 0.01) at the 12th week of experiment, These findings are supported by (40) The study observed that oral dosing of ME in sheep during the transition to high-grain diets initially led to a transient increase in blood lactate levels. The numerically higher lactate in ME-fed lambs likely represents transient accumulation before bacterial conversion to short-chain fatty acids, as ME requires time to metabolize the lactate produced by other rumen microbes, while these results are in contrast that of (43) who investigated ME as a probiotic to mitigate lactate accumulation in cattle ,despite administering high doses (~2×10^{12} cells/day), the study reported that blood lactate level remained unchanged. The non-significant treatment effect (P = 0.13) suggests that both dietary interventions were insufficient to completely prevent lactate production in this intensive feeding as mentioned by (28).

Means having different letters for each parameter within each factor are differed significantly. ns=non-significant (p>0.05); *= significant (p<0.05); **= significant (p<0.01) ± SEM = Standard error Mean TRiG=Triglyceride BUN=Blood urea nitrogen

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		time(week)						P value		
Parameters	treatment	0	4	8	12	overall mean(mg dL^{-1})for treatments	SEM	Treatment	Time	Interaction
Glucose(mg dL^{-1})	control	81.33	89.33	91	94.16	88.95	5.82	0.07 ^{ns}	0.006**	0.45 ^{ns}
	ME	87	110.33	90.2	102.6	97.53				
	SB	80.66	88.2	89.25	100.5	89.65				
	Overall mean(mg dL^{-1}) for weeks	83.00 b	98.58 a	90.50 ab	99.08 a					
TRIG(mg dL^{-1})	control	16.83	22.33	21	14.16	18.58	2.91	0.68 ^{ns}	0.56 ^{ns}	0.58 ^{ns}
	ME	17	21.16	20.2	19.6	19.49				
	SB	17.33	16.8	16.5	19.75	17.59				
	Overall mean(mg dL^{-1}) for weeks	17.05	20.1	19.23	17.83					
BUN(mg dL^{-1})	control	18.85	22.51	20.86	26.51	22.18	4.6	0.22 ^{ns}	0.004**	0.71 ^{ns}
	ME	20.16	32.73	23.2	32.24	27.08				
	SB	20.03	26.76	20.7	39.45	26.73				
	Overall mean(mg dL^{-1}) for weeks	19.68 b	27.34 ab	21.59 b	32.74 a					
Lactate(mg dL^{-1})	control	12	15.11	14.81	17.6	14.88	1.5	0.13 ^{ns}	0.001**	0.70^{ns}
	ME	12.9	16.01	16.02	21.32	16.57				
	SB	13.05	15.66	13.77	16.67	14.79				
	Overall mean(mg dL^{-1}) for weeks	12.65 c	15.59 b	14.87 bc	18.55 a					

Conclusion:

While Administration of Megasphaera elsdenii (ME) or supplementing sodium bicarbonate (SB) did not significantly enhance overall dry matter intake (DMI) or daily weight gain (DWG) as compared to control group, they exhibited distinct temporal effects and critically differed in their impact on feed efficiency. SB supplementation (10g/kg DM) significantly impaired feed conversion ratio, indicating poorer utilization of feed for growth compared to both the control and ME groups. ME supplementation showed relatively potential benefits in the late growth phase, supporting higher DMI. Consequently, SB is not recommended for improving efficiency in Awassi lambs on high-concentrate diets due to its

detrimental effect on FCR. ME administration demonstrated comparable overall performance to the control and may offer advantages for late-phase intake, further investigation into optimized dosing or delivery strategies to more effectively mitigate SARA is recommended.

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