




Effects of diet type and addition of different levels of malic acid on some blood biochemical and rumen parameters of male Arabi lambs

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Abstract

The study was conducted at the Animal Farm of the College of Agriculture/ University of Basrah, to investigate the effects of two types of feed (pelleted and mash) and different levels of malic acid, as well as their interaction, on some biochemical parameters (cholesterol, glucose, total protein, albumin, and urea) and rumen characteristics (papillae length and width). A total of 24 male Arabi lambs, aged 5–6 months with an initial body weight of approximately 22 kg, were used in the experiment. The lambs were randomly allocated into six treatment groups as follows: T1: mash concentrate diet without malic acid supplementation (0 g/kg feed); T2: mash concentrate diet supplemented with malic acid at 4 g/kg feed; T3: mash concentrate diet supplemented with malic acid at 8 g/kg feed; T4: pelleted diet without malic acid supplementation (0 g/kg feed); T5: pelleted diet supplemented with malic acid at 4 g/kg feed; T6: pelleted diet supplemented with malic acid at 8 g/kg feed. The experimental diets were offered twice daily (morning and evening) for a period of three months, preceded by a 10-day adaptation period. The results indicated that the pelleted diet, the supplementation level of 4 g malic acid/kg feed, and their interaction were the most effective in reducing blood cholesterol and urea levels, increasing glucose, total protein, and albumin concentrations, and enhancing rumen papillae length. It was concluded that feeding a pelleted diet supplemented with 4 g malic acid/kg feed improves certain blood biochemical parameters (cholesterol, glucose, total protein, albumin, and urea) and promotes rumen papillae development in Arabi lambs.

I. Introduction

Feed additives have an important role for maintaining and enhancing rumen fermentation, growth, reproduction, and the quality of animal products, but they are not responsible for animal health or efficiency alone (Dhari and Kassim, 2019). In recent years, the use of antibiotics in animal feed has increased, and scientific efforts have focused on developing alternative types of feed additives such as enzymes, propionic and organic acids (Sahoo and Jena, 2014). The use of Organic Acids as feed additives has increased due to antibiotic prevention in the European Union. These carboxylic compounds are commonly present in biological tissues and have been used in livestock production for many years. Some organic acids such as malic acid, formic acid and aspartic acid are found in nature as components of plant and animal tissues (Elmali et al., 2012). One of the characteristics of these acids is that they act as inhibitors of methane formation in the laboratory (Newbold et al., 2005).

Most of the organic acids that have been used as food additives, including formic, malic and propionic acids, have had a significant role in improving the digestion and absorption of nutrients, improving the rumen environment and reducing gases produced (Newbold and Rode, 2006; Sahoo and Jena, 2014). Malic acid is an anti-bacterial energy source that stimulates growth and enhances the physiological properties of animal blood (Ali et al., 2013). Also, Organic acids, including malic acid, have a role in improving some biochemical

parameters in the blood such as glucose, albumin, and total protein, and reducing the levels of cholesterol and triglycerides (Malekkhahi et al., 2015).

This study aimed to know the effect of two types of diet (crushed and pelleted) with the level of malic acid and the interaction between them to examine their effect on some blood biochemical and rumen parameters of male Arabi lambs.

II. Materials and methods

This study was carried out in the animal field of the College of Agriculture, University of Basra, Karma site, from 1/12/2020 to 1/3/2021. Twenty-four Arabi lambs (males) have been used, averaging 5-6 months. Lambs were randomly distributed after their initial weight was recorded, using a digital scale for small animals (sheep and goats), on (6) treatments, (4) lambs for each treatment includes the use of three levels of (L-Malic acid) with two types of diet either crushed or pellet, as follows:

1. (T1) crushed condensed diet without the addition of malic acid (0).
2. (T2) crushed condensed diet with the addition of malic acid (4 g/kg feed).
3. (T3) crushed condensed diet with added malic acid (8 g/kg feed).
4. (T4) pellet feed without adding malic acid (0).
5. (T5) pellet feed with the addition of malic acid (4g/kg feed).
6. (T6) pellet feed with the addition of malic acid (8 g/kg feed).

The lambs were housed in semi-shaded pens of equal size (2 x 2.5 m²), and each pen contains fixed feeders and water basins throughout the experimental period. Where the diet was given twice daily at 7 am and 4 pm at a rate of 3% of bodyweight. Drinking water has been replaced everyday by clean water. The period of feeding on the experimental diets continued for (90) days. The proportions of the food ingredients and their chemical composition are presented in Table (1).

Blood samples were taken from the jugular vein of lambs (8 ml) once a month, using a sterile medical syringe. The samples were distributed into clean and sterile plastic test tubes (without anticoagulant) and left to coagulate for an hour at laboratory temperature, then were placed in a centrifuge (at a speed of 3000 rpm for twenty minutes) for the purpose of separating the blood serum from the rest of the components, and kept by freezing at a temperature (-20 °C) in tightly closed tubes until the determination of the biochemical parameters. After slaughtering the animals, extracting their internal organs and separating their intestines, sections of the rumen were taken from the abdominal area with a length of no more than (2) cm for the purpose of conducting a histological study and detecting the changes that can be obtained in the length and width of the rumen papillae (Alhidary et al., 2016).

Table (1): Ingredients of basal diets and chemical composition

Ingredients (g/ kg DM)	Crushed			Pellet		
	T1	T2	T3	T4	T5	T6
Barley	53	53	53	53	53	53
Wheat bran	36	36	36	36	36	36
Soybean meal	8	8	8	8	8	8
Mineral-vitamin premix	2	2	2	2	2	2
Salt	1	1	1	1	1	1
Malic acid g/kg feed	0	4	8	0	4	8
Chemical composition						
Dry matter	82.8	82.8	82.8	82.8	82.8	82.8
Crude protein	14.02	14.02	14.02	14.02	14.02	14.02
Ether extract	2.86	2.86	2.86	2.86	2.86	2.86
Crude fiber	7.38	7.38	7.38	7.38	7.38	7.38

Ash	3.63	3.63	3.63	3.63	3.63	3.63
Soluble carbohydrates	67.59	67.59	67.59	67.59	67.59	67.59
Metabolic energy MJ/ kg dry matter	12.40	12.40	12.40	12.40	12.40	12.40

The albumin and urea concentrations were measured using a chemical kit from the France Biolabo Company. The glucose concentration was determined using a chemical kit from the England Plamatec Company. The total protein and cholesterol concentrations were measured using a chemical kit from France Biomerieux Company. The data were analyzed as a factorial experiment design to study the effect of different type of diet and the level of malic acid and the interaction between them on the studied traits, and the significant differences between the means ($P < 0.05$) were compared with the statistical program (SPSS, 2019), were used according to the following mathematical model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Y_{ijk} = The value of the observation for any study adjective . μ = Overall mean. A_i = Effect of the type of diet (1, 2). B_j = Effect of malic acid levels (0, 1, 2). AB_{ij} = The effect of the interaction between the levels of malic acid and the type of diet. e_{ijk} = Experimental error that is distributed randomly and naturally with mean equal to zero and variance σ^2e .

III. Results and discussion

Table (2) shows that there were no significant differences ($P < 0.05$) in cholesterol levels during the three months between the pellet and the crushed diet. Significant differences ($P < 0.05$) were also observed in treatment (0 g of malic acid / kg of feed) at the third month in cholesterol concentration, and it was recorded 135.66 mg/100ml compared to treatment (4) and 8 g of malic acid / kg of feed (118.55 and 121.11 mg/100ml), respectively. When studying the interaction between food type and malic acid level, there were significant ($P < 0.05$) differences for the two treatments (powder x malic acid 0 g/kg feed and pellet x malic acid 0 g/kg feed) at the third month in cholesterol concentration, which were recorded (135.43 and 135.20) mg/100ml compared to the other treatments as there are no statistically significant differences between them.

The reason for the decrease in the concentration of cholesterol in the blood serum may be due to its association with an increase in the metabolic rate as a result of the increased secretion of thyroxin hormone from the thyroid gland, as this hormone plays a role in cholesterol metabolism and increases the liver's ability to get rid of cholesterol through bile products (Rasouli et al., 2004). Or it may be due to the fact that the effect of the type of diet contributes to raising the level of insulin, which increases the concentration of propionic acid in the rumen, and that the increase in propionate slows the synthesis of triglycerides and cholesterol in liver cells, and can change the level of fats in blood (Antunović et al., 2005; Kowalik et al., 2012).

The results of the study were in agreement in that there were no significant differences in cholesterol concentration when using pellet and the crushed diet in sheep and goats feeding (Li et al., 2021; Zhong et al., 2018; Islam et al., 2017), and these results were also in agreement in terms of the use of malic acid or its salts with a number of researchers (Malkkhahi et al., 2015; Abas et al., 2007).

Table (2) shows significant differences ($P < 0.05$) for the pellet diet in the glucose concentration at the third month, and it recorded 50.34 mg/100ml compared to the crushed diet, which recorded 47.00 mg/100ml. Those results agreed with the results of Zhong et al., (2018); Islam et al., (2017) and Pi et al., (2005) when sheep and goats fed on a pellet diet compared with crushed diet. It was also noted that there were significant differences ($P < 0.05$) for the treatment (4 g of malic acid / kg of feed) in the concentration of glucose in all months of experiment, and it was recorded (49.65, 43.39 and 63.29) mg/100ml, respectively, compared to the other treatments. This result corresponds to the results of Malekkhahi et al., (2015) and Flores Pérez (2004) when used with malic acid or its salts. When studying the interaction between the type of diet and the level of malic acid, it was noted that there were significant ($P < 0.05$) differences for the treatment (pellet x malic acid 4 g/kg feed) in glucose concentration at the second and third month, and it was recorded (50.33 and 65.11) mg/100ml, respectively, compared to the other treatments. The reason for the



superiority of the pellet diet significantly ($P < 0.05$) in comparison with crushed diet in the concentration of glucose at the third month may be due to the fact that making the feed in the form of pellet increases the intake of feed and increases digestibility and enhances the fermentation of the rumen due to the stay of the feed materials for a longer period in the digestive canal (Li et al., 2021; Lailer et al., 2005), and this leads to increased levels of liberated energy and consequently elevated blood glucose. As for the reason for the increase in glucose concentration when adding malic acid, it may be due to that carboxylic acids activate the transformation of lactic acid into propionic acid by bacteria using the succinate-propionate pathway (Martin et al., 2000), noting that propionic acid has an essential role in glucose synthesis (Bonzanini et al., 2025).

Table (2): Averages of cholesterol and glucose concentrations (mg/100ml) for the different experimental treatments \pm standard error

Treatments		Cholesterol			Glucose		
		1	2	3	1	2	3
type of diet	Crushed	97.84 \pm 1.88	115.93 \pm 6.75	124.67 \pm 2.77	41.61 \pm 0.80	45.60 \pm 1.05	47.00 \pm 0.76 <i>b</i>
	Pellet	99.23 \pm 3.05	117.73 \pm 3.00	125.32 \pm 2.41	42.07 \pm 0.55	47.56 \pm 0.79	50.34 \pm 1.16 <i>a</i>
Malic acid	0 g/kg feed	101.64 \pm 4.47	127.21 \pm 9.69	135.66 \pm 1.89 <i>a</i>	40.43 \pm 0.34 <i>b</i>	43.86 \pm 0.49 <i>b</i>	57.02 \pm 0.54 <i>c</i>
	4 g/kg feed	95.91 \pm 2.72	109.62 \pm 2.06	118.55 \pm 1.69 <i>b</i>	43.39 \pm 0.81 <i>a</i>	49.65 \pm 0.64 <i>a</i>	63.29 \pm 1.15 <i>a</i>
	8 g/kg feed	98.06 \pm 0.84	113.66 \pm 2.60	121.12 \pm 1.46 <i>b</i>	41.70 \pm 0.91 <i>ab</i>	46.23 \pm 1.20 <i>b</i>	60.13 \pm 0.90 <i>b</i>
Interaction		99.54 \pm 1.45	124.77 \pm 20.70	135.43 \pm 3.49 <i>a</i>	40.13 \pm 0.25 <i>b</i>	42.78 \pm 0.46 <i>c</i>	56.04 \pm 0.44 <i>c</i>
crushed \times malic acid 0g / kg feed		96.34 \pm 5.69	108.40 \pm 3.38	117.37 \pm 2.76 <i>b</i>	43.33 \pm 1.34 <i>b</i>	48.97 \pm 0.80 <i>ab</i>	61.47 \pm 1.09 <i>b</i>
crushed \times malic acid 4g/kg feed		97.65 \pm 1.68	114.62 \pm 4.13	121.20 \pm 2.19 <i>b</i>	41.36 \pm 1.85 <i>b</i>	45.06 \pm 2.20 <i>bc</i>	59.18 \pm 0.24 <i>bc</i>
crushed \times malic acid 8g / kg feed		103.75 \pm 9.40	129.66 \pm 2.74	135.20 \pm 2.13 <i>a</i>	40.73 \pm 0.64 <i>b</i>	44.94 \pm 0.37 <i>bc</i>	57.99 \pm 0.72 <i>bc</i>
Pellet \times malic acid 0g/kg feed		95.47 \pm 1.44	110.84 \pm 2.73	119.91 \pm 2.29 <i>b</i>	43.44 \pm 1.14 <i>b</i>	50.33 \pm 0.98 <i>a</i>	65.11 \pm 1.66 <i>a</i>
Pellet \times malic acid 4g/kg feed		98.47 \pm 0.60	112.70 \pm 3.74	121.05 \pm 2.29 <i>b</i>	42.04 \pm 0.61 <i>b</i>	47.40 \pm 0.98 <i>ab</i>	61.08 \pm 1.76 <i>b</i>

* Different letters vertically differ significantly at the 5% level.

Table (3) shows significant differences ($P < 0.05$) for the pellet diet in the concentration of total protein at the third month, and it was (7.29) g/100ml compared to the crushed diet (6.94 g/100ml). These results were in agreement with Zhong et al., (2018) and Pi et al., (2005) when sheep and goats were feeding



on a pellet compared with crushed diet. Also, significant differences ($P < 0.05$) were observed for the treatment (4 g malic acid / kg feed) at the third month in the concentration of total protein, it was recorded 7.43 g/100 ml compared to the treatments (8 and 0 g malic acid / kg feed) which recorded (7.13 and 6.53) g/100ml, respectively. This result corresponds with the results of Malekkhahi et al., (2015) when malic acid or its salts are used. When studying the interaction between the type of diet and the level of malic acid, significant differences ($P < 0.05$) appeared in the treatment (pellet \times malic acid 4 g/kg feed) at the third month in the total protein concentration and recorded 7.56 g/100ml, respectively, compared to the other treatments.

The reason for the superiority of the pellet diet as compared with crushed diet in total protein concentration may be due to that the pellets reduce food particle size intake, which may increase the surface available for microbial degradation and enhance the fermentation of the feed (Bertipaglia et al., 2010), which increases dietary crude protein in the rumen and intestines, and this leads to an increase in protein in the blood after it is absorbed (Dos Santos et al., 2011). Whereas, The addition of malic acid reduces the rumen (pH) and this decrease in pH increases the active proteolytic activity. In addition, organic acids have positive effects on protein hydrolysis, which lowers the pH in the duodenum, thereby increasing nitrogen retention and improving the digestion of nutrients, including protein (Khampa and Wanapat, 2006; Sniffen et al., 2006). The superiority of the treatment (pellet \times malic acid 4 g/kg feed) in total protein concentration was due to the synergistic action of the type of diet malic acid.

There were no significant differences ($P < 0.05$) in the albumin concentration between the pellet and crushed diets in all months of study (Table 3), the result agrees with the results of Pi et al., (2005). While there were significant differences ($P < 0.05$) for the treatment (4 gm of malic acid/kg of feed) in the albumin concentration in the second and third month, and the averages were (5.23 and 5.85) gm/100ml, respectively, compared to the other treatments. The increase in albumin concentration can be explained by the direct relationship between the increase in total protein and albumin in the blood serum (AL-Jassim et al., 2006; Oh et al., 2008). When studying the interaction between the type of diet and the level of malic acid, significant differences ($P < 0.05$) showed in the treatment (pellet \times malic acid 4 g/kg feed) in the albumin concentration in the second and third month, and the concentrations reached (5.34 and 5.92) g/100ml, respectively, compared to the other treatments.

Significant differences ($P < 0.05$) were observed for the crushed diet in the urea concentration at the third month, and it recorded 3.86 mmol/L, compared to the pellet diet, which recorded 3.47 mmol/L (Table 3), this might be due to feeding on crushed diet causes a decrease in the concentration of $\text{NH}_3\text{-N}$ in the rumen, as a possible result of increased incorporation of nitrogen with microbial protein due to microorganisms, and this leads to a decrease in urea in the blood (Paryad and Rashidi, 2009).

Table (3): averages of total protein, albumin (g/100ml) and urea (mmol/L) concentrations for the different experimental treatments \pm standard error

Treatments		Total protein			Albumin			Urea		
		1	2	3	1	2	3	1	2	3
Type of diet	Crushed	2.12	6.00	6.94	4.27	4.65	5.34	7.95	5.57	3.86
		\pm 0.37	\pm 0.20	\pm 0.18	\pm 0.18	\pm 0.13	\pm 0.18	\pm 0.10	\pm 0.17	\pm 0.20
	Pellet	5.41	6.21	7.29	4.49	4.98	5.75	7.74	5.43	3.47
		\pm 0.49	\pm 0.18	\pm 0.14	\pm 0.27	\pm 0.17	\pm 0.12	\pm 0.25	\pm 0.12	\pm 0.16
Malic acid	0 g/kg feed	5.53	5.53	6.79	4.09	4.34	4.92	8.22	5.81	4.33
		\pm 0.71	\pm 0.22	\pm 0.24	\pm 0.19	\pm 0.09	\pm 0.18	\pm 0.13	\pm 0.14	\pm 0.19
	4 g/kg feed	5.57	6.64	7.43	4.58	5.23	5.85	7.55	5.29	3.16
		\pm 0.50	\pm 0.15	\pm 0.18	\pm 0.39	\pm 0.19	\pm 0.12	\pm 0.34	\pm 0.15	\pm 0.13



	8 g/kg feed	5.41 ± 0.35	6.15 ± a 0.18	7.13 ± ab 0.13	4.47 ± 0.24	4.86 ± a 0.16	5.56 ± a 0.07	7.77 ± 0.11	5.40 ± 0.20	3.50 ± b 0.15
Interaction		4.85 ± 0.81	5.46 ± 0.36	6.53 ± b 0.47	4.04 ± 0.12	4.20 ± c 0.07	4.85 ± b 0.38	8.27 ± 0.11	5.97 ± 0.17	4.64 ± a 0.22
crushed × malic acid 0g / kg feed		5.37 ± 0.83	6.46 ± 0.25	7.30 ± ab 0.23	4.41 ± 0.33	5.13 ± ab 0.08	5.79 ± a 0.25	7.67 ± 0.15	5.33 ± 0.16	3.36 ± bc 0.21
crushed x malic acid 4g/kg feed		5.40 ± 0.41	6.09 ± 0.32	6.98 ± ab 0.08	4.35 ± 0.48	4.61 ± bc 0.21	5.38 ± ab 0.06	7.90 ± 0.27	5.41 ± 0.42	3.57 ± bc 0.23
Pellet × malic acid 0g/kg feed		5.04 ± 1.31	5.61 ± 0.30	6.90 ± ab 0.10	4.14 ± 0.39	4.61 ± bc 0.14	4.99 ± b 0.04	8.17 ± 0.73	5.66 ± 0.21	4.30 ± ab 0.24
Pellet x malic acid 4g/kg feed		5.78 ± 0.67	6.81 ± 0.14	7.56 ± a 0.31	4.75 ± 0.76	5.34 ± a 0.40	5.92 ± a 0.05	7.43 ± 0.73	5.26 ± 0.29	2.96 ± c 0.11
Pellet x malic acid 8g/kg feed		5.42 ± 0.64	6.22 ± 0.20	7.28 ± ab 0.26	4.59 ± 0.18	5.10 ± ab 0.18	5.74 ± a 0.03	7.63 ± 0.16	5.39 ± 0.09	3.44 ± bc 0.22

*Different letters vertically differ significantly at the 5% level.

This result is in agreement with the results of Zhong et al., (2018) and Pi et al., (2005) for sheep and goats. It was also noted that there were significant differences ($P < 0.05$) for the treatment (0 gm of malic acid / kg of feed) at the third month in the concentration of urea, and it was recorded 4.33 mmol/L compared to the treatments (8 and 4 g of malic acid / kg of feed) which recorded (3.50 and 3.16). mmol/L, respectively, This result corresponds with the results of Flores Pérez, (2004) and Malekkhahi et al., (2015) when used malic acid or its salts When studying the interaction between the type of diet and the level of malic acid, significant differences ($P < 0.05$) showed for the treatment (pellet x malic acid 0 g/kg feed) at the third month in the urea concentration, and it was recorded at 4.64 mmol/L compared to the other treatments.

The length of the rumen papillae was significantly ($P < 0.05$) in animals of the pellet diet (3.79 mm) compared to the crushed diet (3.15 mm) (Table 4). The reason for this result may be due to the increase in the concentrations of volatile fatty acids resulting from the digestion of carbohydrates when the diet was in the form of pellet compared to the crushed diet (Álvarez-Rodríguez et al., 2012). Or it may be due to the fact that the rumen papillae is associated with increased animal growth, meaning that the larger animal has more developed papillae (Zhong et al., 2018). This result agrees with the result of Malik et al., (2021). Also, treatments (4 and 8 gm malic acid/kg feed) were significantly ($P < 0.05$) in the length of the rumen papillae and recorded (4.11 and 3.67) mm, respectively, compared to the treatment (0 gm malic acid/kg feed) which recorded 2.62 mm. However, organic acids (malic acid) have a positive effect on raising propionic acid levels and pH, and this leads to the growth of rumen papillae (Abd al-Rahman et al., 2019; Suarez et al., 2006). This result agrees with the results of Flores Pérez, (2004) and Abdelrahman et al. al., (2019) on sheep and goats. When studying the interaction between the type of diet and the level of malic acid, significant differences ($P < 0.05$) showed in the treatment (pellet x malic acid 4 g/kg feed and pellet x malic acid 8 g/kg feed) in the length of the rumen papillae and they were recorded (4.37 and 4.02) mm respectively compared to the other treatments. The reason may be due to the interaction effect between the treatment and malic acid. Finally, no significant differences were observed in the width of the rumen papillae.

Table (4): Mean measurements of rumen nipple length and width (mm) for different experimental parameters ± standard error



Treatments		Measurements of the rumen papillae	
		Length	Width
Type of diet	Crushed	3.15± 0.23 <i>b</i>	0.67± 0.04
	Pellet	3.79± 0.19 <i>a</i>	0.75± 0.03
Malic acid	0 g/kg feed	2.62± 0.19 <i>b</i>	0.66±0.03
	4 g/kg feed	4.11± 0.18 <i>a</i>	0.80±0.07
	8 g/kg feed	3.67± 0.17 <i>a</i>	0.67±0.02
Interaction			
crushed × malic acid 0g / kg feed		2.27± 0.30 <i>d</i>	0.62± 0.04
crushed x malic acid 4g/kg feed		3.85± 0.24 <i>ab</i>	0.74± 0.05
crushed x malic acid 8g / kg feed		3.32± 0.22 <i>bc</i>	0.65± 0.05
Pellet × malic acid 0g/kg feed		2.97± 0.10 <i>c</i>	0.70± 0.07
Pellet x malic acid 4g/kg feed		4.37± 0.22 <i>a</i>	0.86± 0.01
Pellet x malic acid 8g/kg feed		4.02± 0.10 <i>a</i>	0.70± 0.03

*Different letters vertically differ significantly at the 5% level.

IV. Conclusions:

We conclude from this study that the use of pellet diet with the level of (4) g of malic acid/kg of feed improves some biochemical parameters of the blood (cholesterol, glucose, total protein, albumin, urea) and the growth of the rumen papillae of Arabi lambs.

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