



Concentrations of silymarin on the rumen activities in lambs

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ABSTRACT: Silymarin is derived from the milk thistle plant, and possesses numerous pharmacological actions, including hepatoprotective, anti-inflammatory, antioxidant, and anticancer properties. The aim of the study was to the determination of influence different concentrations of Silymarin on the rumen performance in 30 local lambs (divided into three groups of 10 lambs each). In the first and second groups, 420 and 210 mg kg⁻¹ of silymarin were administered for 8 weeks, and the third group was the control, which received normal saline solution. The parameters evaluated included the level of volatile fatty acids (VFAs), ammonia and pH in the rumen. Furthermore, the in vitro digestibility of ash, protein, fat and dry matter was investigated. These parameters were examined fortnightly, for eight weeks. The results showed that the level of volatile fatty acids and pH increased in the rumen in G1 and G2 compared to the control group, while the level of ammonia decreased in the rumen in G1 and G2 when compared to G3 ($p < 0.05$). Silymarin increased the in vitro digestibility of crude ash (CA), crude protein (CP), crude fat (CF) and crude dry matter (CDM) in the rumen of lambs. These results indicate that silymarin can improve the digestibility of nutrient elements in the lamb rumen.

Keywords: ammonia; digestibility; crude ash; crude protein; crude fat.

Concentrações de silimarina nas atividades ruminais de cordeiros

RESUMO: A silimarina é derivada da planta do cardo mariano e possui inúmeras ações farmacológicas, incluindo propriedades hepatoprotetoras, anti-inflamatórias, antioxidantes e anticancerígenas. O objetivo do estudo foi determinar a influência de diferentes concentrações de silimarina no desempenho ruminal de 30 cordeiros locais, divididos em três grupos (de 10 cordeiros cada). No primeiro e segundo grupo foi administrado 420 e 210 mg kg⁻¹ de silimarina durante 8 semanas, e, o terceiro grupo foi o controle, que recebeu solução salina normal. Os parâmetros avaliados incluíram o nível de ácidos graxos voláteis (AGVs), amônia e pH no rúmen. Além disso, investigou-se a digestibilidade in vitro de cinzas, proteínas, gorduras e matéria seca. Esses parâmetros foram examinados quinzenalmente, durante oito semanas. Os resultados mostraram que o nível de ácidos graxos voláteis e pH aumentaram no rúmen em G1 e G2, em comparação com o grupo controle, enquanto, o nível de amônia diminuiu no rúmen em G1 e G2 quando comparado com G3 ($p < 0,05$). A silimarina aumentou a digestibilidade in vitro da cinza bruta (CA), proteína bruta (PB), gordura bruta (CF) e matéria seca bruta (CDM) no rúmen dos cordeiros. Esses resultados indicam que silimarina pode melhorar a digestibilidade dos elementos nutrientes no rúmen do cordeiro.

Palavras-chave: amônia; digestibilidade; cinza bruta; proteína bruta; gordura bruta.

1. INTRODUCTION

Silymarin is a compound that is taken from seeds of milk thistle and used for the treatment of liver diseases. Silymarin is composed of flavonolignan isomers (LEE et al., 2007). Silymarin is used in the management and treatment of many diseases for improved the organs' function (VANGAVETI et al., 2021; OMMATI et al., 2021). Silymarin decrease the liver enzymes (GTP) and (AST) in rats (SHAKER et al., 2010).

Silymarin consists mostly of an isomeric combination of six phenolic chemicals, namely silydianin, silychristin, silybin, and isosilybin. Silymarin has been used to treat liver diseases. It inhibits carcinogenesis and has effects as antidiabetic, hypolipidaemic, anti-inflammatory, and cardioprotective (KREN AND WALTEROVA, 2005).

The milk thistle is contains silybin, silychristin, apigenin. The sheep, goats, and cows feed the milk thistle, but there is

low data about its digestibility, health effects, and performance. The rumen fermentation and the dry matter intake were affected by the milk thistle diets. The Milk thistle has essential oils 25-20% oil, which can be increased feed intake. silybum marianum decreased rumen ammonia level (MOJADDAM et al., 2015).

The milk thistle has an active compound if silymarin (POST-WHITE et al., 2007; EL-GHANY et al., 2022). Silymarin contains flavonolignans, such as silychristin, silydianin, isosilybin, dehydrosilybin, and a few flavonoids. Silymarin was used for the treatment of some diseases in cows. silymarin has a role in increasing the efficiency the rumen digestion and ruminal fermentation (KŘÍŽOVÁ et al., 2011).

During the last period of pregnancy, the intake of dry matter decreases. In this case, Silymarin is used to increase the digestibility of dry matter in the rumen, also, used in the

treatment of liver metabolic diseases. Silymarin was work as an antioxidant and decreased liver enzymes (ONMAZ et al., 2017).

The current study aimed to investigate the effects of Silymarin on the level of some rumen parameters in lambs, including levels of VFA, Ammonia, and PH in the lamb's rumen, as well as, *in vitro* Digestibility of Ash, Protein, Fat, and Dry matter in the rumen of lambs after being treated by Silymarin orally at two different doses.

2. MATERIAL AND METHODS

2.1. Animals and Experimental design

30 Awassi lambs (*Ovis aries*) were divided into three groups, each group consisting of 10 lambs. The first and second groups administrated 420 and 201 mg Kg⁻¹ daily of silymarin for 8 weeks, while G3 administrated normal saline at 0.9% concentration. The used parameters in the current study are included the determination of the level of VFA, Ammonia, and pH in the rumen. It also, investigated in Digestibility of Ash, Protein, Fat, and Dry matter in the rumen. The parameters are examined every biweekly for eight weeks. Under the supervision of the approved institutional Ethics Committee for Animal Experimentation at the Faculty of Veterinary Medicine, University of Baghdad, and in strict line with European and National Law for the Care and Use of Animals 575\P.G. Efforts were taken throughout the period of the trial to minimize pain and discomfort. The animals did not receive any sedatives or anesthesia.

2.2. Sample collection

The samples taken in the current study were rumen samples which are collected from the rumen directly by using gastric lavage after fixing the animals to avoid extreme movement. The samples are put in containers and sent to the lab to make an examination. The used parameters in the present work are included volatile fatty acids (VFAs), Ammonia, pH, and the digestibility of ash, protein, fat, and dry matter in the rumen.

2.2.1. VFA level: The volatile fatty acids were determined by the Lepper method (ALÇIÇEK; ÖZKAN, 1996).

2.2.2. Ammonia: it is measured according to the method of (KAPLAN, 1969).

2.2.3. pH: The pH levels were measured immediately by a digital pHmeter (Benchtop pH/ORP Meter, BEP-M210).

2.2.4. Rumen fluids Aspiration:

- 1- The Rumen fluids were collected by a special tube using a manual pump; from the rumen directly (10) mL then placed in a closed plastic tube (50) mL after adding HCl (5) mL then frozen.
- 2- The samples were centrifuged at 3000 RPM (25) minutes after thawing to remove the precipitate substances to yellow fluid.
- 3- The ruminal fluid (0.5) mL is added with (MgO) and (CaCl) (0.5) g. The mixture is measured by the collected tube.
- 4- Dilution of the solution in the flask HCl (0.05%) and mixing until the color of the methylene red is converted

into purple to determine the Ammonia level (AOAC, 2005).

2.3. Digestibility examination:

The *in vitro* digestibility measurements were made using the Ankom Daisy II method according to Damiran et al. (2008). The inoculum was obtained from rumen samples of lamb that were fed 60% roughage and 40% concentrated feed. The rumen fluid was taken after two hours after feed delivery and then put inside the thermos. Mixing of the solutions was done at (a 5:1) ratio, and adding buffer solution (1.800) mL was to the vessels. Vessels were placed in the incubator which was equilibrated to 39°C. After the incubation, the bags were rinsed with water until the water become clear then put in an air oven (105°C) to dry for (120-240) minutes. Feed samples were also analyzed for *in vitro* dry matter, Ash, Protein, and Fat digestibility.

The digestibility of a nutrient is determined by the difference between the nutrient ingested amount minus the nutrient amount extracted from the rumen directly by rumen lavage after two hours, expressed as a percentage of the nutrient ingested: 100 x (intake – samples extracted after two hours from rumen)/intake.

2.4. Statistical analysis:

The data are represented as (mean ± SD). Two-way ANOVA and LSD made the comparisons. (0.05) level of probability was used as the significance. SPSS software (27) is used for performing the comparisons (DANIEL, 2009).

3. RESULTS

Results of the present study showed that the level of volatile fatty acids (VFA) (mmol/100 mL) were highly significant differences in treated groups (G1 and G2) than the control group at a significant level ($p < 0.05$) along the study period (Table 1). The level of volatile fatty acids (VFA) was decreased significantly in G1 at 6th week, while G2 recorded a significant decrease in the last week ($p < 0.05$).

The findings showed that the level of Ammonia (mg/100 mL) decreased in G1 and G2 than in G3 at ($p < 0.05$). While ammonia decreased significantly ($p < 0.05$) in G1 and G2 at the 6th and 8th periods compared with 2nd and 4th weeks of the study (Table 1). The findings showed that pH was increased in G1 than in other groups at ($p < 0.05$). pH was decreased in G1 in the last period however, pH of G2 showed significant ($p < 0.05$) at the 6th week, as shown in Table 1.

Digestibility of ash was increased in G1 than in G2 and G3 at ($P < 0.05$) along all studied periods. The digestibility percentage of Ash was decreased in G1 the 6th and 8th weeks than in the other periods at ($p < 0.05$), while G2 recorded a significantly lower value in the last period of the study as shown in Table 2.

Digestibility of crude protein (CP) was increased in G1 than in G2 and G3 at ($p < 0.05$). Digestibility of Crude Protein % was decreased in G2 significantly ($p < 0.05$) decreased at the sixth week compared with other weeks (Table 2).

Crude fat (CF) digestibility was higher in G1 than in the other groups ($p < 0.05$). From the middle of the second period until the end of the experiment, G1 had a lower crude fat (CF) digestibility than the other groups. As can be seen in the table below, G2 values dropped significantly from 6th week onwards compared to the rest of the research (Table 2).

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Digestibility of Crude Dry matter (CDM) was increased in G1 than in other groups at a significant level ($p < 0.05$). Digestibility of Crude Dry matter (CDM) was decreased in

G3 than in G2 at ($p < 0.05$). however, CDM% showed non significant differences in the different periods as shown in Table 2.

Table 1. Levels of VFAs, ammonia and pH ruminal, in three study groups of lambs, throughout the 8 weeks.

Tabela 1. Níveis de AGVs, amônia e pH ruminal, nos três grupos de cordeiros estudados, ao longo das 8 semanas

Groups	2 nd	4 th	6 th	8 th
VFAs (mmol/100 mL)				
G1	4.60 ± 0.42 Aa	4.90 ± 0.61 Aa	5.20 ± 0.44 Ab	5.60 ± 0.29 Ac
G2	3.10 ± 0.65 Ba	3.20 ± 0.51 Ba	3.30 ± 0.60 Ba	3.40 ± 0.31 Bb
G3	2.85 ± 0.15 Ca	2.82 ± 0.90 Ca	2.81 ± 0.06 Ca	2.87 ± 0.8 Ca
Ammonia (mg /100 mL)				
G1	8.04 ± 1.1 Ca	7.67 ± 0.8 Ca	7.40 ± 2.3 Cb	7.73 ± 1.9 Cb
G2	8.47 ± 1.1 Ba	8.06 ± 1.0 Ba	7.70 ± 2.0 Bb	8.21 ± 2.0 Bb
G3	8.88 ± 1.3 Aa	8.42 ± 0.8 Aa	8.08 ± 2.3 Aa	8.66 ± 2.2 Aa
pH				
G1	6.65±0.05Aa	6.67±0.23Aa	6.70±0.18Aa	6.75±0.10Ab
G2	6.59±0.05Ba	6.61±0.20Ba	6.65±0.21Bb	6.70±0.09Ac
G3	6.51±0.10Ca	6.53±0.22Ca	6.52±0.17Ca	6.54±0.11Ca

The capital letters are used for comparing the cells vertically, while the small letters are used for comparing the cells horizontally at ($p < 0.05$).

As letras maiúsculas são usadas para comparar as células verticalmente, enquanto as letras minúsculas são usadas para comparar as células horizontalmente em ($P < 0,05$).

Table 2. Digestibility of ash, crude protein (CP), crude fat (CF) and crude dry matter, in three study groups of lambs, throughout the 8 weeks.

Tabela 2. Digestibilidade de cinzas, proteína bruta (PB), gordura bruta (CF) e matéria seca bruta, nos três grupos de cordeiros estudados, ao longo das 8 semanas

Groups	2 nd	4 th	6 th	8 th
Ash (%)				
G1	76.2 ± 2.2 Aa	77.2 ± 1.2 Aa	78.2 ± 1.5 Ab	79.2 ± 1.7 Ab
G2	73.2 ± 1.9 Ba	74.2 ± 1.8 Ba	74.2 ± 1.7 Ba	75.2 ± 2.2 Bb
G3	70.2 ± 3.2 Ca	70.3 ± 3.0 Ca	70.6 ± 2.9 Ca	71.0 ± 2.1 Ca
Crude protein – CP (%)				
G1	77.4 ± 1.3 Aa	78.6 ± 1.0 Aa	77.9 ± 1.1 Aa	78.9 ± 1.0 Aa
G2	73.6 ± 1.6 Ba	74.6 ± 1.1 Ba	75.7 ± 1.3 Bb	74.9 ± 1.7 Ba
G3	71.3 ± 1.5 Ca	70.8 ± 1.2 Ca	71.6 ± 1.7 Ca	70.9 ± 1.3 Ca
Crude fat – CF (%)				
G1	84.4 ± 1.0 Aa	87.6 ± 1.0 Ab	87.9 ± 1.1 Ab	86.9 ± 1.1 Ab
G2	81.6 ± 1.3 Ba	82.6 ± 1.3 Ba	83.7 ± 1.5 Bb	82.9 ± 1.4 Ba
G3	78.3 ± 1.1 Ca	78.8 ± 1.2 Ca	78.6 ± 1.7 Ca	78.9 ± 1.3 Ca
Crude dry matter – CDM (%)				
G1	78.2 ± 2.6 Aa	78.4 ± 1.3 Aa	78.4 ± 1.9 Aa	79.2 ± 1.3 Aa
G2	74.4 ± 1.3 Ba	73.7 ± 2.2 Ba	75.7 ± 1.4 Ba	74.3 ± 1.8 Ba
G3	70.3 ± 2.1 Ca	70.3 ± 1.1 Ca	70.3 ± 1.4 Ca	70.3 ± 1.2 Ca

The capital letters are used for comparing the cells vertically, while the small letters are used for comparing the cells horizontally at ($p < 0.05$).

As letras maiúsculas são usadas para comparar as células verticalmente, enquanto as letras minúsculas são usadas para comparar as células horizontalmente em ($P < 0,05$).

4. DISCUSSION

The study was conducted for the first time in Iraq and the world by studying the effect of silymarin on some functional profiles of lamb rumen and their digestibility.

When compared to the control group, animals given Silymarin had significantly higher levels of volatile fatty acids in both G1 and G2 ($p < 0.05$). The volatile Fatty Acids formed in the rumen are butyric acid, propionic acid, and acetic acid. Rumen microbes can ferment carbohydrates into volatile fatty acids by the corresponding enzymes (WANG et al., 2020).

VFAs formed carbohydrates fermentation (CEBRA et al., 2014). VFAs are formed for energy providing (DIJKSTRA, 1994). VFAs are important for anaerobic digestion, decomposing organics, and forming methane and CO₂ (ANDERSON; YANG, 1992).

The level of Ammonia was decreased in G1 and G2 in the rumen treated with silymarin than in the control group at ($p < 0.05$). Rumen microbes trans NPN to ammonia by urease. The ammonia is combined with ketoacid to form the amino acids. Rumen microbes have a role in urea hydrolysis, for form ureases that degrade the urea to CO₂ and ammonia. Ammonia is used for the synthesis of proteins. The rumen microbes break down the protein into ammonia and amino acids (GETAHUN et al., 2019).

Decreasing the ammonia level in the lamb rumen in our study may attribute to an increased level of pH (acidosis and alkalosis meter) due to an increased level of Volatile fatty acids that neutralize the alkalinity of the ammonia. pH was increased in G1 and G2 in the rumen treated with Silymarin than in the control group at ($p < 0.05$). The watery solution pH of Silymarin ranged (5-6), in addition to silymarin stimuli

to the generation of volatile fatty acids; all these factors stimuli increase PH (SURAI et al., 2015).

Digestibility of ash, protein, fat, and dry matter was increased in G1 and G2 in the rumen treated with Silymarin than the control group at ($p < 0.05$). The digestibility of the silymarin was perfect in the rumen (KŘÍŽOVÁ et al., 2011). Silymarin in the broiler diet has enhanced growth, digestibility of the nutrient, microflora, meat quality, and blood profile (SHANMUGAM et al., 2022).

Adding micelle silymarin 0.2% to the diet is enhancing the growth performance, fecal gas emission, digestibility, meat quality, and microbial content, in the pigs (KOO et al., 2022). The digestibility of silymarin in hours increases digestible energy, protein, fat, and ash. Silymarin increases the digestibility of flavonolignans when it is provided daily (DOCKALOVA et al., 2021).

Milk thistle seeds contain 75% flavonolignans and 25% polymeric and oxidized polyphenolic compounds. Milk thistle seeds are used orally to promote liver health, stimulate milk production, and reduce the risk of gallstones. Body weight, serum total protein, and liver enzymes were all improved after milk thistle was given to Japanese quail. In quail, milk thistle has been shown to boost immunity, growth, and feed conversion (KHAZAEI et al., 2022). Silymarin increases the general growth, digestibility of nutrients, and meat quality in pigs. The total digestibility of nitrogen was increased (ZHANG; KIM, 2022). A study by FARYADI et al. (2021) found that nano-silymarin improved hen performance and egg quality as asserted by our results.

5. CONCLUSIONS

Silymarin increases VFAs, and PH and decreases ammonia in the rumen. Also, the study proved that Silymarin could improve the digestibility of the nutrient elements in the rumen of the lamb.

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Data Availability Statement (how the data can be made available): Study data can be obtained by request to the corresponding author or the second author, via e-mail. It is not available on the website as the research project is still under development.

Conflicts of Interest: The author affirms that she has no financial or personal affiliations that could unduly affect or skew the paper's content.