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Effects of Saponins on Rumen Fermentation, Nutrients Digestibility, Performance, and Plasma Metabolites in Sheep and Goat Kids

M. H. Aazami[1], A. M. Tahmasbi[1], M. H. Ghaffari[1*], A. A. Naserian[1], R. Valizadeh[1] and A. H. Ghaffari[1]

[1]Faculty of Agriculture, Excellence Center in Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author MHA designed the study, performed the statistical analysis. Authors AMT and AAN managed the analyses of the study. Author MHG managed the literature searches, wrote the protocol, and the draft of the manuscript. Authors RV and AHG assisted with this project. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Two *in vivo* experiments were carried out to investigate the effects of saponins on rumen fermentation, plasma metabolites, nutrients digestibility, and performance in small ruminant.
Study Design: In the experiment 1, three Baluchi sheep (48± 4.3 kg, body weight) were randomly assigned to three experimental diets in a 3×3 Latin square design to determine the effects of saponins on digestibility, ruminal fermentation characteristics and plasma metabolites. Saponins were added at levels of 0, 100 and 200 mg/kg dry matter intake to diet. In the experiment 2, eighteen Saanen kids (6–7-month) were used in a completely randomized design to determine the effects of saponins on ruminal fermentation, plasma metabolites, and body measurements. Saponins were added at levels of 0, 36 and 54 mg/kg dry matter intake.
Place and Duration of Study: The trials were conducted at the Research Farm of Agriculture Faculty of Ferdowsi University of Mashhad (Iran), between February 2011 and October 2011.

**Corresponding author: Email: morteza.h.g@gmail.com;*

Methodology: Ammonia-N concentration was determined using the distillation method and blood metabolites were determined by an automated biochemical analyzer using commercial kits.
Results: In the experiment 1, saponins administration had no effect on apparent nutrient digestibility, ruminal pH, ruminal ammonia-N concentrations. Results showed that sheep receiving saponins had lower ($P<0.05$) plasma cholesterol concentrations than the control. In addition, saponins administration decreased plasma cholesterol concentrations, but did not affect the concentration of blood urea nitrogen, glucose, and triglycerides. In the experiment 2, saponins administration had no effect on DMI, ruminal pH, ammonia-N concentrations, body measurements and plasma metabolites except cholesterol. In goat kids we observed significant reduction in plasma cholesterol concentrations.
Conclusion: Administration of saponins in the diets of small ruminants did not improve nutrient digestibility, ruminal fermentation and growth performance, but reduced blood cholesterol concentration.

Keywords: Sheep, Kids, Rumen fermentation, Blood metabolites.

1. INTRODUCTION

Numerous research studies have been conducted to assess the potential of plant secondary metabolites as natural manipulating agents for ruminal fermentation [1,2]. Chemically, saponins are phytochemical compounds composed of steroids, triterpenoids, and steroid alkaloids commonly found in plants [3]. Two main types of saponins are known, monodesmoside saponins and bidesmoside saponins. Saponins that have one sugar molecule attached at the C3 position are called monodesmoside saponins, and those that have a minimum of two sugars, are called bidesmoside saponins [3]. Saponins as feed supplements could have the potential to modulate ruminal fermentation and improve nutrient utilization in ruminants [4]. Saponins have variable effects on ruminal fermentation [5,6]. It has been shown in both *in vitro* [7,8] and *in vivo* [9,10] that saponins might reduce the pH and ammonia–N concentration in the rumen. Saponins have been reported to have mixed effect on feed intake decreasing [11,12], or increasing [13,14] or no effect on feed intake in ruminants [15,16,17]. In the study of Lu & Jorgensen [18], the administration of saponins in the diet of sheep increased organic matter and fibre digestibility in the total digestive tract. Moreover, addition of saponin-rich plants, such as *Yucca schidigera* has been found to improve growth, feed efficiency and health in ruminants [19]. According to Wina et al. [3], many different factors affect the response of ruminants to saponins including sources, levels of supplementation, and diet composition. The objective of the present investigation was to determine the effect of saponins on plasma metabolites, feed digestibility, rumen fermentation, performance and body measurements of small ruminants. The hypothesis tested was that using saponins in the diet would modulate ruminal fermentation and, improve performance, and nutrient utilization in small ruminants.

2. MATERIALS AND METHODS

Two *in vivo* experiments were conducted at the Research Farm of Agriculture Faculty of Ferdowsi University of Mashhad (Iran) in 2011. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. In both experiments, commercial saponins (CAS 8047-15-2) from *Quillaja saponaria bark* were purchased from Merck KGaA, Darmstadt, Germany.

2.1 Animals, Diets and Experimental Design

Experiment 1: three Baluchi sheep (48± 4.3 kg, body weight) fitted with ruminal cannulas (2.5 cm i.d.) were housed in individual metabolism cages (0.5×1.2×1m) in a temperature controlled building (approximately 22 ºC). Treatments consisted of saponins administered to animal during d 1 to d 21 (d 0 represents the day before saponins administration) providing saponins at 0, 100, and 200 mg/kg dry matter intake (DMI) in a 3×3 Latin square design to diet. The basal diet was 300 g alfalfa hay/kg dry matter (DM), 200 g wheat straw/kg DM, 125 g canola meal/kg DM and 300 g barley/kg DM based concentrate. The ingredients and chemical composition of the basal diet is shown in Table 1. All diets were supplied as total mixed ration (TMR), at maintenance level twice daily in equal portions at 08:00 and 20:00 hour. Saponins was daily prepared as a liquid drench by dissolving Quillaja powder/kg DMI in 10 ml of distilled water [24] and they were drenched with 10 ml water solution for different treatments. Clean water was freely available ad-libitum at all times. The experiment consisted of 3 periods. Each period lasted 21 days, comprising 14 days of adaptation to the experimental diet followed by 7 days of data collection.

Table 1. Ingredients and chemical composition of experimental diets [a] (DM basis)

Item	Experiment 1	Experiment 2
Ingredient (%)		
Alfalfa hay	30	-
Corn silage	-	45
wheat straw	20	-
Barley	30	25
Wheat bran	12.5	14
Canola meal	6	14
Mineral and vitamin premix	1	1
NaCl	0.3	0.7
Limestone	0.2	0.3
Chemical composition [b]		
DM	90.4	85.9
CP	11.9	14.5
EE	2.4	3
NDF	40.8	39.5
ADF	25.2	22.4
NFC	40.4	39
Ca	0.8	0.8
P	0.5	0.6

[a] *Ration were formulated to supply nutrient requirement at maintenance level in experiment 1 and growing level for experiment 2 (NRC, 2001).*

[b] *Chemical composition was calculated based on tabulated composition of individual feedstuffs (Ministry of Agriculture, MOA, PRC, 2004). DM=Dry Matter; EE= Ether Extract; CP=Crude Protein; NDF=Neutral Detergent Fibre; ADF=Acid Detergent Fibre.*

Experiment 2: eighteen male Iranian Saanen weaned kids (6–7-month) were used in a completely randomized design. Each kid was confined in a separate digestion and metabolism crate (1.5×0.8 m) during the entire experiment. The kids were allowed a 10 days adjustment period in the crates, before starting a 63-day growth trial followed by 7 days for data collection. Kids were randomly divided into three groups of six each. The basal diet was

450 g corn silage/kg DM, 140 g wheat straw/kg DM, 140 g canola meal/kg DM and 250 g barley/kg DM base concentrate. Treatments consisted of saponins administered to an animal as a liquid drench (dissolved in 10 ml of water), during the entire experiment, provided saponins at 0 (treatment 1), 36 (treatment 2), and 54 (treatment 3) mg/kg DM. Body weight of each kid was registered at the beginning of adaptation and the average body weight for treatment 1, 2, and 3 were 22.34, 22.54, and 22.46 kg, respectively. The ingredients and chemical composition of the basal diet is shown in Table 1. All diets were supplied as TMR, and offered at growing level twice daily in equal portions at 08:00 and 20:00 h. Clean water was freely available *ad-libitum* at all times.

2.2 Measurements and Sampling

During the sampling days in both experiments, rumen fluid samples were collected at 3 h after morning feeding for the determination of ruminal pH and ammonia-N concentrations. The rumen fluid samples were filtered through four layers of cheesecloth and immediately used to measure its pH using a glass electrode pH-meter (691 Metrohm, Herisau, Switzland). The ruminal fluid was subsequently acidified with 10mL of 0.2 N HCl solution (50%, vol/vol) and stored frozen before ammonia-N analysis. Blood samples were collected from the jugular vein (10-mL into sterile tubes containing EDTA solution) at 3 h after the morning feeding. The samples were immediately placed on ice for processing in the laboratory. Blood samples were centrifuged (3000 g for 15 min at 5ºC); plasma was harvested and frozen at 20ºC for later analysis. During the 7 days of the collection period in the experiment 1, the amount of feed given and the feed left over after feeding were weighted and individual samples were collected daily and composited by animal for DM determination. Composited samples were dried (60ºC) and ground to pass through a 1-mm screen (Retsch Cutting Mill, Retschmule, Germany) and then used for analysis. Fecal samples (0.1 daily outputs) were collected and composited by animal over the 7-day collection period. The composited fecal samples were then mixed well and duplicate batches were dried to a steady mass at 60ºC, over 48 h, for determination of DM content, and then ground to pass through a 1-mm screen for later chemical analyses. Urine from each animal was collected daily in plastic vessels containing 100 mL sulfuric acid solution (0.1, v/v) to maintain a pH level below three, weighed, mixed well and a 0.1 daily aliquot was pooled over the 7-day collection period per animal. The bulked urine samples were stored at -20ºC until chemical analysis. Apparent digestibility of nutrients was estimated by the marker ratio technique using acid-insoluble ash as an internal marker [40]. In experiment 2, the body measurements (i.e., neck girth, heart girth, withers height, rump height, cannon bone length, hip width, pin-hip and body length) as growth performance were determined every 15 days.

2.3 Laboratory Analyses

The dry matter content of feed ingredients was determined by oven-drying at 60ºC for 48 h then analyzed for concentrations of DM, crude protein (CP), ether extract (EE) [20], neutral detergent fiber (NDF) and acid detergent fiber (ADF) [21]. Samples of feeds offered and individual refusals were also retained for dry matter determination after drying in a forced drought oven at 80℃ for 48 h. Sub-samples of feed offered, feed refusals and faeces were dried at 50ºC then ground to pass through a 1-mm dry mesh screen and stored until analyzed. Rumen ammonia-N concentrations were determined using the distillation method (Kjeltec Auto 1030 Analyzer, tecator, Hoganas, Sweden). The concentrations of cholesterol, triglyceride, blood urea nitrogen (BUN), total protein, albumin and glucose were determined by an automated biochemical analyzer (Biotecnica, Targa 3000, Rome, Italy) using

commercial kits (Pars Azmoon Co., Tehran, Iran) according to the manufacturer's instructions.

2.4 Statistical Analyses

Experiment 1: the statistical model was:

$Y_{ijkl} = \mu + T_i + SQ_j + \text{Period (SQ)}_{kj} + \text{Sheep (SQ)}_{lj} + \varepsilon_{ijkl}$
where Y_{ijkl} = observation ijkl; μ = the overall mean; T_i = the effect of treatment i; SQ = the effect of square j; Period (SQ) k_i = the effect of period k within square j; Sheep $(SQ)_{li}$ = the effect of sheep l within square j and ε_{ijkl} = random error with mean 0 and variance 2. Data were analyzed using the MIXED procedure of SAS (Version 9.1; Cary, NC). Before statistical analysis data were tested for normality using Proc UNIVARIATE in SAS (Version 9.1; Cary, NC).

Experiment 2: The data was analyzed as a completely randomized design using the PROC MIXED procedure of SAS 9.1.3 (SAS Institute Inc. Cary, NC, USA), with the animal as the experimental unit according to the following model:

$$Yij = \mu + Ti + \varepsilon ij$$

Where yij = dependent variable; μ = overall mean of the population; Ti = treatment, and εij = unexplained residual element assumed to be independent and normally distributed. All the data were presented as least squares means obtained through the LSMEANS (least-squares means) statement. Mean separations were determined using the PDIFF statement. Least significant difference at $P<0.05$ was used to determine significant differences among means.

3. RESULTS AND DISCUSSION

3.1 Experiment 1: Effects of Saponins on Rumen Fermentation Parameters, Plasma Metabolites and Feed Digestibility in Baluchi Sheep

The mean of apparent nutrients digestibility including DM, organic matter (OM), CP, NDF, and ADF are presented in Table 2. In the present study, apparent digestibility of DM, OM, CP, NDF and ADF did not differ ($P > 0.05$) among the treatments (Table 2). Previous experiments have shown a variety of responses in apparent digestibility to the administration of saponins in diet. Some studies have demonstrated that saponins extracts or saponins-containing plants did not affect nutrient digestibility [22,23]. Our results are consistent with reports of Saïda-Nasri et al. [24], who reported that the administration of 30, 60 or 90 mg of *Quillaja saponarie* per kg DMI had no effects on feed digestibility in Barbarine lambs. Similarly, the administration of Y. schidigera and Q. saponaria had no effect on NDF and ADF digestibility in dairy *cows [14].* In contrast, Makkar & Becker, [25], considered that the presence of *Quillaja saponin* caused a significant reduction in the apparent and true digestibility of the substrate in an *in vitro* fermentation. This inconsistency suggests that responses to saponins in terms of intake and digestibility in sheep could depend upon a diet composition, saponin source and their level of inclusion in the diet.

Table 2. Effects of saponins on nutrients digestibility in Baluchi sheep

Variable	Doses of saponins administered (mg/kg DMI)			S.E.M	p-Value
	0	100	200		
Nutrients (%)					
DM	62.0	62.1	62.4	0.81	0.49
OM	64.0	64.2	64.6	0.83	0.45
CP	59.2	59.5	59.4	0.94	0.14
NDF	52.7	54.0	54.2	1.20	0.41
ADF	47.2	45.9	48.4	1.12	0.09

S.E.M= standard error of the mean; DM= Dry matter; OM= Organic Matter; CP= Crude Protein; NDF= neutral detergent fiber; ADF= acid detergent fiber.

The mean of rumen fermentation parameters and plasma metabolites are presented in Table 3. Saponins administration had no effect on rumen fluid pH and ammonia-N concentrations ($P > 0.05$). Table 3 shows that sheep receiving saponins had lower ($P<0.05$) concentrations of plasma cholesterol than the control. Concentrations of plasma glucose, blood urea nitrogen (BUN) and triglycerides were similar among all the treatments ($P > 0.05$). The concentrations of ammonia-N and ruminal pH were not changed ($P > 0.05$) when sheep were fed diets supplemented with 100 or 200 mg/kg DMI saponins showed that saponins had no effects on the pattern of rumen fermentation, just as Zhou et al. [26], reported. Saponins have variable effects on ruminal pH and ammonia-N concentrations [5,6]. Pen et al. [27] concluded that ruminal ammonia-N concentrations decreased with increasing saponins in diet, which is in contrast with our results. In our study, saponins supply had an anticholesterol effect as reported by other authors [28,29]. One of the expected effects of saponins in this study was a reduction of plasma cholesterol concentration. This cholesterol-lowering effect of saponins is attributed to their ability to form insoluble micelle complexes with sterols such as cholesterol in the intestine [30]. Moreover, Saponins can inhibit absorption of cholesterol from the small intestinal and lowers plasma cholesterol in animals [31].

Table 3. Effects of saponins on rumen parameters and plasma metabolites in Baluchi sheep

Variable	Doses of saponins administered (mg/kg DMI)			S.E.M	p-Value
	0	100	200		
Rumen parameters					
pH	6.39	6.38	6.33	0.02	0.13
ammonia-N (mg/dl)	8.72	8.40	8.65	0.37	0.83
Blood metabolites (mg/dl)					
Cholesterol	81.2^{a}	76.3^{ab}	70.3^{b}	2.73	0.04
BUN	18.9	18.6	19.2	1.14	0.92
Glucose	63.7	61.8	64.2	2.35	0.76
Triglycerides	18.2	20.7	20.3	2.92	0.81

Within rows, means with different letters are significantly different ($P < 0.05$). S.E.M= standard error of the mean;

3.2 Experiment 2: Effects of Saponins on Rumen Fermentation Parameters, Plasma Metabolites and Performance of Saanen Kids

The mean of DMI, body weight, average daily gain (ADG), and feed conversion rate (FCR) are presented in Table 4. The body weight, ADG, DMI and FCR of sheep at the days 15, 30 and 45 of the experiment remained unaffected (P > 0.05) by saponins supply (Table 4). The present study showed no differences in feed intake among treatments (P > 0.05). Some studies have reported that saponins extract or saponin-rich plants were responsible for reduced feed intakes [11,12], while other researchers have found little effects of saponins on feed intake in ruminants [27,16,24]. Patra et al. [4], considered that dietary saponins could not largely affect feed intake in ruminants. Similar result was reported by Saïda-Nasri et al. [24], who administrated different levels of saponins (30, 60 or 90 mg/kg DMI) from Quillaja saponarie saponins did not improve growth performance in Barbarine sheep. Our results are inconsistent with reports of Mader & Brumm, [32], who reported that the administration of Y. schidigera plant extracts (saponin-rich plants) improved growth, feed efficiency and health in ruminants. Moreover, Hu et al. [33], reported that the goats given diets containing 3 g of tea saponins per day had higher ADG and feed conversion rate than those on 0 and 6 g of tea saponins.

Table 4. Effects of saponins on DMI, body weight, ADG and FCR in Saanen kids

Variable	Dose of saponins administered (mg/kg DMI)			S.E.M	p-Value
	0	36	54		
DMI (kg/d)					
15 d	1.00	1.05	1.05	0.39	0.14
30 d	1.16	1.18	1.17	0.28	0.74
45 d	1.13	1.15	1.15	0.34	0.76
Body weight (kg)					
15-d	23.6	23.4	23.5	1.13	0.99
30-d	25.4	24.7	24.8	1.22	0.90
45-d	27.2	26.3	27.2	1.34	0.94
Average daily gain (kg/day)	0.09	0.08	0.10	0.02	0.22
Feed conversion rate	10.2	12.2	10.1	2.07	0.19

DMI= dry matter intake; S.E.M= standard error of the mean;

The mean of rumen fermentation parameters and plasma metabolites are presented in Table 5. Consistent with our findings in experiment 1, saponins administration in the diet of Saanen kids had no affect (P > 0.05) on rumen pH and ammonia-N concentration (Table 5). Table 6 shows that goat kids receiving saponins had lower (P<0.05) blood cholesterol concentration than the control. In both sheep and Saanen kids, we observed statistically significant reduction in blood cholesterol concentration. Concentrations of plasma glucose, and BUN were similar (P > 0.05) among the treatments, but triglycerides concentrations increased (P<0.10) in goat kids fed the saponins-supplemented diet compared with goat kids fed the control diet (Table 5). In agreement with Benchaar et al. [34], ruminal pH and ammonia-N concentrations were not affected by saponins. Previous research on the effects of saponins on ruminal ammonia-N concentrations has been inconsistent. Some researchers reported that ruminal ammonia–N concentrations decreased linearly with increasing level [24,35], while others found no effect on ruminal ammonia-N concentrations [11,36,9]. However, the lack of effect on rumen fermentation parameters due to the administration of saponins to the

diet of Saanen kids indicates that saponins did not interrupt the rumen fermentation function. Serum cholesterol-lowering properties of saponins from different sources have been observed in a number of studies in a variety of animals [29,37,38]. This effect is likely a result of Inhibitory effects of saponins on absorption of cholesterol or reabsorption of bile acids from the small intestine [31]. The absence of effect of saponins on plasma glucose and BUN in the present study might be due to the low levels of saponins administered to Saanen kids.

Table 5. Effects of saponins on rumen parameters and plasma metabolites in Saanen kids

Variable	Doses of saponins administered (mg/kg DMI)			S.E.M	p-value
	0	36	54		
Rumen parameters					
pH	6.46	6.52	6.53	0.05	0.48
Ammonia-N (mg/dl)	9.38	8.96	8.03	0.54	0.23
Blood metabolites (mg/dl)					
Cholesterol	75.6[a]	69.9[ab]	69.2[b]	1.76	0.03
BUN	16.5	17.7	17.9	0.65	0.56
Glucose	67.0	64.5	66.7	1.65	0.50
Triglycerides	19.3	17.9	22.1	1.30	0.08

Within rows, means with different letters are significantly different (P < 0.05). BUN= Blood Urea Nitrogen; S.E.M= standard error of the mean;

The mean of body measurements are presented in Table 6. Saponins administration had no effect (P > 0.05) on body measurements (i.e., neck girth, heart girth, withers height, rump height, cannon bone length, hip width, hip-pin interval and body length) (Table 6). There are very few studies on the effect of saponins or saponin-containing plants on body measurements. Using body measurements can be useful in defining performance traits of animals [39]. In this study, saponins administration had no effect on body measurements. However, this effect seems to depend on saponins type and dose (exposure concentrations) [41].

Table 6. Effects of saponins on body measurements of Saanen male kids

Variable	Doses of saponins administered (mg/kg DMI)			S.E.M	p-Value
	0	36	54		
Body measurements (cm)					
Neck Girth	31.4	32.8	31.8	1.11	0.66
Heart Girth	67.2	67.0	66.2	1.41	0.87
Withers height	53.0	54.8	52.6	1.41	0.52
Rump height	57.2	56.4	57.2	1.14	0.85
Cannon bone length	12.4	12.6	12.8	0.23	0.49
Hip width	12.8	12.6	12.4	0.39	0.77
Hip-Pin interval	12.4	12.8	13.2	0.28	0.17
Body length	39.2	39.0	38.2	1.02	0.77

S.E.M= standard error of the mean;

4. CONCLUSION

Saponins administration to Baluchi sheep and goat kids had no effect on total intake, nutrient digestibility, ruminal fermentation or most plasma metabolites, but decreased the blood cholesterol concentration. Goat kids did not make benefit from the administration of saponins since their body weight, ADG and body measurements were unchanged. Further research is required to understand the impact of higher levels of saponins on digestibility, growth performance and meat quality of small ruminant receiving different diets.

ETHICAL APPROVAL

Principles of animal care" (Ferdowsi University of Mashhad (FUM) publication No. 8716.21, revised 2011) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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