Research Article

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Dietary supplementation of sunflower oil and quebracho tannins in sheep feeding: *in vivo* nutrient digestibility, nitrogen utilization and *in vitro* ruminal degradation kinetics

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Abstract

BACKGROUND: The effect of the inclusion of sunflower oil (SF) and quebracho tannin (QT) in a sheep diet was evaluated. Nutrient digestibility and nitrogen (N) utilization, as well as *in vitro* ruminal degradation kinetics, were evaluated at three levels [0, 20 and 40 g kg^{-1} of diet dry matter] of SF and QT in a 3^2 arrangement. The treatments were 0 (control); 20 and 40 g of QT and/or SF kg^{-1} of the diet. Four intact male sheep (45 ± 1.3 kg) for each treatment were used in the digestibility trial and kept individually in metabolic cages.

RESULTS: Nutrient digestibility and N balance were not affected by SF. However, QT at 40 g kg^{-1} of dry matter decreased ($P < 0.05$) nutrient digestibility and also increased the proportion of absorbed N. Both SF and QT reduced ($P < 0.05$) the slowly degraded fraction and rate for organic matter and N. Even though the QT had a negative ($P < 0.05$) effect on nutrient digestibility, this effect was mild ($P > 0.05$) when SF was included in the QT-added diets. Moreover, an interaction ($P < 0.05$) of SF × QT was observed on the synchronization index as an indicator of the efficiency of rumen microbial protein synthesis.

CONCLUSION: Supplementation of either SF or QT to sheep diets reduced ruminal organic matter and N degradability, reflecting the compensatory digestion in the post-ruminal track for organic matter feed utilization.

Keywords: tannins; sunflower; digestion; degradation kinetics; sheep

INTRODUCTION

Ruminant meat and milk are the predominant natural sources of the *cis*-9, *trans*-11 conjugated linoleic acid, accounting for almost 90% of total linoleic acid in milk fat from cows fed typical diets.[1] Vargas *et al.* [2] reported that the contents of polyunsaturated fatty acids in ruminant meat can achieved by two strategies: (i) feeding concentrates supplemented with unsaturated fat rich in linoleic acid or polyunsaturated fatty acids and (ii) feeding pasture-based diets. Moreover, the reduction of microbial biohydrogenation using condensed tannins (CT) would increase muscle Δ^9-desaturase protein expression in sheep.[3,4] Recently, Kamel *et al.* [5] reported that the inclusion of quebracho tannins (QT) and sunflower oil (SF) in lamb diets improved the meat contents with respect to healthy fatty acids without a negative effect on animal performance.

The anti-nutritive effects of CT could be the result of an interaction with the protein or extra-cellular enzymes in the rumen, decreasing the attachment of ruminal microorganisms to feed particles.[6] In general, CT could reduce the activity of ruminal microorganisms.[7]

Dietary supplementation with fat has variable effects on nutrient digestion as a result of both level and type. Ben Salem *et al.*[8] reported no effect of 7 g kg^{-1} rapeseed oil supplementation to a grass hay-based diet [forage:concentrate (F:C), 60:40], although rapeseed oil decreased organic matter (OM) and fiber digestion in cows fed a corn silage-based diet (F:C, 65:35). By contrast, Martin *et al.* [9] found that supplementing 5.7 g kg^{-1} linseed oil to a forage-based diet (F:C, 65:35) of dairy cows decreased the digestion of dry matter (DM), OM and fiber. Doreau *et al.*[10] supplemented 2.6 g kg^{-1} linseed oil to a dairy cow diet rich in forage (F:C, 75:25) and found that linseed oil did not depress the digestion of DM, OM and fiber compared to the basal diet. However, Ueda

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et al.[11] reported that fiber and OM digestion increased in 3 g kg^{-1} linseed oil supplemented dairy cows fed a high-forage diet (F:C, 65:35), although digestion of these components decreased when cows were fed a high-concentrate diet (F:C, 35:65). Al-Dobaib and Kamel[12] found that the digestion of DM, nitrogen (N), neutral detergent fiber (NDF) and acid detergent fiber (ADF) was not affected when SF was added at a level of 2 and 1.7 g kg^{-1} of DM when the diet contained a high concentrate ratio (F:C, 40:60), respectively. However, digestion was reduced when the level of SF increased up to 4 g kg^{-1} of DM.[12] Benchaar *et al.*[13] reported that the digestion of DM, OM, crude protein (CP), NDF and ADF was not different compared to a basal diet when linseed oil was increased gradually up to 4 g kg^{-1} of DM in cows fed a diet with a F:C ratio of 50:50. Taken together, these results suggested that effects of unsaturated fat supplementation, including SF, on nutrient digestion vary with the amount of fat added and the F:C ratio of the diet. In the present study, we have investigated the effect of different levels of SF and/or QT supplementation on nutrient digestion and *in vitro* ruminal degradation kinetics.

MATERIALS AND METHODS

Experimental diets

Different levels (0, 2 and 4 g kg^{-1} of DM) of either SF or QT were studied in a 3^2 arrangement (nine experimental diets). The SF was acquired from the local supermarket. A commercial tannin source (Unitan ATO, Saica, Argentina) from the quebracho plant (*Schinopsis* spp.) was used as a CT additive. The QT contained 75 g kg^{-1} CTs in DM.[14] The treatments were 0 (control: 0 QT and 0 SF) and 20 and 40 g kg^{-1} DM of the diet of QT or/and SF. Diets were formulated to be isoenergetic and isonitrogenous (Table 1).

Nutrients digestibility and N balance

Four intact male sheep aged approximately 2 years old with a body weight of 45 ± 1.3 kg were used in the digestibility trial. Animals were kept individually in metabolic cages. Over a 21-day period, animals were acclimated to the treatment diet received to allow the rumen bacteria population to be adjusted to the diet. Samples of the tested diet, orts, faces and urine for individual animals were collected daily throughout data collection period (5 days for each diet). They were then dried at 60 °C for 48 h and ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA, USA), and stored for subsequent analyses. Analytical DM content of samples was determined by oven drying at 135 °C for 3 h; OM was determined by ashing, and N content was determined using a Foss-Kjeltec 8200 (Foss Analytical AB, Hoganas, Sweden) (AOAC[15]). The NDF and ADF concentrations were sequentially determined using a Fibertec 2010 Analyzer (Foss Analytical AB) in accordance with the manufacturer's instructions, based on the methods described by Van Soest *et al.*[16] Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat-stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St Louis, MO, USA). Experimental diets were subsequently examined using the same animals. The duration period for the digestion trials was 9 months.

In vitro study and synchronization index (SI)

Three animals were fed the same diet for 70 days and then killed. The rumen content of each animal was squeezed through four layers of cheesecloth into pre-warmed flasks to separate the liquid from solid fractions. An automatic incubator (Daisyll incubator; ANKOM Technology, Macedon, NY, USA) with three-glass bottles was used for the *in vitro* study. To begin the *in vitro* experiment, each glass was filled with 360 mL of rumen fluid and 1440 mL of artificial saliva[17] and was kept in an incubator adjusted at 39 °C. Six bags (pore size of 45 µm; Swiss Nylon Monofilament, Luzern, Switzerland) were used for each bottle. One bag was removed at intervals of 3, 6, 12, 24, 48 or 72 h. After incubation, bags and residues were washed by running tap water until the water became clear, and then they were squeezed gently. Microorganisms attached to the residual samples were eliminated by freezing–rethawing technique as described by Kamel *et al.*[18] To determine washing loss fraction, another one bag was used. Residuals of OM and N were determined in each bag. Degradability coefficients were calculated by fitting the data for OM and N disappeared to model of Ørskov and McDonald[19]:

$$P = a + b\left(1 - e^{-ct}\right)$$

where P is the cumulative amounts of OM and N degraded at time t, a is the readily degraded fraction, b is the fraction potentially degraded in the rumen, c is the rate constant of degradation of b and t is the incubation time (h). The quantity degraded per hour was calculated as the difference between the cumulative amounts degraded at successive hours and allocated to the appropriate hour of the day.

From hourly of OM and N degraded, a SI of nitrogen to organic matter was then calculated using:

$$SI = \frac{25 - \sum_{1-24} \frac{\sqrt{\left(25 - \frac{hourlyN}{OM}\right)^2}}{24}}{25}$$

as proposed by Sinclair *et al.*,[20] where N represents the amount of N (g) degraded per unit of OM (kg) degraded at a certain time.

The value of 25 represents 25 g of N kg^{-1} of OM truly digested in the rumen, which is assumed to be the optimal ratio.[21] A SI of 1.0 represents perfect synchrony between nitrogen and energy supply through the day, whereas values < 1.0 refer to the degree of asynchrony.[20] The formulation assumed that the animals were fed on two equal amounts at 09.00 h and 16.00 h; DM intake was 1 kg days^{-1} and ruminal outflow rate was 0.05 h^{-1}.[22]

Statistical analysis

Data for digestion and in vitro trials were analyzed by using the mixed model procedure of the SAS[23], which included the fixed effects of SF, QT at the levels 0, 2 and 4 g kg^{-1} of DM and the interaction between SF and QT in the diet. In addition, the effect of sampling time was included using the repeated measurements. Animal was the term of the random statement. Residual errors were used to test main fixed effects (SF and QT) and the interaction between SF × QT. Differences were considered significant at $P \leq 0.05$. When the interaction between SF level in the diet and QT level was $P < 0.05$, least square means were separated by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Nutrients digestibility and N balance

Supplementation of SF had no effect ($P > 0.05$) on the digestion of OM, N and ADF, although the effect tended toward significance ($P = 0.11$) for NDF (Table 2). The digestion of OM, N, NFD and ADF of the experimental diets differed ($P < 0.05$) in response to QT supplementation in the diet. In general, diets containing 40 g QT kg^{-1}

Table 1. Ingredients and chemical composition of the experimental diets

		Quebracho tannins (QT)		Sunflower oil (SF)		QT + SF			
Level (g kg^{-1})	Control (0)	20	40	20	40	20 + 20	40 + 40	20 + 40	40 + 20
Ingredients (g kg^{-1} DM)									
Alfalfa hay	144	149	153	149	153	153	161	157	157
Palm kernel meal	150	150	150	150	150	150	150	150	150
Barley grain	426	396	367	396	367	367	308	338	338
Wheat bran	100	100	100	100	100	100	100	100	100
Canola meal	70	70	70	70	70	70	70	70	70
Soybean meal	32	37	42	37	42	42	53	47	47
Molasses	50	50	50	50	50	50	50	50	50
Lime-stone	10	10	10	10	10	10	10	10	10
Salt	5	5	5	5	5	5	5	5	5
Vitamins + minerals[a]	13	13	13	13	13	13	13	13	13
Sunflower oil (SF)	–	–	–	20	40	20	40	40	20
Quebracho tannins (QT)	–	20	40	–	–	20	40	20	40
Chemical composition (g kg^{-1})									
Organic matter	931	918	918	927	932	919	927	926	927
Crude protein	139	144	150	145	138	142	142	143	141
Ether extract	25	25	21	37	60	40	64	61	38
NDF[b]	351	348	336	340	352	336	360	357	334
ADF[c]	174	172	171	165	177	173	186	179	184
ADL[d]	34	33	34	34	39	40	40	34	45
GE (MJ kg^{-1})[e]	18.2	18.2	18.1	18.4	18.9	18.5	19.0	18.9	18.5

[a] Contained (per kg) 90.2 g of Ca, 49.0 g of P, 48.9 g of Mg, 17.6 g of S, 140 g of Na, 14.3 g of K, 2.07 g of Fe, 1.9 g of Mn, 2.7 g of Zn, 447 mg of Cu, 69 mg of I, 7 mg of Co, 20 mg of Se, 452 IU of vitamin A, 58 IU of vitamin D, and 2,692 IU of vitamin E.
[b] NDF, ash free neutral detergent fibre.
[c] AFD, acid detergent fibre.
[d] ADL, acid detergent lignin (in sulphuric acid).
[e] GE, calculated gross energy.

had low ($P < 0.05$) digestion for all nutrients *versus* 0 g QT kg^{-1} of diet. However, 20 g QT kg^{-1} diet had a similar ($P > 0.05$) digestion compared to the 0 g QT kg^{-1} diet. No interaction between treatments and sampling time was found for any of the nutrient digestion measured; therefore, only averages over time are presented. The digestion of OM, N and ADF was not affected ($P > 0.05$) by the interaction effect SF × QT, except that the digestion of NDF tended to be effected ($P = 0.08$).

The QT supplementation tended to reduce ($P = 0.07$) nitrogen intake (NI, g days^{-1}) and the effect was significant ($P < 0.05$) for fecal N (FN, g kg^{-1} of NI), urinary N (UN, g kg^{-1} of NI) and retained nitrogen/digestible nitrogen (RN/DN). The results showed that the RN/DN increased ($P < 0.05$) in the 2 g kg^{-1} QT diet compared to the 0 g kg^{-1} QT diet, although no significant ($P > 0.05$) differences were detected between the 4 g kg^{-1} QT diet and other levels of QT (i.e. 0 or 2 g kg^{-1}). The values for RN/DN as influenced by QT levels were 37.4, 48.4 and 47.1 for control, 20 and 40 g QT kg^{-1}, respectively. The interaction effect of SF × QT on NI, FN, UN RN and the RN/DN ratio was not significant ($P > 0.05$).

The effects of unsaturated fat, including SF supplementation, on nutrient digestion have been variable among studies. For example, Ben Salem *et al.*[8] reported no effect of 7 g kg^{-1} rapeseed oil supplementation to a grass hay-based diet (F:C, 60:40), although rapeseed oil decreased OM and fiber digestion in cows fed a corn silage-based diet (F:C, 65:35). By contrast, Martin *et al.*[9] found that supplementing 5.7 g kg^{-1} linseed oil to a forage-based diet (F:C, 65:35) of dairy cows decreased the digestion of DM, OM and fiber. Doreau *et al.*[10] supplemented 2.6 g kg^{-1} linseed oil to a dairy cow diet rich in forage (F:C, 75:25) and found that linseed oil did not depress digestion of DM, OM and fiber compared to the basal diet. However, Ueda *et al.* [11] reported that fiber and OM digestion increased in 3 g kg^{-1} linseed oil supplemented dairy cows fed a high-forage diet (65:35, F:C), although the digestion of these components decreased when cows were fed a high-concentrate diet (35:65, F:C). Al-Dobaib and Kamel[12] found that the digestion of DM, N, NDF and ADF was not affected when SF was added at a level of 2 and 1.7 g kg^{-1} of DM when the diet contained a high concentrate ratio (F:C, 40:60), respectively. However, the digestion of nutrients was reduced when the level of SF increased up to 4 g kg^{-1} of DM.[12] The results of the present study found no effect for the different amounts of SF on the digestion of OM, NDF, N and ADF in sheep fed a diet with a F:C ratio of 30:70. Benchaar *et al.* [13] reported that the digestion of DM, OM, CP, NDF and ADF was not different compared to the basal diet when linseed oil was increased gradually up to 4 g kg^{-1} of DM in a cow a fed diet with a F:C ratio of 50:50. Taken together, these results suggested that the effect of unsaturated fat supplementation, including SF, on nutrient digestion varies with the amount of fat added.

Reduction of ruminal degradation (rate, c and extent, $a + b$) of OM in the present study (Table 3) could be attributed to the negative effect of fat on the degradation of NDF, which consisted of approximately 35 g kg^{-1} of DM in the experimental diets. Doreau and Ferly[24] reported that different experiments carried out *in vitro* on bacterial culture showed a negative effect of fatty acids on bacterial growth. This action is greater with the unsaturation of

Table 2. Nutrient digestibility and N balance (intake nitrogen, IN; fecal nitrogen, FN; urinary nitrogen, UN; and retained nitrogen, RN) as influenced by different levels of sunflower oil (SF) and (or) quebracho tannin (QT) in sheep (n = 4)

	Digestibility coefficient (g kg^{-1})				N balance				
Level (g kg^{-1})	OM	N	NDF	ADF	IN (g d^{-1})	FN (g kg^{-1} of NI)	UN (g kg^{-1} of NI)	RN (g kg^{-1} of NI)	RN/DN
Control (0)	78.2	68.4	59.4	48.8	28.5	31.6	45.1	23.3	34.2
QT									
20	73.4	67.2	47.7	34.9	26.7	32.8	36.4	30.8	45.2
40	68.3	59.6	36.5	28.9	24.3	40.4	34.1	25.6	43.1
SF									
20	74.0	66.0	49.3	36.0	28.7	34.0	42.9	23.0	35.1
40	78.3	66.3	45.7	37.3	25.5	33.7	34.4	28.9	42.9
QT + SF									
20 + 20	71.4	56.9	46.7	27.9	27.6	43.1	27.1	29.8	52.5
40 + 40	67.3	53.9	40.0	28.9	24.7	46.1	29.0	24.2	44.6
20 + 40	69.1	53.8	43.3	30.6	26.5	46.2	25.0	28.8	53.6
40 + 20	74.4	62.9	43.7	31.3	24.3	37.1	33.0	30.0	47.7
SEM	2.14	1.66	3.03	4.47	0.95	1.66	1.65	1.31	1.91
Probability (*P*-value)									
SF effect	0.630	0.269	0.108	0.304	0.128	0.269	0.111	0.948	0.361
QT effect	0.005	0.026	0.042	0.028	0.077	0.026	0.003	0.328	0.038
SF × QT	0.400	0.912	0.082	0.647	0.809	0.912	0.622	0.783	0.608
Means for main effect									
SF									
0	73.3	66.1	47.9	37.6	26.5	34.9	38.5	26.6	40.8
20	71.5	58.9	46.4	31.5	27.5	41.1	31.7	27.2	47.0
40	73.3	61.1	43.1	32.5	24.8	38.9	33.4	27.7	44.6
QT									
0	75.6^{x}	66.9^{x}	51.5^{x}	40.7^{x}	27.6	33.1^{y}	41.8^{x}	25.1	37.4^{y}
20	74.2^{x}	62.3^{xy}	46.1^{xy}	31.4^{xy}	26.1	37.7^{xy}	32.1^{y}	30.2	48.4^{x}
40	68.2^{y}	55.8^{y}	39.9^{y}	29.5^{y}	25.2	44.2^{x}	29.6^{y}	26.2	47.1^{xy}

Means in the same column within main effect with no superscript letters after them or with common superscript letters are not significantly different ($P < 0.05$).

long-chain fatty acids and with the *cis* form rather than the *trans* form.[25] The effect of fatty acids on bacterial growth could be a result of their absorption on the cell wall of the substrate, resulting in a slower capitation of amino acids and ATP production by bacteria, [26] which would lead to a reduction of its activity.

Moreover, the effect of SF supplementation on ruminal digestion of OM varied with the level of SF added to the diet. At a level of linseed oil supplementation of 2.6 g kg^{-1} in dairy cows[10] and 3 g kg^{-1} in growing steers[27], as well as 2 g kg^{-1} of SF in camels,[12] no effects were observed on ruminal digestion. However, at higher levels of oil supplementation (i.e. 6 g kg^{-1} linseed oil of dietary DM[28] and 4 g kg^{-1} of SF[12]), a decrease in the ruminal digestion of OM was found. The inhibitory effect for SF noted on the ruminal degradation parameters of OM in the present study appears to be in contradiction with the insignificant effect of SF supplementation on total tract digestion of OM, suggesting an increase in post-ruminal digestion. This hypothesis can be supported by the data from Benchaar *et al.*[13] and Ueda *et al.*,[11] who observed an increase in apparent total-tract digestion of fiber despite ruminal digestion in cows fed a concentrate-based diet and supplemented with linseed oil, suggesting a compensatory digestion at post-ruminal sites. The addition of SF at different levels had no significant effects on NI, FN, UN, RN or RN/DN.

Total tract digestion of nutrients as influenced by tannins was found to be dose-dependent; Komolong *et al.*[29] observed a liner decline of apparent digestion for DM, OM and N when sheep were fed on a high quality of lucerne hay supplemented with QT at levels of 0, 20, 40 and 60 g kg^{-1} DM. No significant effects were detected for a low level of QT on the digestion of OM, NDF, ADF and N (1 and 2 g kg^{-1} QT of DM[14]) and on NDF, CP and ADF (0.64 g kg^{-1} QT of DM[13]). By contrast, at a higher level of QT, the digestion of nutrients (OM, NDF, ADF and N) was decreased (3 g kg^{-1} QT of DM[14]; 2.5 g kg^{-1} QT of DM[30]). Total tract digestion of OM and N in the present study is analogous to that of ruminal degradation parameters found in an *in vitro* trial showing a negative dose response as a result of the level of QT added to the diet (Table 4). In general, CT could reduce the activity of ruminal microorganisms[7] and microbial N synthesis in the rumen.[14] Tannins were frequently observed to reduce structural carbohydrate degradation by reducing the number of cellulolytic microbes in the rumen fluid,[31] inhibiting cellulase,[6,32] preventing adhesion of microbes onto feed particles,[33] and combining with dietary protein and to form CT–protein complexes, thus reducing ruminal N digestion[6] and the degradation of N. Moreover, increasing the QT level in the diet leaded to increase CT–protein complexation. Taking into consideration that NDF and N consisted of more than 50 g kg^{-1} of the OM in the tested diets, this would explain the inhibitory effect of QT on the ruminal and post-ruminal digestion of nutrients. The compensatory digestion of OM and N at the post-ruminal tract for QT-added diets was not

Table 3. Ruminal degradation parameters of OM (rapidly degraded fraction, a; slowly degraded fraction, b; degradation rate, c; ruminally degraded fraction, a + b and undegradable fraction, u) as influenced by different levels of sunflower oil (SF) or (and) quebracho tannin (QT) in sheep (n = 3)

	OM				
Level (g kg^{-1})	*a* (g kg^{-1})	*b* (g kg^{-1})	*c* (h^{-1})	*a* + *b* (g kg^{-1})	*u* (g kg^{-1})
Control (0)	17.7	53.3	0.110	71.0	29.0
QT					
20	17.0	55.0	0.097	72.0	28.0
40	16.5	50.0	0.097	66.5	33.5
SF					
20	16.7	51.0	0.107	67.7	32.3
40	16.3	49.3	0.067	65.7	34.3
QT + SF					
20 + 20	15.9	48.0	0.083	63.9	36.1
40 + 40	16.4	41.7	0.057	58.1	41.9
20 + 40	15.7	48.0	0.080	63.7	36.3
40 + 20	16.5	45.0	0.067	61.5	38.5
SEM	0.505	0.922	0.0036	1.001	1.001
Probability (*P*-value)					
SF effect	0.127	< 0.001	< 0.001	< 0.001	< 0.001
QT effect	0.332	< 0.001	< 0.001	< 0.001	< 0.001
SF × QT	0.796	0.319	0.079	0.117	0.117
Means for main effect					
SF					
0	17.1	52.8^{x}	0.101^{x}	69.8^{x}	30.2z
20	16.1	49.0^{y}	0.090^{y}	65.1^{y}	34.9^{y}
40	16.4	45.3z	0.063z	61.8z	38.2^{x}
QT					
0	16.9	51.2^{x}	0.094^{x}	68.1^{x}	31.9^{y}
20	16.5	49.3^{x}	0.082^{y}	65.8^{x}	34.2^{y}
40	16.2	46.6^{y}	0.078^{y}	62.8^{y}	37.2^{x}

Means in the same column within main effect with no superscript letters after them or with common superscript letters are not significantly different ($P < 0.05$).

Table 4. Ruminal degradation parameters of N (rapidly degraded fraction, a; slowly degraded fraction, b; degradation rate, c; ruminally degraded fraction, ab and undegradable fraction, u) and synchronization index (SI) as influenced by different levels of sunflower oil (SF) and (or) quebracho tannin (QT) in sheep (n = 3)

	N					
Level (g kg^{-1})	*a* (g kg^{-1})	*b* (g kg^{-1})	*c* (h^{-1})	*a*+*b* (g kg^{-1})	*u* (g kg^{-1})	SI*
Control (0)	14.0	38.0^{a}	0.075	52.0^{a}	48.0^{e}	0.67^{cde}
QT						
20	13.3	36.6^{ab}	0.074	49.9^{abc}	50.1^{cde}	0.63^{f}
40	13.2	35.3^{bcd}	0.071	48.5^{bcd}	51.5^{bcd}	0.67^{def}
SF						
20	14.3	34.0^{de}	0.073	48.3^{bcd}	51.7^{bcd}	0.65^{ef}
40	13.7	34.3^{cde}	0.068	48.0^{cd}	52.0^{bc}	0.70^{bcd}
QT + SF						
20 + 20	13.7	36.0^{bc}	0.079	49.7^{abc}	50.3^{cde}	0.74^{ab}
40 + 40	12.9	29.3^{f}	0.054	42.2^{f}	57.8^{a}	0.70^{bcde}
20 + 40	13.3	33.0^{e}	0.061	46.3^{d}	53.7^{b}	0.72^{abc}
40 + 20	13.7	37.0^{ab}	0.070	50.7^{ab}	49.3^{de}	0.76^{a}
SEM	0.476	0.486	0.0029	0.656	0.656	0.014
Probability (*P*-value)						
SF effect	0.375	< 0.001	< 0.001	< 0.001	< 0.001	
QT effect	0.201	0.009	0.039	0.005	0.041	
SF × QT	0.992	< 0.001	0.275	< 0.001	0.001	
Means for main effect						
SF						
0	13.5	36.6^{x}	0.074^{x}	50.1^{x}	49.9^{y}	0.66^{y}
20	13.9	35.7^{x}	0.074^{x}	49.6^{x}	50.4^{y}	0.72^{x}
40	13.3	32.2^{y}	0.061^{y}	45.5^{y}	54.5^{x}	0.71^{x}
QT						
0	14.0	35.4^{x}	0.072^{x}	49.4^{x}	50.6^{y}	0.68^{y}
20	13.4	35.2^{x}	0.072^{x}	48.6^{x}	51.4^{x}	0.69^{y}
40	13.2	33.9^{y}	0.065^{y}	47.1^{y}	52.9^{x}	0.71^{x}

$^{a-f}$Means with different lowercase letters within the diets are different ($P < 0.05$). $^{x-y}$Means in the same column within main effect Means in the same column within main effect with no superscript letters after them or with common superscript letters are not significantly different ($P < 0.05$).

observed, and the adverse effect of QT on ruminal degradation of OM and N had been extended in the post-ruminal sites. This finding might be a result of (i) the QT level added to the diet; (ii) constant digestion for N in small intestine; and (iii) the relevant negative effects of CTs on digestion within the internal environment of the large intestine (pH > 3.0), which all require further investigation.

The decline in whole tract digestion of N and urinary N excretion, as well as the increment of fecal N responding linearly to the QT dose, is consistent with previous studies regarding the action of tannin in the digestive tract of animals.[34,29] A reduction of N digestion could be attributed to the impaired ruminal N degradation observed in the present study as a result of the negative effects of CTs on the proteolysis and growth of proteolytic rumen microorganisms. Butter *et al.*[35] suggested that high fecal-N excretion could be a result of one or more of the following factors: (i) digesta protein bound to tannin; (ii) decreasing ruminal and intestinal digestive enzyme activity because of tannin; (iii) impaired intestinal function; and (iiii) increasing secretion of endogenous proteins. Komolong *et al.*[29] noted a marked decline in urinary N excretion counteracting the elevated fecal N excretion in the diets containing CTs. Theodoridou *et al.*[36] concluded that a reduction in urinary N excretion is generally attributed to low ruminal degradation of N and ammonia loss. Although the decline in urinary N excretion had been associated with the presence of QT, the RN was not affected ($P > 0.05$) among the diets with different QT levels. These results are similar to those reported by Komolong *et al.*[29] who concluded that non-ammonia-nitrogen apparently absorbed from the small intestine was constant or slightly reduced in response to the increase of dietary CTs. Our results are in agreement with those of Woodward and Reed[37] who found a quadratic response in RN as a result of the increase of *Acacia brevispica* in the diet.

Ruminal degradation of OM and N, and SI

The *b*, *c*, *a* + *b* and *u* of OM for the tested diets varied ($P < 0.05$) as a result of the levels of either SF or QT (Table 3). Increasing the level of SF added to the diet reduced ($P < 0.05$) the *b* fraction, degradation rate (*c*) and *a* + *b* fraction of OM. Subsequently, the *u*

fraction of OM [100 – ($a + b$)] was increased ($P < 0.05$) in response to an increasing level of SF added to the diet. Addition of QT at the level of 20 g kg^{-1} of DM had no effect ($P > 0.05$) on a, b, $a + b$ and u fractions of OM compared to the 0 g kg^{-1} QT diet, although the reductions were significant ($P < 0.05$) for the 40 g kg^{-1} QT diet. QT-added diets (i.e. QT 20 and 40 g kg^{-1} DM) had a lower ($P < 0.05$) degradation rate compared to the control diet (QT 0 g kg^{-1} of DM). The response of the u fraction to QT showed a similar pattern to that of SF supplementation, with the u fraction being increased ($P < 0.05$) as a result of QT at 40 QT g kg^{-1} supplementation compared to the 0 g QT kg^{-1} diet. Parameters of ruminally degradable OM (a, b, c, $a + b$ and u) for the tested diets were not affected ($P > 0.05$) by the interaction effect of SF × QT.

Levels of SF added to the diets had a significant ($P < 0.001$) effect on the b, c and $a + b$ fractions of N that were ruminally degraded. The results in Table 4 showed that the diet supplemented with 4 g kg^{-1} SF had lower ($P < 0.001$) b, c and $a + b$ fractions for N compared to SF at 0 and 20 g kg^{-1} of DM, and no significant differences were found between diets containing 0 or 20 g SF kg^{-1} DM. Levels of QT supplemented to the diets had an adverse effect ($P < 0.05$) on the b, c and $a + b$ fractions of N that were ruminally degraded. The interaction effects of SF × QT on a and c in the rumen for N were not significant ($P > 0.05$). However, these effects were significant ($P < 0.05$) for b, $a + b$ and u (Table 4).

The SI between N and OM released in the rumen was enhanced ($P < 0.05$) with the addition of either SF or QT at the level of 40 g kg^{-1} of DM compared to non-supplemented diets, with values of SI of 0.66, 0.72 and 0.71 (SF) and 0.68, 0.69 and 0.71 (QT) for the levels of 0, 20 and 40 g kg^{-1} of DM, respectively (Table 4). Moreover, SI was affected ($P = 0.001$) by the interaction of SF × QT; among the tested diets, the 20 g SF + 40 g QT kg^{-1} of DM had the highest ($P < 0.05$) value of SI compared to the control diet.

The addition of both 20 and 40 g of SF had a negative effect on the degradation rate (c) of OM, although the negative effect on the c rate of N degradation was noted only with a high level of SF supplementation (Table 4). Previous results have shown that not all types of ruminal microorganisms are modified as a result of fat supplementation in the same way. Galbraith and Miller[26] and Maczulak *et al.*[25] reported that growth of cellulolytic strains is reduced more compared to amylolytic strains, with Gram-positive strains being more sensitive than Gram-negative strains. Moreover, Mackie and White[38] concluded that the predominant ruminal proteolytic microorganisms are Gram-negative and are less sensitive to fatty acids supplementation.

The addition of either SF or QT at 20 g kg^{-1} of DM significantly reduced ($P < 0.05$) the c rate of OM, although this level of supplementation had no significant ($P > 0.05$) effect on the c rate of N. Moreover, the addition of either SF or QT at a level of 20 g kg^{-1} of DM showed enhanced ($P < 0.05$) microbial nitrogen synthesis as measured as the SI. At a higher level of supplementation (i.e. 40 g kg^{-1} of DM), the c rate of both OM and N was decreased ($P < 0.05$) compared to 0 g kg^{-1}, with a significant increase of SI. This finding emphasises that the release of energy from OM (degradation rate) has a greater effect on microbial nitrogen synthesis than on N release. Kamel *et al.*[39] reported that the mass of microbial-N and its efficiency were increased as a result of enhancing the SI when yeast (*Saccharomyces cerevisiae*) was added to berseem hay (*Trifolium alexandrinum*) as a result of the higher releasing of energy from OM and as long as N release had no effect. However, the ruminally energy released was much higher than the optimal amount recommended by Czerkawski,[21] which is a result of the high concentrate:forage ratio (70:30) in the tested diets.

The quantity of protein flowing to the post-ruminal tract is a major factor determining the productivity of ruminant. The protein reaching the abomasum consists of a mixture of dietary and microbial protein (metabolizable protein). An increasing flow of protein from the rumen depends on decreasing the proteolysis by rumen microorganisms and increasing microbial nitrogen synthesis. The results of the the present study concerning the DMI, OM g kg^{-1} of DM, ruminally degraded OM, microbial N synthesis (19.2 g microbial N kg ruminally degraded OM^{-1}), NI and ruminally undegraded N lead to the calculation of metabolizable protein. The ratio of metabolizable protein to NI was found to be constant, ranging from 1.05 to 1.0 in control and 20 g SF + 40 g QT kg^{-1}, respectively. However, the diet comprising 20 g SF + 40 g QT kg^{-1} was found to have the highest average daily gain ($P = 0.11^5$) among the diets tested. This finding shows that the quality of metabolizable protein might have a greater effect on the average daily gain compared to the quantity of metabolizable protein, which requires further investigation.

CONCLUSIONS

Supplementation of with either SF or QT reduced the ruminal degradability of OM and N, although the inhibitory effect was compensatory in the post-ruminal track for OM. Ruminal release of energy from OM had a pronounced effect on the SI as an indicator for microbial N synthesis. Even though the QT had negative ($P < 0.05$) effect on nutrient digestibility, this effect was mild ($P > 0.05$) when SF was included in the QT-added diets (i.e. interaction effect of QT × SF). The results emphasise that oil supplementation might have modulated the effect on CT-containing diets.

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Availability of data and materials

The datasets generated or analyzed during the present study available from the first author (H E M Kamel) on reasonable request. The datasets supporting the conclusions of this article are included within the article.

Author contributions

HEMK and SNA-D carried out the experiment and analyzed the data. AZMS helped with the data statistical analysis and the preparation of the tables of results. AZMS and HEMK drafted, revised and prepared the manuscript for submission. All authors read and approved the final manuscript submitted for publication.

Ethical approval

All animal experiments were reviewed and approved by the Institutional Animal Care and were performed in accordance with the ethical standard laid down in the 1996 Declaration of Helsinki and its later amendments and with the Guidelines for Experimental Animals.

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