

Influence of grape pomace powder as a feed supplement on feed intake, digestibility and rumen ecology of dairy steers

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ABSTRACT: Four rumen fistulated dairy steers with initial body weight of 220 ± 15 kg were randomly assigned according to a 4×4 Latin square design to examine the effect of dietary supplementation with grape pomace powder (GPP) levels on feed intake, nutrient digestibility and rumen fermentation characteristic. The four dietary treatments were GPP supplementation at 0, 2, 4 and 6 % of dry matter intake (DMI), respectively. The concentrate diets offered to steers at 0.5 % body weight and 3% urea-treated rice straw (UTRS) was fed *ad libitum* as roughage source. Results revealed that UTRS intakes was unchanged ($P>0.05$) by GPP supplementation, while total dry matter intakes were significantly higher than those in control groups ($P<0.05$). Moreover, DM digestibility tended to be increased ($P=0.07$). Ruminant temperature, pH, and $\text{NH}_3\text{-N}$ were similar among treatments ($P>0.05$) while BUN tended to be decreased ($P=0.07$). Based on this study it could be concluded that grape pomace powder is potential to be used as an alternative feed supplement in the concentrate diet which resulted in improved digestibility and maintained rumen ecology.

Keywords: Grape pomace powder, nutrient digestibility, rumen ecology, dairy steers

Introduction

Manipulation of the rumen fermentation by enhancing fibrous feed digestibility in ruminants as well as improve their performance are some of the most important goals for animal nutritionists (Patra et al., 2006). The use of antibiotics as feed additives has proved to be a useful tool to reduce energy and nitrogen losses from the diet and improve feed intake and efficiency (Patra and Saxena, 2011). However, the use of antibiotics in ruminants has been increasing concern due to the potential appearance of residues in milk, meat and bacterial resistance. Furthermore, it has been banned by the European Union. Therefore, research has been greatly focused to exploit plant

secondary compound as natural feed additives to improve rumen fermentation such as improving rumen ecology, animal health and productivity, inhibit protozoa and decreasing methane production (Chanthakhoun et al., 2011). Recently, plants containing secondary compounds, inclusion of condensed tannins and saponins have shown potential to manipulate rumen fermentation by enhancing the efficiency of utilization of feed energy such as grape pomace (GP; *Vitis vinifera* Linn.), which is a high tannin feed by-product produced in large amounts in many parts of the world (Spanghero et al., 2009). Bahrami et al. (2010) studied effect of diet with varying levels of dried grape pomace in male lambs and found that the growth performance was

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significantly improve by reducing FCR after adding 5 to 10% of dried GP to diets while adding more than 10 % of dried GP would increase FCR. However, there are limited studies on the influences of dried-red grape pomace powder levels in dairy steers. Therefore, the aim of this study was to investigate the effect of grape pomace powder levels on feed intake, nutrient digestibility and rumen fermentation in dairy steers.

Materials and methods

Four rumen fistulated dairy steers with initial body weight (BW) of 220 ± 15 kg were randomly assigned according to a 4×4 Latin square design. All animals were kept in the individual pens, the mineral block and water were available for *ad libitum* consumption. The steers were offered the concentrate diet at 0.5% BW and 3% urea-treated rice straw (UTRS) was fed *ad libitum*. The four dietary treatments were GPP supplementation at 0, 2, 4 and 6% of dry matter intake (DMI), respectively. These rations already tested by *in vitro* technique by Foiklang et al. (2015) and aimed to investigate by *in vivo* trial using dairy steers to know the effects of GPP supplementation. The experiment was conducted for four periods, and each period lasted 21 days. During the first 14 days all animals were fed with their respective diets, while last 7 days they were moved to metabolism crates for total collection of feces. Feeds and feces were collected and dried at 60°C for 48 hours and ground to pass through a 1 mm sieve in the feed mill using Cyclotech Mill,

Tecator, Sweden for chemical analysis using standard methods of AOAC (1990) for DM, organic matter (OM), ash and crude protein (CP). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were estimated according to Van Soest et al. (1991) with addition of α -amylase without sodium sulphite and the result was expressed with residual ash. Contents of condensed tannins (CT) in GPP was analyzed by using the modified vanillin-HCl method (Burns, 1971), while crude saponins (CS) was measured by using methanol extraction modified by Pongchompu et al. (2009). At the end of each period, rumen fluid was collected at 0, 2, 4 and 6 h-post feeding using a 60 ml hand syringe at 0, 2, 4 and 6 h-post feeding. Ruminal temperature and pH were measured immediately using a portable pH and temperature meter (Hanna Instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth and centrifuged at $16,000 \times g$ for 15 min for $\text{NH}_3\text{-N}$ analysis using the micro-Kjeldahl methods (AOAC, 1990). A blood sample was collected from the jugular vein at the same time as rumen fluid sampling for analysis of blood urea-nitrogen (BUN) according to Crocker (1967). All obtained data were subjected according to a 4×4 Latin square design using the GLM procedures of the Statistical Analysis System Institute (SAS, 1998). Differences among means with $P < 0.05$ was accepted as representing statistical differences. Treatment means were compared by orthogonal polynomials.

Table 1 Ingredients and chemical composition of concentrate, urea treated rice straw and feed supplements used in the experiment

Items	Concentrate diet	UTRS ¹	GPP
Ingredient (kg of DM)			
Cassava chip	63.6	-	-
Rice bran	10.0	-	-
Coconut meal	11.8	-	-
Palm kernel meal	7.4	-	-
Urea	3.0	-	-
Molasses	2.0	-	-
Mineral premix	1.0	-	-
Salt	1.0	-	-
Sulfur	0.5	-	-
Total	100	-	-
Chemical composition			
Dry matter, %	89.2	50.6	89.5
..... % of DM			
Organic matter	92.2	86.1	84.2
Crude protein	14.2	5.5	12.8
Condensed tannins	-	-	12.3
Crude saponins	-	-	14.6
Neutral detergent fiber	18.3	72.3	47.5
Acid detergent fiber	15.2	57.5	30.6
Total digestible nutrients ²	76.0	52.2	-

¹UTRS = 3% urea treated rice straw; ²Calculated value.

Results and Discussion

The experimental feed and their chemical compositions are shown in **Table 1**. The mixture of concentrate consisted of 14.2% CP and 76% TDN. Concentrate ingredients were based on local feed resources. GPP contained 12.8% CP, 12.3% CT, 47.5% NDF and 30.6% ADF on a DM basis while the UTRS contained 5.5% CP, 72.3%

NDF and 57.5% ADF on a DM basis. The results of UTRS, concentrate, total DM as well as apparent nutrient digestibility as influenced by GPP supplementation are shown in **Table 2**. Under the current study, it was found that GPP supplementation did not affect the UTRS intake ($P>0.05$) in dairy steers. Increasing levels of GPP supplementation increased total feed intakes ($P<0.05$).

Table 2 Effect of grape pomace powder (GPP) supplementation on dry matter intake, nutrient intake and nutrient digestibility in dairy steers

Item	GPP (% of DMI)				SEM	Contrasts		
	0	2	4	6		lin	qua	cub
UTRS ¹ intake, kg/day	4.1	4.1	4.2	4.2	0.04	NS	NS	NS
%BW	1.94	1.94	1.95	1.95	0.01	NS	NS	NS
g/kg BW ^{0.75}	73.7	74.1	74.6	74.5	0.38	NS	NS	NS
Concentrate intake, kg/day	1.1	1.1	1.1	1.1	0.01	NS	NS	NS
%BW	0.5	0.5	0.5	0.5	0.001	NS	NS	NS
g/kg BW ^{0.75}	19.2	19.1	19.2	19.2	0.06	NS	NS	NS
GPP intake, kg/day	0.00 ^a	0.09 ^b	0.18 ^c	0.27 ^d	0.03	***	*	NS
%BW	0.00 ^a	0.04 ^b	0.08 ^c	0.13 ^d	0.01	***	**	*
g/kg BW ^{0.75}	0.00 ^a	1.61 ^b	3.21 ^c	4.8 ^d	0.46	***	**	NS
CT intake, g/day	0.0 ^a	11.01 ^b	22.02 ^c	33.03 ^d	3.18	***	*	NS
CS intake, g/day	0.0 ^a	13.07 ^b	26.13 ^c	39.20 ^d	3.78	***	*	NS
Total intake, kg/day	5.2 ^a	5.3 ^a	5.4 ^{ab}	5.5 ^b	0.06	*	NS	NS
%BW	2.43 ^a	2.49 ^a	2.54 ^b	2.58 ^b	0.02	*	NS	NS
g/kg BW ^{0.75}	92.9 ^a	94.9 ^a	97.0 ^{ab}	98.5 ^b	0.66	*	NS	NS
Nutrient intake, kg/day								
Organic matter	4.5 ^a	4.6 ^a	4.7 ^{ab}	4.8 ^b	0.05	*	NS	NS
Crude protein	0.38	0.39	0.40	0.42	0.01	NS	NS	NS
Neutral detergent fiber	3.2	3.2	3.3	3.3	0.03	NS	NS	NS
Acid detergent fiber	2.5	2.6	2.6	2.6	0.03	NS	NS	NS
Nutrient digestibility, %								
Dry matter	58.9	60.3	63.3	64.4	1.71	0.07	NS	NS
Organic matter	65.0	65.0	67.4	67.8	1.46	NS	NS	NS
Crude protein	65.2	68.9	69.9	71.3	1.72	NS	NS	NS
Neutral detergent fiber	48.7	49.6	57.0	58.5	1.31	NS	NS	NS
Acid detergent fiber	45.0	46.5	52.8	53.2	1.22	NS	NS	NS

^{a,b,c,d}Values within the row with different superscripts are significantly different.

¹UTRS=3% urea treated rice straw; *P<0.05; **P<0.01; ***P<0.001.

Moreover, organic matter intake was increased when increasing levels of GPP supplementation ($P < 0.05$). Nutrient digestibility in terms of dry matter tended to be increased ($P = 0.07$) when the levels of GPP was increased while others were similar among treatments ($P > 0.05$).

The pattern of ruminal fermentation included temperature, pH, $\text{NH}_3\text{-N}$ and overall means are presented in **Table 3**. Ruminal temperature and ruminal pH were similar among treatments ($P > 0.05$). In this current study, ruminal $\text{NH}_3\text{-N}$ concentrations were 12.8 to 14.8 mg/dL. In addition, BUN concentration tended to be decreased when GPP was supplemented ($P = 0.07$).

The CP content of GPP was within the range (11 to 13% CP) which reported by Yu and Ahmedna (2013) but higher than that reported by Greenwood et al. (2013) at 6.35% CP. The total protein content of grape seed protein may vary significantly depending on the variety of grape, location and fertilization conditions (Yu and Ahmedna, 2013). The inclusion of the grape marc into livestock rations provides an opportunity not only to use a waste byproduct resourcefully, but also to induce beneficial metabolic changes in animals (Moate et al., 2014). Moreover, grape pomace contains condensed tannins (CT) that could alter N metabolism, which would be beneficial from an environmental perspective (Greenwood et al., 2012) and the CT content in GPP was also higher than the value reported by Abarghuei et al. (2010). Increasing levels of GPP supplementation could increase total feed intakes

means that GPP did not depress feed intake of the steers. The CT intake of 6% of GPP supplementation was 33.03 g/day did not show significant effect on feed intake which similar with Pilajun and Wanapat (2011) who supplemented mangosteen peel at 30 g/kg DM (23.36 g/day of CT) to the diet had no effect on feed intake. Beauchemin et al. (2007) found that adding 2% quebracho tannin extract to the diet had no effect on either DM or NDF digestibility in cattle. Ngamsaeng et al. (2006) also found that adding 3% of mangosteen peel (16.4% CT) to the diet had no effect on feed intake. However, other authors have found that feeding high levels of dietary saponins and/or tannins decreased apparent digestibility (Pilajun and Wanapat, 2011). Markar (2003) stated that the high levels of CT intake could be more than 40 g/kg DM which could decrease DMI. Ruminal pH and temperature were stable at pH 6.5 to 6.6 and temperature of 38.8 to 39.0 °C, and the pH was within the range considered optimal for microbial digestion of fiber and protein [6.0 to 7.0; Firkins (1996)].

In addition, $\text{NH}_3\text{-N}$ concentrations were close to those previously reported by Wanapat and Pimpa (1999) for improving rumen ecology, digestibility and voluntary feed intake. Concentrations of BUN are highly correlated to the concentration of $\text{NH}_3\text{-N}$ production in the rumen. Normally, when protein degradation is more rapid than synthesis, NH_3 will accumulate in the rumen liquor and is absorbed into the blood carried to the liver but when CT was supplemented it could be formed with macro molecule as protein to be tannin-protein complex which

protect protein degradation by the microbes (Markar, 2003). Bach et al. (2005) stated that an elevated concentration of BUN could result from ruminal $\text{NH}_3\text{-N}$ concentration because up to 50% of $\text{NH}_3\text{-N}$ produce from rumen fermentation was absorbed into the blood via the rumen wall and influences the urea nitrogen concentration.

However, nitrogen in blood stream came from other site such as ammonia nitrogen from small intestine and colon fermentation, as well as endogenous nitrogen is also importance impact on urea synthesis in the liver (Lapierre and Lobley, 2001).

Table 3 Effect of grape pomace powder (GPP) supplementation on rumen fermentation and blood urea nitrogen in dairy steers

Item	GPP (% of DMI)				SEM	Contrasts		
	0	2	4	6		lin	qua	cub
Ruminal pH	6.50	6.56	6.49	6.56	0.02	NS	NS	NS
Temperature, °C	38.8	39.0	38.9	39.0	0.09	NS	NS	NS
$\text{NH}_3\text{-N}$, mg/dL	14.8	12.9	12.8	13.1	0.89	NS	NS	NS
BUN, mg/dL	10.4	10.9	9.5	8.9	0.51	0.07	NS	NS

Conclusion and Recommendations

Based on the present study, it could be concluded that grape pomace powder is potential to be used as an alternative feed supplement in the concentrate diet for dairy steers which resulted in improving rumen fermentation efficiency and digestibility. Therefore, using of GPP in ruminant feeding is recommended to be feed supplement and this study suggested that GPP supplementation could be at 2 to 6% of DMI. However, future research should be conducted on comparison the effect of GPP and others alternative feed supplements and/or plant containing secondary compounds in various ruminant species.

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