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EFFECT OF HYDROLYSABLE TANNIN SUPPLEMENTATION ON PRODUCTION PERFORMANCE OF DAIRY CROSSBRED COWS

M. Ali[1,*], H. A. Mehboob[1], M. A. Mirza[1], H. Raza[1] and M. Osredkar[2]

[1]Institute of Animal Sciences, University of Agriculture, Faisalabad, 38040, Pakistan
[2]Tanin Sevnica D.D., Hermanova Cesta 1, 8290 Sevnica, Slovenia
[*]Correspondence E-mail address: drmubarak434@gmail.com

ABSTRACT

Nowadays, hydrolysable tannin has been introduced to improve the protein utilization in ruminants. The objective of this study was to determine the effect of hydrolysable tannin on production performance (milk production and composition) of dairy crossbred cows. In this experiment, 12 crossbred lactating cows (average milk production 10 liters/day) were used in this experiment. The cows were randomly divided into four groups (A, B, C and D). In group A, animals were fed basal diet without supplementation of hydrolysable tannin, however; the animals in groups B, C and D fed basal diet with 20, 30 and 40 g hydrolysable tannin per day, respectively. Duration of the study was 55 days including 10 days of adaptation period. Milk yield was daily (morning and evening) recorded during the experimental period. For milk composition analyses, the milk sample was collected fortnightly from each cow. Milk samples were analyzed for milk protein, fat, lactose, total solids and milk urea contents. Results showed that the milk production was found significantly higher ($P<0.05$) in group D. The differences in milk fat and total solids were non-significant ($P>0.05$) between groups. However, the milk protein and lactose (%) were increased with supplementation of hydrolysable tannin. There was reduction in milk urea content of animals fed diet with hydrolysable tannin supplementation but highest drop was observed in group B. The supplementation of hydrolysable tannin also results in the reduction of somatic cell count. From results of this study, it is concluded that hydrolysable tannin supplementation improves milk production and udder health.

Keywords: Hydrolysable tannin, dairy cows, milk yield, milk composition, somatic cell count.

INTRODUCTION

The objective of livestock production system is to meet energy and protein requirements of animals for better health of animals and maximum economic benefits. The economics of feeding depends on feed intake, cost of feed as well as feed efficiency. Different approaches are being used to enhance feed efficiency. Tannin is considered to increase feed efficiency in animals. There are two types of tannin; condensed tannin and hydrolysable tannin. Within a pH range of 3.5 to 7.0, tannins bind to certain proteins. At pH <3.5 or >8.5, the tannin-protein complexes are dissociated. The hydrolysable tannin consists of a carbohydrate moiety in which the hydroxyl groups are esterified to gallic acid or m-digallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins) (Mangan, 1988). The hydrolysable tannin supplementation in diet of dairy animals maximizes amino acids supply in the intestine and prevents degradation of protein in the rumen. It ultimately minimizes losses of protein in form of methane and urinary nitrogen. Tannins may improve protein utilization in ruminants (Mueller-Harvey, 2006). Tannins make bond with protein in range of pH 3.5-7, as increase intestinal dietary protein and overall animal performance in sense of body weight, milk production and reproductive performance. Tannins also caused slow degradation of plant protein in the rumen which improves dietary N utilization (Min and Hart 2003). Addition of chestnut tannins at 4g/kg of CP in grass silage fed to steers reduced ruminal degradability, 10 % increase in the duodenal non-amino acid N flow and more CP apparent digestion in the intestine (Decruyenaere *et al*., 1996). Tannins from *Lotus corniculatus* also caused increase in absorption of essential amino acid upto 62% in small intestine (Waghorn *et al*., 1987). Losses of urinary nitrogen were lower but higher fecal nitrogen losses in feces for silage contained tannins. After adaptation, hydrolysable tannin may provide nutrients for absorption but the condensed tannin limit the availability of DM for digestion (Lowry *et al*., 1996). Alfalfa treated with 4-6% tannins cause more protein digestibility in intestine which might be due to reduction in proteolysis in rumen (McSweeney *et al*., 2001).

Nowadays, hydrolysable tannin from chestnut is used as a feed additive to increase the nutrients digestion and absorption in the intestinal tract of dairy cows. Forages with low condensed tannin have positive effects on milk yield and composition due to efficient utilization of essential amino acids. Degradation of protein in rumen results in production of ammonia which is converted into urea and may cause alkalosis. There may be degradation of 70% of soluble forage protein (Barry and Manley

1984), converted into ammonia by deamination of amino acids. There is no scope in organic animal farming of formaldehyde (IFOAM, 2006).

The present study was conducted with objective of investigating the effect of different levels of hydrolysable tannin on milk yield and composition of crossbred dairy cows.

MATERIALS AND METHODS

Product Information: Farmatan SCC® is a mixture of mainly hydrolysable tannin (castalagin and vescalagin) extracted from chestnut tree, essential oils (eugenol and cinnamaldehyde) and zinc in chelated form (Tanin Sevnica, Slovenia).

Animals and Diet: This study was conducted at Livestock Experiment Farm, University of Agriculture Faisalabad, Pakistan during early summer months (March-April, 2015). Twelve cross-bred (Friesian and Sahiwal/ Cholistani cross) dairy cows (average milk yield 10 liters/day, average lactation number 3-4, average age 6±2 years and average body weight 400±30 kg) were selected. Selected cows were divided into four groups; A, B, C and D (3 cows per group) and each cow act as replicate. Group A (control) was fed diet without hydrolysable tannin supplementation. Hydrolysable tannin was supplemented in the diet of groups B, C and D at 20, 30 and 40 g /day, respectively. Each cow was fed berseem mixed with wheat straw (*ad-libitum*), 2 kg concentrate (1kg/milking time), 2 kg maize oil cake (1kg/ milking time) and 1 kg corn gluten (0.5 kg/ milking time). Hydrolysable tannin was mixed with concentrate to ensure its intake. Ingredient and chemical composition of concentrate is presented in Table 1.

Chemical Analysis: Chemical analyses were performed in laboratory of Institute of Animal Sciences, University of Agriculture, Faisalabad. Feed samples were analyzed for DM, CP and NDF. The DM content was determined by oven drying at 104°C for 24 h. Nitrogen (N) was determined by Kjeldhal method and CP was calculated as N × 6.25. The NDF was determined following method of Van Soest *et al*. (1991) with sodium sulphite. Milk yield was recorded daily at morning and evening. Fortnightly, milk samples were collected from each cow for composition analysis. Milk samples were analyzed for protein, fat, lactose, milk urea and total solids contents. Fat was determined by Gerber method as described by Pearson (1976). Milk lactose analysis was done following Lane and Eynon titration method as described by Egan *et al*. (1981) and milk protein was determined according to the method described by Davide (1977). Total milk solids were determined by formula devised by Richmond *et al*. (1942). The concentration of milk urea was determined in an auto analyzer using the method of Talke and Schubert (1965). Somatic cell count (SCC) was determined on day 5 and 45 of experiment as explained by Barratt *et al*. (2003).

Statistical Analysis: Data of milk yield and composition were statistically analyzed using GLM procedure of SAS 9.2 (SAS, 2009).The model used was:

$$Y_{ij} = \mu + TD_i + \epsilon_{ij}$$

where, Y_{ij} is the dependent variable; μ the overall mean; TD_i is the fixed effect of treatment doses (i = 1-3) of hydrolysable tannin; ϵ_{ij} is the residual error.

RESULTS

Milk production: Milk production data of experimental groups is presented in table 2. Milk production was not different (P>0.05) in groups during adaptation (Table 2). However, the highest milk production (12.50 kg) was found in group D on day 15. The difference in milk production between groups was found significant at day 15 (P<0.05), day 30 (P<0.01) and day 45 (P<0.001) for each period. The difference in milk production between groups was statistically significant (P<0.05) at day 15 (Table 2), whereas the difference among groups for milk production at day 30 of research trial was highly significant (P<0.01). Milk production at end of experiment was highly different (P<0.001) between groups.

Milk Composition: Table 3 shows data on milk composition of dairy cows fed diets supplemented with different levels of hydrolysable tannin. There was an increase (upto 0.3%) in milk protein at day 15 and 30 in group D but was lowest in that group on day 45 than other days (Table 3). Milk lactose data of experimental groups is presented in Table 3. There is no statistically significant (P>0.05) difference between groups in milk lactose content (Table 3). There was increase in milk lactose on day 45 (Table 3). Highest increase in milk lactose was found in group B and D. There was increase (day 15, day 45) in milk lactose content for group B, but milk lactose content was decreased on day 30 (Table 3). There was no difference (P>0.05) in milk total solids for milk samples analyzed fortnightly (Table 3). The differences in milk total solids were non-significant (P>0.05) in all experimental groups.

Milk Urea and Somatic Cell Count: Table 4 shows **m**ilk urea data of experimental groups. Milk urea content was decreased in supplemented groups (Table 4). There was difference (P<0.05) in milk urea content between groups at day 1, 15 and 45 (Table 4). Difference in milk urea content was highly significant (P<0.01) between groups on day 30 (Table 4). Milk urea was highest (45.67 mg/dl) on day 1 in group B. Data on milk somatic cell count of dairy cows fed different levels of hydrolysable tannin is presented in Table 5. Somatic cell count of milk decreased in hydrolysable tannin supplemented groups whereas it was unchanged in control group.

Table 1. Ingredients and chemical composition of concentrate.

Ingredients	%
Maize oil cake	15
Corn Gluten 30%	20
Maize Grain	15
Wheat bran	30
Canola Meal	2
Molasses	15
Common salt	1
DCP	1
Urea	1
Chemical composition	
Dry matter	92
Crude protein	15
Total digestible nutrients	70

Table 2. Average milk production of research animals at day 15, 30 and 45 of trial.

Days	Experimental groups[1]				SEM[2]	Significance
	A	B	C	D		
Day 15	11.71 [ab]	11.08 [a]	12.09 [ab]	12.50 [b]	0.19	*
Day 30	10.47 [a]	10.22 [a]	11.60 [ab]	12.20 [b]	0.21	**
Day 45	10.10 [a]	10.44 [a]	11.04 [ab]	12.12 [b]	0.18	***

[1]Experimental groups: A = Control; B = Farmatan SCC 20 g; C = Farmatan SCC 30 g; D = Farmatan SCC 40 g.
[2]SEM = Standard error of mean.
*, ($P < 0.05$); **, ($P < 0.01$); ***, ($P < 0.001$)

Table 3. Milk protein, fat, lactose and total solids of milk samples collected at day 15, 30 and 45 of trial.

Parameters	Experimental groups[1]				SEM	Significance
	A	B	C	D		
Milk protein (%)						
Day 15	3.9	3.6	3.7	3.8	0.06	NS
Day 30	3.4	3.5	3.1	3.8	0.15	NS
Day 45	2.6	2.9	2.7	3.1	0.10	NS
Milk Fat (%)						
Day 15	3.3	3.3	3.5	3.3	0.06	NS
Day 30	2.9	3.3	3.1	3.0	0.08	NS
Day 45	2.9	3.5	3.3	3.2	0.12	NS
Milk Lactose (%)						
Day 15	3.8	4.1	3.6	4.2	0.14	NS
Day 30	4.0	3.8	4.2	4.2	0.09	NS
Day 45	4.2	4.4	4.6	4.5	0.09	NS
Milk Total solids (%)						
Day 15	11.5	11.8	11.3	11.9	0.14	NS
Day 30	11.8	11.6	11.8	11.8	0.04	NS
Day 45	11.7	12.5	12.0	12.5	0.19	NS

NS, non-significant ($P>0.05$)

Table 4. Milk urea (mg/dl) of samples collected on day 1, 15, 30 and 45 of research trial.

Days	Experimental groups[1]				SEM[2]	Significance
	A	B	C	D		
Day 1	41.33	45.67	34.33	30.00	3.50	*
Day 15	40.00	26.00	30.33	26.33	3.26	*
Day 30	37.33	20.00	23.67	19.00	4.23	**
Day 45	32.33	21.00	24.33	20.67	2.71	*

[1]Experimental groups: A = Control; B = Farmatan SCC 20 g; C = Farmatan SCC 30 g; D = Farmatan SCC 40 g.
[2]SEM = Standard error of mean.
**, ($P < 0.01$); *, ($P < 0.05$)

Table 5. Somatic cell count in milk samples of experimental animals at day 5 and 45 of trial.

Groups	Experimental groups[1]	
	At day 5	At day 45
A	1083333	1083333
B	200000	100000
C	416667	150000
D	366667	150000

[1]Experimental groups; A = Control; B = Farmatan SCC 20 g; C = Farmatan SCC 30 g; D = Farmatan SCC 40 g.

DISCUSSION

Milk Production: Total milk production was found highest in group D compared to other treatment groups Difference (P<0.01) was more in milk production at day 30 of research trial. Milk production between groups was highly different (P<0.001) at day 45. This is in accordance to Dubey (2007) who reported significant increase in milk yield from 9.44 to 10.35 kg/day/cow by adding 3% tannin from *Acacia nilotica* pods in crossbred cows. Kushwaha *et al*. (2012) reported no effect of condensed tannin in ewes grazing *Lotus corniculatus* in early lactation but there was increase in milk yield by 2 liters during mid and late lactation. No change in milk production and milk composition was observed by addition of Quebracho Condensed Tannin reported by Benchaar *et al*. (2008). In the study of Colombini *et al*. (2010), milk yield was also not affected by offering silage treated with tannin. Similar results were also reported by Benchaar *et al*. (2008) but Colombini *et al*. (2010) reported slight increase in fat corrected milk (P<0.10). Supplementation of chestnut tannin (120 g/day) in diet of cows increased milk yield (Errante *et al*., 1998). Colombini *et al*., 2010 reported that treatment of lucerne silage with 4-5% tannin on DM basis was found effective and important in shifting protein supply from rumen to small intestine. Rai and Shukla (1979) reported that there was no effect on milk production of adding tannin (11%) form Sal seed meal in concentrate mixture. Lavrencic *et al*. (2006) also explained that the addition of tannin upto 5% have positive effect on dairy animal performance. Makkar (2003) described that improved performance in sense of milk yield by animals is due to protection of protein from rumen microbes which in turn cause increase supply of amino acids to small intestine along with more absorption through it.

Milk Composition: Milk protein was found non-significant (P>0.05) among all groups for milk samples collected at day 15, 30 and 45. However milk protein was increased in hydrolysable tannin supplemented groups (C and D). Similar trend observed by Barman (2004) who reported inclusion of 4% tannin from *Acacia nilotica* increased milk protein (%) in lactating crossbred cows. Similarly, feeding of *Lotus corniculatus* than perennial grass increased milk protein by 10% (Harris *et al*., 1998). Orešnik (1996) found increased in milk protein by 0.15% by inclusion of chestnut tannin in diet, which is in accordance to the results of Bhatta *et al*. (2000) who reported significant difference (P<0.07) in milk protein content between control and treatment group (containing condensed tannins 7.5% seed husks of *Tamarindus indica*). An increased flow of amino acids to the duodenum causing an increase in milk protein may be justifiable, because Barry *et al*. (1986) have reported an increased flow of non-ammonia nitrogen to the duodenum in sheep fed with lotus-containing condensed tannin. Milk protein was decreased in all groups at day 45. However, at day 45 milk protein was maximum (3.1%) for group D and lowest (2.6%) for control group. That decrease may be due to intense environmental temperature. Lavrencic *et al*. (2006) observed no significant difference in milk protein between control (3.14%) and treatment (3.24%) groups, respectively.

There was decrease in milk fat % upto 0.5% in all groups but no drop was found in group C. Dubey (2007) also found no increase in fat (%) by 3% inclusion of tannin. Fat (%) was also not different by adding *Acacia nilotica* as a source of tannin (Kushwaha *et al*., 2012). Addition of babul pods as source of tannin upto 20% caused no increase in fat (%) but there was decrease in fat (%) by addition of pods upto 40% (Barman and Rai, 2008). However, Lavrencic *et al*. (2006) reported 3.15 and 4.15 milk fat % for control and treatment groups respectively, although this difference was not significant but treatment group has more fat (4.15%) than control (3.15%).

In the present study, there was an increase (0.6%) in milk lactose for all treatment groups except control group (A). This increase was not significant (P>0.05) between groups. Bhatta *et al*. (2000) reported no difference in milk lactose for control and treatment groups (supplemented with tamarind seed husk at 2.5% and 7.5%). Milk total solids were constant in all treatment groups but were reduced about 1% in control group on day 45. This difference was not significant (P>0.05) between groups. No change in milk solids was observed in 3 and 4.5% tannin (Kushwaha *et al*., 2012). Same trend was observed on inclusion of 4% (Barman and Rai 2008) and 3% (Dubey 2007) babul pods in total mixed ration of lactating cows.

Milk Urea and Somatic Cell Count: In the present study, the lowest milk urea (19 mg/dl) was in group D on day 30 but maximum drop (24 mg/dl) was observed in milk urea of group B at the end of trial. Milk urea content was highly different (P<0.01) between groups on day 30. Lavrencic *et al*. (2006) reported difference in milk urea was not significant but there was decrease in milk urea in chestnut tannin supplemented group which indicated decrease in rumen protein degradability, resulting in lowered ammonia. Lavrencic *et al*. (2006) explained that addition of tannin upto 5% have positive effect on animal performance.

In the present study, milk somatic cell count was found lower in all treatment groups as these may be due to inclusion of zinc mineral in supplemented product as zinc has role in optimizing cellular immune response and for better health of epithelial and mammary tissues (Cook-Mills and Fraker, 1993). Zinc also play role in the prevention of bacterial entry within teat canal by formation of keratin which entrap these bacteria (Craven and Williams, 1985).

Conclusions: Hydrolysable tannin incorporation in the concentrate mixture of crossbred lactating cows showed significant improvement in milk yield, milk quality and udder health status, whereas there was no effect on milk protein, lactose, fat and total solids. Supplementation of hydrolysable tannin at 40 g indicated better performance of dairy crossbred cows. Therefore, it may be concluded that hydrolysable tannin can be used to get optimum performance from lactating cows.

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