

EFFECT OF CHOP LENGTHS OF BERSEEM (*Trifolium alexandrinum* L.) FODDER ON PRODUCTIVE PERFORMANCE AND NUTRIENT DIGESTIBILITY OF SAHIWAL COWS

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The objective of this study was to investigate the effect of chop lengths of berseem fodder on dry matter intake (DMI), water intake, milk production, milk composition and nutrients digestibility in Sahiwal cows. For this purpose, twelve Sahiwal mid-lactating cows of almost same weight and milk production were randomly assigned to three treatments in such a way that each treatment contained four cows. Cows received ad-libitum berseem fodder with: whole plant (WP) and two different chop lengths: medium chop length (MCL) (3.8 cm) and small chop length (SCL) (1.9 cm). Experimental period was 40 d long and consisted of 14 d of adaptation. Daily feed intake and water intake of individual animal were measured. In the last week digestibility was determined by using internal marker (acid insoluble ash). Results delineated that reducing the chop length of berseem fodder increased ($P<0.05$) DMI 9.24 to 11.25 kg/day. However, milk production (7 L/day) was not affected ($P>0.05$) by the varying chop lengths of berseem. Feeding WP berseem fodder increased ($P<0.05$) percentage of fat, total solids and ash compared to other ones. The digestibility of DM, protein, fat and neutral detergent fiber was increased ($P<0.05$) by feeding SCL berseem. However, there was no significant difference in fiber and acid detergent fiber digestibility, with all groups having 71% (mean) and 69% (mean), respectively. In conclusion, feeding WP berseem fodder could increase milk quality in term of increased milk fat, total solids and ash contents. As fodder chop length had no impact on milk production, WP berseem can be a useful option for improving milk quality in future.

Keywords: Sahiwal cow, berseem fodder, chop length, milk quality, digestibility.

INTRODUCTION

Milk production is the main objective of dairy producers which mainly depends upon dry matter intake (DMI) of cows. Various factors can affect the DMI, such as cow feeding behavior, chop length of fodder and feeding frequency (Grant and Albright, 2001; deVries *et al.*, 2005). Spending more time on eating can improve fiber digestibility and performance of lactating dairy cows (Aikman *et al.*, 2008). Forages provide fiber and energy to maintain proper ruminal functions and milk fat concentration. As little as 25 to 28% of dietary neutral detergent fiber (NDF) is required by animal for adequate fiber and out of which 75% should be provided through forages (NRC, 1989).

Different strategies like increased milking, feeding frequencies, artificial lighting, cooling system, manipulation of diet composition, forage size and feedstuffs processing (Shabi *et al.*, 2005; Zhang *et al.*, 2015; Muhammad *et al.*, 2016; Wang *et al.*, 2016; Niu *et al.*, 2017; Xia *et al.*, 2018a; Xia *et al.*, 2018b; Xia *et al.*, 2018c; Muhammad *et al.*, 2019; Khan *et al.*, 2020; Zaman *et al.*, 2020) along with disease prevention programs have been used to improve milk production and composition (Mani, 2019; Marwan *et al.*,

2019; Mousa and Marwan, 2019). Milk fat percentage response, time spent chewing and ruminal pH were used to determine the efficacy of fiber in dairy cows' diet (Sudweeks *et al.*, 1981; Armentano and Pereira, 1997; Mertens, 1997). Rumen pH is responsible for the production of lactic acid and volatile fatty acids, which partly buffered by saliva. Acetic acid (precursor of milk fat) production is reduced by alteration of rumen pH. Fat synthesis is also decreased by generating intermediate products through modification of fatty acid bio hydrogenation (Shingfield *et al.*, 2010).

The physical form of diet is a sword of two sides when considering the profitability of dairy cows. Forage chop length effect the fodder intake, digestibility and milk yield in dairy cattle, but it depends on the forage type, amount and storage (Nasrollahi *et al.*, 2015). There is a direct relationship among chop length of fodder, and intake, saliva production, the number of mastication's and rumination time of animal (Yansari *et al.*, 2004; Beauchemin and Yang, 2005).

Extra longer chopped diets may lead to more sorting, filling of rumen, reduce DMI and milk yield but low NDF diets with fine chop size may lead to acidosis, laminitis, displaced

abomasum, decreased salivary buffer secretion, reduced rumen pH and milk fat depression but increase DMI (Esmaeili *et al.*, 2016; Muhammad *et al.*, 2016; Muhammad *et al.*, 2019). Intake of dietary forage is important to consume physical effective NDF (peNDF), which is responsible for rumination and fiber digestibility. The stimulated to ruminate more as the level of peNDF is increases in the diet (Zebeli *et al.*, 2012). As under consumption of peNDF due to sorting behavior of cattle and over consumption of easily fermentable carbohydrates may lead to an increased risk of sub-acute ruminal acidosis (SARA) which can lead to decrease in DMI, fiber digestibility and milk production (Muhammad *et al.*, 2016; Miller-Cushon and de Vries, 2017). Optimum chewing time is required to minimize the risk of rumen acidosis, improve fiber digestion and promote high feed intakes in dairy cows (Beauchemin, 2018).

Although considerable research has been conducted to explore the impact of chop length on rumen function and milk production in exotic breed (Holstein Friesian and Jersey) (Bhandari *et al.*, 2008). But only few reports are available on Sahiwal cows regarding chop length of fodder (Fayyaz *et al.*, 2019). Sahiwal cattle is considered as best Zebu cattle for milk production to play the same position for the tropics as the Holstein (Rehman *et al.*, 2014). Due to lack of breeding programs in Pakistan, feeding and management strategies play an important role in improving animal performance. Interactions between several factors, such as reduced forage particle size, forage source, forage to concentration ratio, range of particle lengths and forage NDF content can lead to discrepancies among studies (Rehman and Khan, 2011). At farmer level a lot of variation existed regarding particle size of fodders. That's why there is dire need to optimize chopping size of fodder (berseem) in Sahiwal cows. In this study, our objective was to determine the effect of various chop lengths of berseem fodder on DMI, water intake, milk yield, composition and nutrient digestibility in lactating Sahiwal cows.

MATERIALS AND METHODS

Experimental animals and diets: Twelve Sahiwal mid-lactating cows with almost same weight (345 ± 5.1) parity were selected from the herd maintained at Livestock Experiment Station, University of Agriculture, Faisalabad in 2019. The cows were divided into three treatments, Whole plant (WP), medium chop length (MCL) and small chop length (SCL) with four cows in each group. Average initial milk production of cows fed WP, MCL and SCL were 7 ± 0.12 L, 7.3 ± 0.39 L and 7 ± 0.34 L, respectively. The experiment duration was of 40 days with 14 days as adaptation period. For individual feeding all the animals were housed individually in the separate stall under natural ventilation and day light in the same shed. All animals were

fed with ad-libitum berseem fodder during the entire experiment varying in chop size (WP, MCL (3.8 cm) and SCL (1.9 cm). To manipulate berseem particle size, daily fresh fodder was used to cut down with the help of chopper equipped with two different number of teeth's (11 and 13) of cogwheel. The amount of feed offered was adjusted daily to obtain approximately 5% of orts. For the determination of fodder composition, proximate analysis of fodder was conducted on weekly basis. The overall composition of berseem fodder is depicted in Table 1. The concentrate ration having 20% crude protein and 68% total digestible nutrients was given according to their milk production, i.e. of 1 kg for each 2.5 kg of milk production to all animals. The composition of concentrate is shown in Table 1.

Table 1. Chemical composition of berseem fodder and concentrate

Nutrients (%)	Fodder	Concentrate
Dry matter	19.20	91.0
Fat	2.00	3.5
Crude protein	20.96	20.0
Crude fiber	23.20	5.6
Ash	11.93	10.0
NDF	44.20	-
ADF	26.90	-

Data collection

Dry matter intake: Daily DMI of each cow was calculated. For this purpose, a weighed quantity of fodder was offered two times in a day to each cow in the experiment. In the next morning the orts samples of each cow were also weighed and recorded 1 h before the feeding of fresh fodder.

Water intake: Daily water intake of each animal was recorded. Animals were given clean and measured quantity of ad-libitum water three times daily. Total water intake was measured by subtracting the left-over water from the offered.

Milk yield and composition: Cows were milked two times daily at morning and evening with equal interval and weight of milk was recorded in the parlor to calculate the daily milk yield of each cow separately. Weekly composite milk samples of morning and evening milking were collected from individual animal and used for determining milk constituents: (Fat % by Gerber's technique, protein percentage by formal titration technique, lactose by difference method, ash percentage by Gravimetric technique, and total solids percentage, solid not fat percentage and specific gravity by Fleischman's formula described by AOAC (2000).

Nutrients digestibility: Percentage of nutrients digestibility was determined in the last week of experiment by using internal marker (AIA) present in fodder. Feces samples of 3 consecutive days were collected from each animal and make a composite sample. During collection care was taken to avoid mixing of dust and feed particles in feces samples. The

excreta samples were placed in a plastic bag and stored at -20°C until further analysis. The samples of offered fodder was also collected. Before chemical assessment, specimens of excreta and feed were dried for 72 h at 57°C, after which they were ground to passed by a one-mm screen. Feed and excreta content of moisture, nitrogen, fat, fiber, NDF and ADF were determined according to the methods of the AOAC (2000) as described in recent studies (Hussain *et al.*, 2018; Demir *et al.*, 2019; Raza *et al.*, 2019; Hussain *et al.*, 2020). Determination of AIA was performed after ashing the samples and treating the ash with boiling HCL (Viveros *et al.*, 2002).

Digestibility was calculated according to equation

$$\% \text{ Digestibility} = \left(100 - \frac{\% \text{ marker in feed} \times \% \text{ nutrient in feces}}{\% \text{ marker in feces} \times \% \text{ nutrient in feed}} \right) \times 100$$

Statistical analysis: All data were analyzed under Completely Randomized using Analysis of Variance (ANOVA) technique of Minitab Statistical Software 19 (Minitab, 2010). Tukey's test was used to separate difference means among treatments (Keunen *et al.*, 2002).

Data were assumed to be statistically significant when $P < 0.05$.

RESULTS

Effect of chop length on feed intake and water intake:

Reducing chop length of berseem fodder increased DMI from 9.24 to 11.25 kg/day in Sahiwal cows. Highest DMI was observed by feeding of SCL berseem followed by MCL and WP (Table 2). The mean water intake from drinking water was 26 ± 1.08 L/day kg (mean $\pm$ SD) with no significant difference between treatment groups. Likewise, daily water intake from feed were also similar in result of chop length of berseem. Chop length of berseem showed no effect ($P > 0.05$) on total water intake in Sahiwal cows. Reducing chop length of berseem had no effect ($P > 0.05$) on water intake and DMI ratio in Sahiwal cows.

Effect of chop length on milk production and composition:

Summary data on milk production and composition are given in Table 3. Milk production was similar in all groups during experiment with 7 ± 0.39 L/day (mean $\pm$ SD) in

Table 2. Effect of chop size of berseem fodder on green fodder intake

Variable	WP*	MCL†	SCL‡	SEM	P-value
Green fodder intake (kg/day)	33.42	33.12	34.175	1.01	0.710
DMI (kg/day)	9.24 ^c	10.05 ^b	11.25 ^a	0.38	0.001
Water intake from drinking water (L/day)	23.82	25.62	29.46	1.08	0.090
Water available from fodder (L/day)	27.04	27.77	26.26	0.81	0.780
Total water intake (L/day)	51.93	54.09	56.69	1.27	0.340
Water: Dry matter	5.75	5.25	5.18	0.13	0.140

drinking water intake and total water intake in Sahiwal cows; *whole plant; †medium chop length; ‡short chop length; SEM, standard error of mean

Table 3. Effect of chop length of berseem on milk production and composition

Variable	WP*	MCL†	SCL‡	SEM	P-value
Milk production (L/day)	6.98	7.00	7.14	0.39	0.98
Fat %	4.50 ^a	3.60 ^{ab}	3.60 ^b	0.17	0.03
Protein (%)	3.24	3.20	3.27	0.04	0.87
Lactose (%)	4.60	4.51	4.28	0.10	0.48
Ash (%)	0.90 ^a	0.84 ^{ab}	0.70 ^b	0.03	0.03
Total solids (%)	13.26 ^a	12.16 ^{ab}	11.86 ^b	0.25	0.04
Solid not fat (%)	8.75	8.56	8.26	0.10	0.16
Specific gravity	1.029	1.031	1.030	0.0006	0.64

*whole plant; †medium chop length; ‡short chop length; SEM, standard error of mean

Table 4. Effect of chop length of berseem on nutrients digestibility

Variable	WP*	MCL†	SCL‡	SEM	P-value
Dry matter digestibility (%)	75.69 ^b	83.62 ^a	84.12 ^a	1.32	0.001
Fat digestibility (%)	64.66 ^b	76.49 ^a	77.49 ^a	2.28	0.010
Protein digestibility (%)	66.41 ^b	73.55 ^{ab}	79.94 ^a	2.17	0.010
Fiber digestibility (%)	66.54	76.13	71.08	1.75	0.060
NDF digestibility (%)	64.53 ^b	72.97 ^a	73.30 ^a	1.59	0.010
ADF digestibility (%)	62.76	69.66	74.19	2.26	0.100

*whole plant; †medium chop length; ‡short chop length; SEM, standard error of mean

Sahiwal cattle ($P<0.05$). Cows in the WP group had higher milk fat percentage $4.50 \pm 0.17\%$ compared with $3.60 \pm 0.17\%$ in the SCL group ($P<0.002$). Total milk solid increased by $13.26 \pm 0.25\%$ (WL) vs $11.86 \pm 0.25\%$ (SCL) ($P<0.05$) and milk ash by $0.90 \pm 0.03\%$ (High) vs $0.70 \pm 0.03\%$ (Low) ($P<0.05$). However, there was, no significant difference in protein, lactose, solid not fat and specific gravity percentage of milk in all groups.

Effect of chop length of berseem on nutrients digestibility:

Reducing chop length of berseem increased digestibility of DM by $84.12 \pm 1.32\%$ (SCL) vs $75.69 \pm 1.32\%$ (WP), fat by $77.49 \pm 2.28\%$ (SCL) vs $64.66 \pm 2.28\%$ (WP), protein by $79.94 \pm 2.17\%$ (SCL) vs $66.41 \pm 2.17\%$ (WP) and NDF by $73.30 \pm 1.59\%$ (SCL) vs $64.53 \pm 1.59\%$ (WP) in Sahiwal cows ($P<0.05$). However, there was, no significant difference in fiber and ADF digestibility, with all groups having $71 \pm 1.75\%$ (mean $\pm$ SD) and $69 \pm 2.26\%$ (mean $\pm$ SD), respectively (Table 4).

DISCUSSION

Intake of dry matter depends on the physical form of diet, digestion rate, digestibility, rate of passage and reduction of particle size in the rumen. These associations suggest that the intake of dry matter limits with rumen fill but if the filling of rumen was the only factor which limits intake of dry matter than the cows fed with finely chopped fodder should have increased intake of dry matter because they have a lower filling of rumen than the cows fed with long particles (Fernandez *et al.*, 2004). Reducing fodder length increased DM intake compared to whole plant, and this result agrees with these earlier studies (Yansari *et al.*, 2004; Kmicikewycz *et al.*, 2015; Tayyab *et al.*, 2019). This differences in DMI may be due to increased gut fill associated with bulk rumen fill, as also noted in other studies (Leonardi *et al.*, 2005). The reduced intake of DM for the cows fed on longer chop length forages can be associated with two potential mechanisms. First, by feeding of longer chop length forage the digesta mean retention time was increased in the reticulorumen second, the higher activity of sorting in favors of smaller chop length particles of diet might contributing to the limitation of intake in cows fed on longer chop length diets (Zebeli *et al.*, 2007). The result of present study was also supported by the recent studies (Jiang *et al.*, 2018; Kahyani *et al.*, 2013; Alamouti *et al.*, 2014). Whereas the intake of dry matter and organic matter was reduced 1.7 and 1.8 kg/day respectively when the forage chop size reduced (2.8 mm) (Krause and Combs, 2003). The finding of present study was in contrast with previous studies whose reported that intake of dry matter was not affected by the feeding of varying chop lengths fodder (Krause *et al.*, 2002; Soita *et al.*, 2000; Maulfair *et al.*, 2011; Fredin *et al.*, 2015).

Production of milk was not affected by the varying chop length of berseem fodder in present study. These results were supported by Schwab *et al.* (2002) and Ramirez *et al.* (2016). Earlier Couderc *et al.* (2006) reported that the increase in milk production was not associated with increased intake of nutrients by cows. Whereas the results of present study were in contrast with the results from Krause and Combs (2003), Kmicikewycz *et al.* (2015) and Tayyab *et al.* (2019) who reported that the production of milk and fat corrected milk (FCM) was increased 1.3 kg/day and 2.5 kg/day by decreasing the forage chop size.

Change in milk composition is associated with the physical form of diet. In present study the percentage of milk fat, total solids and ash were increased by feeding of WP followed by MCL and SCL. Results of present study were in line with previous studies (Tayyab *et al.*, 2019). The effects of particle length on fat contents in milk were likely to be observed when the level of NDF were below minimum requirements recommended by (NRC, 1989). So, the fat contents are most affected component of milk by the chop length of forage whereas the other contents like protein and lactose was less affected and not changed by the chop size of forage. There are some factors such as long-chain *trans* fatty acids and ratio of acetate to propionate are the major components which controlling the production of milk fat. Indeed, Increase of TMR chop length along with homogenization in rumen-stabilized feeding particle length, enables the increase in rumen fermentation and production of acetate and decrease in the propionate and *trans* fatty acid formation. These factors increase the production of fat (Mertens, 1997; NRC, 2001). Alternatively, a higher fiber diets may increase pH of rumen and reduces ruminal production of *trans*-10, *cis*-12 conjugated linoleic acid that has been associated with the reduction in fat contents of milk (Harvatine and Bauman, 2011). Reduced chop length of forage decreases the milk fat by reducing the rumen pH, possibly through the increase in *trans* fatty acid in the rumen, or by decreasing the supply of butyrate and acetate (Mertens, 1997; Griinari *et al.*, 1998). Results of present study were in contrast with Schwab *et al.* (2002) study suggested that percentage of fat in milk and total yield of fat was reduced by 0.25% and 0.08 kg/day by feeding of longer chop length silage. In the present study the percentage of protein, lactose and solid not fat were not changed by feeding of varying chop lengths. Previously, Kahyani *et al.* (2013) reported that milk protein and lactose percentage was not changed due to chop size of alfalfa hay. Findings of present study was in contrast with Yang *et al.* (2001) who concluded that the percentage of protein and lactose was increased by the longer chop length of forage. Bal *et al.* (2000) reported that the percentage of milk fat was not increased when whole plant corn silage was fed to cows. In the present study the digestibility of DM, protein, fat and NDF was increased by feeding of small chop length berseem in Sahiwal cows whereas, the digestibility of fiber and ADF

was not affected by the varying chop lengths of berseem feeding. Digestibility in ruminants is a function of competition between digestion and passage rates (Yansari and Primohammadi, 2009). Results of present study was in line with (Jarrett *et al.*, 2014) studies concluded that the digestibility of DM and protein was increased by feeding of smaller chop lengths than longer chop lengths whereas the digestibility of OM was increased linearly by feeding of small chop length. Similar results were observed in previous studies of Maulfair *et al.* (2011) who reported that short chop length (14 mm) of Lucerne silage increase total tract digestibility of organic matter and dry matter (both +4%). Longer chop length (19 mm) decreases DM digestibility by 4.3%, NDF digestibility. Krause and Combs (2003) concluded that the apparent digestibility in the total tract of DM and ether extract were tended to greater in animals fed with long chop size of forage. Whereas some previous studies reported that the digestibility of DM, OM, ADF and NDF was not affected by the varying chop sizes of corn silage (Zebeli *et al.*, 2007). The differences in results was may be due to variation in the chop lengths of forages in different studies. Beside feeding fodder to cows we offered a fix amount of concentrate to all treatments according to their milk production. That's why, concentrate affect the DMI and digestibility in same manner in all treatments.

Conclusions: Feeding whole plant berseem fodder could increase milk quality in term of increased milk fat, milk total solids and ash contents. As fodder chop length had no impact on milk production, whole plant berseem can be a useful option for improving milk quality in future.

Conflicts of interest: The authors declare no conflicts of interest.

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