Indian Journal of Animal Sciences **92** (5): 599–603, May 2022/Article
https://doi.org/10.56093/ijans.v92i5.114512

Amelioration in the quality traits of forage pearl millet *(Pennisetum glaucum)* by application of liquid microbial inoculants

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Received: 27 August 2021; Accepted: 17 February 2022

ABSTRACT

Pearl millet *(Pennisetum glaucum)* is a promising dual purpose (fodder and grain) crop. The present investigation was carried to evaluate the effect of liquid microbial inoculants on the quality traits of forage pearl millet. Experiment was laid out in randomized complete block design with a total of 12 treatment combinations of liquid microbial inoculants (*Azotobacter* sp., *Sphingobacterium* sp., *Stenotrophomonas maltophilia* and *Burkholderia seminalis*) with 100% Recommended Dose of Fertiliser (RDF) and replicated thrice. The application of the liquid microbial inoculants showed improvement in the quality traits over the uninoculated control at both the locations. The treatment T_9 (RDF + *B. seminalis* + *S. maltophilia)* showed significant increase in the total sugars, ash content, CP (crude protein) and IVDMD (*in vitro* dry matter digestibility), while significant decrease in the ADF (acid detergent fibre) and NDF (neutral detergent fibre). Further, very strong positive and significant correlation was observed between ADF, NDF and the various antinutrients while they were recorded to be negatively correlated with rest of the quality parameters. Therefore, it can be concluded that liquid microbial inoculants could play a predominant role in improving the forage quality of forage pearl millet.

Keywords: *Azotobacter* sp., *Burkholderia seminalis*, Liquid microbial inoculants**,** Pearl millet, Quality traits, *Sphingobacterium* sp., *Stenotrophomonas maltophilia*

Livestock being an integral part of Indian agriculture plays a crucial role in the bucolic economy, contributing about 9% to the National GDP and 25% to the agricultural GDP. India possesses largest livestock population in the world. Nonetheless, the cultivated area under fodder crops is only 4.9% resulting in total forage production of about 978.7 mMT annually while the annual requirement is 1325.7 mMT. Therefore, currently, the country is facing a net shortage of 44% concentrate feeds, 10.95% dry roughages and 35.6% green fodder (IGFRI Vision 2050). Thus, it becomes imperative to put organized efforts to enhance the availability of good quality fodder to dwindle the vast gap between demand and supply.

Undersander and Cosgrove (2011) described forage quality as a measure of the potential of a forage to produce a desired response. The two main factors that determine animal performance are forage intake and forage nutritive value, specifically, dry matter and fibre digestibility (Oskey 2020).

In this context, pearl millet (*Pennisetum glaucum* L.) is a promising dual purpose (fodder and grain), drought tolerant, warm season crop. The grain is rich in protein (11.6%), fat (5%) and with very high carbohydrates (67%), commonly used as green fodder for animals and chapatti making. To improve the yield and quality of the forage, it is imperative to determine the fertilizer requirement of the crop which will not only increase yield but also enhance quality of fodder particularly protein contents (Ayub *et al*. 2007). Chemical fertilizers have been extensively used, but their indiscriminate use has resulted in drastic changes in the environment. Thereupon, the need of the hour is the use of organic interventions such as microbial inoculants which are not only eco-friendly but also maintain the soil fertility. In this regard, rhizospheric bacteria known as plant growth promoting rhizobacteria (PGPR) can serve as a promising choice for use as microbial inoculants. Thus, the present study was conducted to evaluate the influence of liquid microbial inoculants on quality traits of forage pearl millet.

MATERIALS AND METHODS

Procurement of test cultures: Pure cultures of *Azotobacter* sp., *Sphingobacterium* sp., *Stenotrophomonas maltophilia* and *Burkholderia seminalis* were procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.

Weather during the crop season: The meteorological data for the crop season was obtained from Agro-meteorological field unit (AMFU) located at Punjab Agricultural University, Regional Research Station, Bathinda and Punjab Agricultural University, Ludhiana during the crop growing season (*kharif* 2020).

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Experimental details: The field experiment was conducted at Punjab Agricultural University, Regional Research Station, Bathinda and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *kharif* 2020.The experiment was laid out in a randomised complete block design (RCBD) with 12 treatments and replicated thrice at both the locations i.e Bathinda and Ludhiana respectively. The different treatments were as follows:

T_1, RDF; T_2, RDF + *Azotobacter* sp.; T_3, RDF + *Burkholderia seminalis*; T_4, RDF + *Stenotrophomonas maltophilia*; T_5, RDF + *Sphingobacterium* sp.; T_6, RDF + *Azotobacter* sp. + *Burkholderia seminalis*; T_7, RDF + *Azotobacter* sp. + *Stenotrophomonas maltophilia*; T_8, RDF + *Azotobacter* sp. + *Sphingobacterium* sp.; T_9, RDF + *Burkholderia seminalis* + *Stenotrophomonas maltophilia*; T_{10}, RDF + *Burkholderia seminalis* + *Sphingobacterium* sp.; T_{11}, RDF + *Stenotrophomonas maltophilia* + *Sphingobacterium* sp. and T_{12}, RDF + Consortium (Commercially available biofertilizer from the Department of Microbiology, Punjab Agricultural University, Ludhiana).

The land preparations were done mechanically with proper care to avoid mixing of soil from adjacent plots. The pearl millet cultivar 'FBC-16' was sown at the rate of 8 kg/acre. Pearl millet seeds were inoculated with liquid microbial inoculants of *Azotobacter* sp., *Sphingobacterium* sp., *S. maltophilia* and *B. seminalis* as per treatments @ 100 ml/acre. Inoculated seeds were air dried in shade and planted within 2 h. Weeding and hoeing was done so as to avoid weeds and appropriate control measures were taken to prevent insects and pests. Other cultural operations and plant protection measures were followed as per the recommendations.

Forage quality traits: The quality parameters of the forage crop were analysed by first sun drying the plant samples and later oven dried to obtain a constant weight. The dried samples were then finely ground and used for the estimation. The quality traits studied in the present study were total sugars (Dubois *et al*.1956), ash content (AOAC 2007), crude protein (CP) content (AOAC 2007), acid detergent fibre (ADF), neutral detergent fibre (NDF) by the method of Vansoest (2015), *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry 2006), total phenols (Swain and Hillis 1959), oxalate content (Abeza *et al*. 1968) and tannins content (Sadasivam and Manickam 1992).

Statistical analysis: Randomized complete block design was employed for the data analysis. All statistical analysis were performed by the procedure as described by Snedecor and Cochran (1994). The comparisons were made at 5% level of significance. Correlation studies were carried out using IBM SPSS Statistics V22.0 (2013).

RESULTS AND DISCUSSION

Total ash content: Application of liquid microbial inoculants manifested a significant effect on the ash content at both the locations (Table 1). The highest ash content in the fodder pearl millet was recorded with T_9 (15.97 and 14.97%) and the lowest by T_1 (13.47 and 11.81%) at Bathinda and Ludhiana respectively.

The improvement in the ash percentage might be attributed to the elevation in the available nitrogen which led to the accumulation of more nutrients and soluble salts for the growth and as a consequence, augmenting the quality of forage crop.

The results of present study corroborates with Oskey (2020) who recorded ash content of the order 12.49 to 14.21% in conventional and BMR (brown midrib) pearl millet in combination with cowpea.

Crude protein: The samples of pearl millet were analyzed for crude protein content at harvest and the data regarding the same is given in Table 1.

At Bathinda, significant effect of liquid microbial inoculants on the crude protein was evident. The highest amount of crude protein was exhibited by T_9 (9.62%) while the lowest by T_1 (8.02%). At Ludhiana, the influence of liquid microbial inoculants was statistically non-significant on the crude protein content in forage pearl millet. However, a numeric increase in the same was observed in all the treatments as compared to control. Maximum amount of crude protein was obtained when the inoculation of seed was done with T_9 (8.17%), followed by T_{11} (7.98%).

The higher crude protein content as compared to the

Table 1. Influence of liquid microbial inoculants on quality attributes of forage pearl millet

Attribute	Location	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	PSE	C.D. (5%)
Ash (%)	BTI	13.47	13.58	13.61	14.12	13.54	14.34	14.74	14.17	15.97	15.56	15.77	13.53	0.26	1.64
	LDH	11.81	12.28	12.56	12.44	12.56	13.41	13.69	12.67	14.97	14.23	14.64	12.14	0.30	1.21
CP (%)	BTI	8.02	8.25	8.48	8.55	8.25	9.08	9.12	8.85	9.62	9.22	9.38	8.22	0.15	1.00
	LDH	7.15	7.48	7.68	7.68	7.68	7.85	7.85	7.75	8.17	7.85	7.98	7.28	0.08	NS
NDF (%)	BTI	68.66	67.66	65.14	62.76	67.10	60.89	60.66	61.74	59.59	60.16	59.70	68.30	1.03	6.24
	LDH	69.21	61.49	61.09	61.23	66.45	60.78	60.59	60.87	58.55	60.43	60.27	68.44	1.00	5.96
ADF (%)	BTI	39.82	38.36	38.23	37.71	38.25	36.92	36.18	37.21	32.86	35.32	34.78	38.50	0.55	NS
	LDH	40.12	39.17	37.66	37.58	37.92	36.49	36.32	37.12	32.69	34.16	34.67	39.29	0.63	3.74
IVDMD (%)	BTI	53.10	53.89	53.84	53.98	53.75	56.60	59.84	54.33	64.89	61.67	63.95	53.32	1.27	7.22
	LDH	51.65	52.48	53.61	54.85	53.53	56.57	58.10	56.40	61.01	58.83	58.85	51.85	0089	5.08

Details of treatments are given under Materials and Methods. BTI, Bathinda; LDH, Ludhiana.

Table 2. Influence of liquid microbial inoculants on total sugars and various anti-nutrients of forage pearl millet

Parameter	Location	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	PSE	CD (5%)
Total Sugars (mg/g)	BTI	14.69	15.99	16.42	16.43	16.27	17.48	17.99	16.89	18.85	17.81	18.34	15.95	0.34	2.19
	LDH	13.84	15.71	16.16	16.13	15.29	16.56	16.82	16.20	17.74	18.10	18.14	14.51	0.38	1.88
Total phenols (mg/g)	BTI	2.42	2.36	2.31	2.28	2.34	2.16	2.10	2.26	2.01	2.07	2.05	2.40	0.04	NS
	LDH	2.40	2.24	2.16	2.11	2.18	2.09	2.07	2.10	2.00	2.04	2.01	2.27	0.03	NS
Tannins (mg/g)	BTI	0.871	0.862	0.850	0.840	0.857	0.832	0.829	0.834	0.807	0.824	0.813	0.865	0.006	NS
	LDH	0.761	0.748	0.746	0.743	0.750	0.742	0.741	0.743	0.729	0.737	0.737	0.759	0.002	NS
Oxalates (%)	BTI	1.00	0.96	0.90	0.89	0.94	0.86	0.85	0.88	0.73	0.78	0.77	0.97	0.02	0.14
	LDH	0.94	0.90	0.81	0.77	0.88	0.76	0.74	0.76	0.71	0.73	0.72	0.93	0.21	NS

Details of treatments are given under Materials and Methods. BTI, Bathinda; LDH, Ludhiana.

control might be due to the higher amount of nitrogen accumulated due to biological nitrogen fixing ability of inoculated bacterial cultures. The results of the present investigation are in close association with Damame *et al*. (2013) who also reported crude protein in forage pearl millet to fall in the range of 7.92-9.33%.

Neutral detergent fibre: Forages with low NDF will have higher intake than those with high NDF, therefore, low percentage of NDF is desirable in the fodder crops. The data concerning the NDF content of forage pearl millet was recorded at harvest at both the locations (Table 1).

The liquid microbial inoculants significantly decreased the NDF over the control. Application of T_9 recorded the minimum NDF (59.59 and 58.55%) while the maximum was obtained with T_1 (68.66 and 69.21%) at Bathinda and Ludhiana respectively.

The decrease in the NDF content due to the liquid microbial inoculants application might be ascribed to the increased availability of nutrients, particularly nitrogen which imparts succulence to the green plants. Thus, the contribution of liquid microbial inoculants in lowering the NDF and improving the quality of fodder pearl millet is noteworthy.

Acid detergent fibre: ADF values are inversely related to digestibility, so forages with low ADF concentrations are usually higher in energy. The data concerning ADF content at both the locations is given in Table 1.

A non-significant but numeric decline in the ADF content of the fodder pearl millet by the application of liquid microbial inoculants with respect to control was observed at Bathinda. The maximum ADF content was recorded in T_1 and the minimum with the application of T_9 (32.86%). Likewise, at Ludhiana, a significant reduction in the ADF content was observed with the application of liquid microbial inoculants with respect to the control. The highest ADF content was obtained in T_1 (40.12%), followed by T_{12} (39.29%). The lowest ADF content was recorded with the application of T_9 (32.69%).

The decreased values of ADF % in the treatments with liquid microbial inoculants might be attributed to increase in the phosphorous (P) uptake, and improved root growth and development by the inoculated bacterial cultures. The results observed are in close agreements with the findings of Makarana *et al*. (2018) who recorded similar results in fodder pearl millet.

Total sugars: A significant improvement in total sugars with the application of liquid microbial inoculants was noted at both the locations (Table 2). At Bathinda, the maximum amount of total sugars was obtained with T_9 (18.85 mg/g) while the minimum was recorded with T_1 (14.69 mg/g). At Ludhiana also, significant escalation in the total sugars content was observed with the highest amount of total sugars exhibited by the treatment T_{11} (18.14 mg/g) and the lowest by T_1 (13.84 mg/g).

The sugars accumulated in the plants play a role in stress mitigation such as osmoprotection, carbon storage and scavenging of reactive oxygen species, and an increase in the sugars content with the application of liquid microbial inoculants indicates better stress tolerance of fodder pearl millet thus improving its quality. Our results are in close association with Anju *et al*. (2019) who observed similar values of total soluble sugars in the pearl millet under salinity stress.

In vitro *dry matter digestibility*: Application of liquid microbial inoculants had a significant influence on IVDMD of forage pearl millet at both the locations (Table 1). The highest IVDMD was exhibited by the application of T_9 (64.89 and 61.01%) and the lowest by T_1 (53.10 and 51.65%) at Bathinda and Ludhiana respectively.

The observed elevation in the *in vitro* dry matter digestibility might be due to the synergistic influence of nitrogen fixation and phosphate solubilisation by the microbial inoculants, thus, improving the digestibility of the fodder. The results of the present study are in accordance with Kaur *et al*. (2017) who recorded similar values of *in vitro* dry matter digestibility in napier bajra hybrid.

Total phenols: Phenols are known to lower the digestibility of minerals, proteins, carbohydrates and fats (Othman *et al*. 2007). These anti-quality factors are harmful or indigestible when consumed regularly in large amounts over a prolonged duration. Hence, lower amount of phenols would imply better nutritional characteristics, thus its lower quantity is desired in the fodder samples.

The application of liquid microbial inoculants influenced

the total phenols content in the forage pearl millet non-significantly but a numeric decline was observed with inoculation in all the treatments with respect to control (Table 2). The highest amount of phenols was registered with T_1 (2.42 and 2.40 mg/g) while the lowest with T_9 (2.01 and 2.00 mg/g) at Bathinda and Ludhiana respectively.

The decline in the total phenolic content with the application of liquid microbial inoculants might be attributed to the ability of the plant growth promoting bacteria to protect the plant from various types of abiotic stresses by the synthesis of ACC deaminase enzyme under stressful conditions.

Total phenolic acid in pearl millet is reported to be about 147.8 mg/100 g by Dykes and Rooney (2007) which is in close association with the results of the present investigation.

Tannins: The incorporation of low levels of tannins (2-4%) in the diet elevated the proportion of protein escaping rumen degradation and the absorption of essential amino acids whereas higher tannin levels (4-10%) declined forage intake (Barry and McNabb 1999). Thus, it can be implied that the low tannin containing cultivars are preferred in terms of nutritional attributes.

A non-significant influence of liquid microbial inoculants was observed wherein the amount of tannins decreased in contrast with the uninoculated treatment (Table 2). The minimum amount of tannins was recorded with the application of T_9 (0.807 and 0.729 mg/g) and the maximum with T_1 (0.871 and 0.761 mg/g) at Bathinda and Ludhiana respectively. There is only a slight variation in the different treatments.

The amount of tannins obtained by the application of different treatments i.e. microbial inoculants in the present study are far less than 4% thus indicating no compromise on the quality. The resistance of liquid microbial inoculants against the toxic effects of tannins could also play a major role or the tannic acid might have been degraded into resorcinol, pyrogallol and gallic acid by the increased action of microbes. Goel *et al.* (2011) also reported degradation of tannins by the action of microbes.

Oxalate content: If feed containing high concentration of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the gut lining (Yilkal 2015). The range of oxalates in the fodder pearl millet which is safe for consumption is reported to be 0.63-1.98% (Kaur *et al*. 2012).

At Bathinda, the application of liquid microbial inoculants induced a significant decrease in the oxalate content as compared to control (Table 2). The highest oxalate content was reported with the use of T_1 (1.00%) while the lowest with T_9 (0.73%). At Ludhiana, it was observed that the liquid microbial inoculants had a non-significant impact on the oxalate content of forage pearl millet. The application of all the bio-inoculants resulted in the decline in oxalate content with respect to the control. The minimum oxalate content was recorded with T_9 (0.71%) and the maximum with T_1 (0.94%).

Table 3. Correlation between quality traits of forage pearl millet as influenced by various liquid microbial inoculants treatments at Bathinda (BTI) and Ludhiana (LDH)

	Ash		ADF		NDF		CP		IVDMD		Phenols		Tannins		Oxalate	
	BTI	LDH	BTI	LDH	BTI	LDH	BTI	LDH	BTI	LDH	BTI	LDH	BTI	LDH	BTI	LDH
Total Sugars	0.907*	0.915*	-0.957*	-0.929*	-0.915*	-0.872*	0.973*	0.912*	0.902*	0.914*	-0.968*	-0.942*	-0.961*	-0.945*	-0.954*	-0.918*
Ash			-0.961*	-0.968*	-0.872*	-0.700**	0.944*	0.888*	0.977*	0.954*	-0.957*	-0.861*	-0.938*	-0.883*	-0.964*	-0.834*
ADF					0.860*	0.754*	-0.956*	-0.929*	-0.950*	-0.969*	0.949*	0.910*	0.949*	0.935*	0.970*	0.889*
NDF							-0.957*	-0.856*	-0.803*	-0.778*	0.942*	0.864*	0.970*	0.923*	0.927*	0.878*
CP									0.916*	0.924*	-0.984*	-0.965*	-0.984*	-0.967*	-0.972*	-0.922*
IVDMD											-0.945*	-0.914*	-0.891*	-0.932*	-0.929*	-0.931*
Phenols													0.969*	0.949*	0.971*	0.954*
Tannins															0.980*	0.933*

*, Correlation is significant at 0.01 level; **, Correlation is significant at 0.05 level.

The results obtained in the present study indicate that the oxalate content lies well within the prescribed range. Moreover, oxalate can be degraded into carbon dioxide and formic acid by the microbes which could result in the lower oxalate content (Rahman *et al*. 2012).

Correlation analysis of quality traits: The correlation analysis of different quality attributes was performed at both the locations so as to evaluate the degree of association between them (Table 3). At Bathinda, all the quality attributes were significantly correlated with each other. This suggests that an increase in the desirable characters could result in a simultaneous decrease in the negative characters. Likewise, at Ludhiana, significant correlation was observed within the various quality traits.

Kaur *et al*. (2017) also observed negative correlation between ADF and IVDMD (r=-1.000*) in a study conducted on Napier Bajra hybrid. The inverse relationship between the desirable and undesirable characters is quite evident from the correlation studies suggesting that the application of liquid microbial inoculants resulted in an elevation in the total sugars, ash content, crude protein and IVDMD, and a simultaneous decline in the ADF, NDF and the various antinutrients. Therefore, selection for these traits may aid in the enhancement of the green fodder yield. Thus, it can be presumed that liquid microbial inoculants could play a paramount role in improving the quality of fodder crops.

The results conclusively revealed that RDF + *B. seminalis* + *S. maltophilia* treatment was the best. Thus, these microbial inoculants could play an essential role in ameliorating the quality of forage pearl millet thus, enriching the animal's diet with requisite nutrients and that may help in increasing the animal productivity.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Punjab Agricultural University, Ludhiana, Punjab, India.

REFERENCES

AOAC. 2007. *Official Methods of Analysis*. 18th edn. Association of Official Analytical chemists, Gaithersburg.

Abeza R H, Blake J T and Fisher E T. 1968. Oxalate determination. Analytical problems encountered with certain plant species. *Journal of the Association of Official Agricultural Chemists* **51**: 963–65.

Anju U L, Doddagoudar S R, Pattanashetti S K, Gowda B and Vijaykumar K. 2019. Influence of seed priming on seed germination, seedling growth, peroxidase activity, proline and total soluble sugar content of pearl millet (*Pennisetum glaucum* L.) under salinity stress. *International Journal of Chemical Studies* **7**(5): 508–14.

Ayub M, Nadeem M A, Tanveer A, Tahir M and Khan R M A. 2007. Interactive effect of different nitrogen levels and seeding rate on fodder yield and quality of pearl millet. *Pakistan Journal of Agricultural Sciences* **44**: 592–96.

Barry T N and McNabb W C. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* **81**: 263–72.

Damame S V, Bhingarde R N and Pathan S H. 2013. Effect of different nitrogen levels on nutritional quality and nitrate nitrogen accumulation in forage pearl millet genotypes grown under rainfed conditions. *Forage Research* **39**(2): 93–95.

Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350–56.

Dykes L and Rooney L W. 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World* **52**(3): 105-11.

Goel G, Kumar A, Beniwal V, Raghav M, Puniya A K and Singh K. 2011. Degradation of tannic acid and purification and characterization of tannase from *Enterococcus faecalis*. *International Biodeterioration and Biodegradation* **65**(7): 1061–65.

IGFRI Vision 2050. Indian Grassland and Fodder Research Institute, Jhansi (UP).

Kaur G, Mandhania S and Chaudhary D P. 2012. Calcium oxalate in Napier Bajra Hybrid: Trends. *Indian Journal of Animal Nutrition* **29**: 58–62.

Kaur R, Goyal M and Tiwana U S. 2017. Yield and quality attributes with seasonal variation in Napier Bajra hybrid (*Pennisetum purpureum* × *Pennisetum glaucum*) under different nitrogen environments. *Journal of Applied and Natural Science* **9**(3): 1350–57.

Makarana G, Yadav R K, Kumar R, Kumar A, Soni P G, Kar S and Rajvaidya S K. 2018. Fodder and grain quality of Pearl millet (*Pennisetum glaucum* L.) under cutting management in saline irrigation water. *Journal of Pharmacognosy and Phytochemistry* **7**(3): 1251–57.

Oskey M A. 2020. 'Yield and nutritional composition of conventional and bmr pearl millet under different management conditions'. PhD Dissertation, Clemson University.

Othman A, Ismail A, Ghani N A and Adenan I. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry* **100**(4): 1523–30.

Rahman M M, Abdullah R B and Khadijah W E W. 2012. A review of oxalate poisoning in domestic animals: tolerance and performance aspects. *Journal of Animal Physiology and Animal Nutrition* **97**: 605–14.

Sadasivam S and Manickam A. 1992. Phenolics. *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Ltd, New Delhi, India.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. IOWA State University Press. Ames, IOWA, USA.

Swain T and Hillis W E. 1959. The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture* **10**: 63–68.

Tilley J M A and Terry R A. 2006. A two-stage technique for the *in vitro* digestion of forage crops. *Grass and Forage Science* **18**: 104–11.

Undersander D and Cosgrove D. 2011. *Alfalfa management guide.* American Society of Agronomy Crop Science Society of America Soil Science Society of America.

Van Soest P J. 2015. *The detergent system for analysis of foods and feeds*. Cornell University.

Yilkal T. 2015. Important anti-nutritional substances and inherent toxicants of feed. *Food Science and Quality Management* **36**: 40–47.