



# Effect of Zinc Fertilization on Nutritional Quality of Cowpea Cultivars

Manisha, Rakesh Kumar, Hardev Ram, Nitin Tyagi, Rajesh Kumar Meena,  
Dinesh Kumar, Rakesh Kumar, Kuldeep Singh, Doohong Min<sup>1</sup>

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## ABSTRACT

**Background:** The improvement in livestock productivity may be possible by availing better quality fodder in adequate quantity to the dairy farmers. Zinc deficiency might be a major factor for lower quality fodder of cowpea.

**Methods:** The experiment was laid out in factorial randomized block design; comprised of three cowpea cultivars viz., C-152, MFC-08-14 and MFC-09-1 and five zinc management practices viz., control; 10 kg ZnSO<sub>4</sub> as basal; 20 kg ZnSO<sub>4</sub> as basal; 0.5% ZnSO<sub>4</sub> as foliar spray at 20 DAS; 0.5% ZnSO<sub>4</sub> as foliar spray at 20 and 40 DAS.

**Result:** Results revealed that C-152 showed significantly better quality in terms of higher dry matter, crude protein and total ash; and lower acid insoluble ash, neutral detergent fibre, acid detergent fibre and acid detergent lignin amongst all the three varieties. Though, remarkably higher ether extract was obtained with MFC-08-14. Among the zinc management practices, 20 kg ZnSO<sub>4</sub> as basal (Zn<sub>5</sub>) and foliar application of 0.5% ZnSO<sub>4</sub> at 20 and 40 DAS (Zn<sub>5</sub>) recorded significant improvement in fodder yield and quality traits of cowpea.

**Key words:** Cowpea, Fodder quality, Total digestible nutrients, Zinc.

## INTRODUCTION

The demand for milk and other dairy products has been increased worldwide in the era of globalization. To avoid vitiation in the current growth rate in this sector, there is a need to increase milk production and productivity of fodder crops. This can be achieved through better quality fodder in adequate quantity. Fodder crops contributing growth to the livestock sector paves the way for protein and minerals to animals. At present, dry fodder production is lagging about 23.4% and this scarcity was steepest in Jharkhand (67%) followed by Uttarakhand (55%) and Odisha (44.8%) (Roy *et al.*, 2019). The shortage of feed and fodder is one of the major challenges of animal husbandry sector. Leguminous fodder crops are more nutritious than cereals. Cowpea is an excellent fodder amongst the annual leguminous crops. Its green biomass is rich in crude protein (CP); and contains lower neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Low productivity of livestock owing to malnutrition or under nutrition is a major concern to Indian dairy farmers. Zinc is a vital trace element in animal nutrition and plays an important role in several biological functions. Yasothai (2014) emphasized the role of zinc in reproductive capacity and semen quality in dairy cattle. Its deficiency also causes parakeratosis in pigs. Although food/ fodder fortification and supplementation are likely to offer a way in some countries to mitigate the Zn deficiencies in humans and animals, but alternative agricultural strategies (*e.g.*, fertilization) appear to be more useful in improving micronutrient concentration in fodder crops.

The direct linkage between soil micronutrient and micronutrient of forage and fodders and therefore, a positive effect on crop yield/ animal produce is possible due to zinc

ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

<sup>1</sup>Department of Agronomy, Kansas State University, Manhattan-KS, USA.

**Corresponding Author:** Rakesh Kumar, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

Email: drdudi\_rk@rediffmail.com.com

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(Nube and Voortman, 2006). Since fodder quality is a function of genetic makeup and environment hence, increased efficiency of uptake and utilization of soil nutrients will help to reduce chemical usage in agriculture. With respect to physiological aspect, nutritional efficiency is the ability of the genotype to absorb the nutrient from the soil, distribute it and use it internally (De Oliveira *et al.*, 2012). Cowpea varieties responded differently in uptake of zinc under different level of zinc (Mfeka, 2017). Proximate composition differs among genotypes in terms of protein, dietary fibre contents and various other quality parameters (Carvalho *et al.*, 2012). Cultivars that are efficient in the absorption and use of zinc can be a modality to enhance quality. Therefore, exploiting the genetic potential of varieties with high zinc efficiency could be an ecological way to produce quality fodder in problematic areas.

## MATERIALS AND METHODS

The experiment was conducted at Research Farm of Agronomy

Section, ICAR-National Dairy Research Institute, Karnal located at 29°45' N latitude, 76°58' E longitudes. The experiment was laid out in factorial randomized block design with fifteen treatment combinations and replicated thrice. The treatment consisted of three cultivars of cowpea viz., C-152, MFC-08-14, MFC-09-1 and five management practices of zinc viz., control, 10 kg ZnSO<sub>4</sub>/ha, 20 kg ZnSO<sub>4</sub>/ha, foliar application of 0.5% ZnSO<sub>4</sub>/ha at 20 DAS, foliar application of 0.5% ZnSO<sub>4</sub>/ha foliar spray at 20 and 40 DAS. Cowpea crop was harvested manually at 60 DAS. The oven-dried samples of plants were ground to pass through 40 mesh sieve in a Macro-Wiley Mill and used for chemical analysis. Finally milled sample were analysed for dry matter (DM), total ash (TA), acid insoluble ash (AIA), ether extract (EE) and Kjeldahl Nitrogen using AOAC (2005) method. Crude protein (CP) content in cowpea was determined by multiplying the N concentration by 6.25. Cell content and cell wall constituents were analysed as suggested by Van Soest *et al.* (1991). Insoluble protein fractions from fibre viz., neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were estimated as Licitra *et al.* (1996) method. Dry matter intake (DMI), dry matter digestibility (DMD), net energy of lactation (NEL) and relative feed value (RFV) were determined by using following formulae (Horrocks and Vallentine, 1999):

$$\text{DMI (\%)} = \frac{120}{\text{NDF}}$$

$$\text{DMD (\%)} = 88.9 - (0.779 \times \text{ADF})$$

$$\text{TDN (\%)} = (-1.291 \times \text{ADF}) + 101.35$$

$$\text{NEL (Mcal/kg)} = [1.004 - (0.0119 \times \text{ADF})] \times 2.205$$

$$\text{RFV} = \text{DMI} \times \text{DDM} \times 0.775$$

Relative feed quality (RFQ) (Undersander *et al.*, 2010), digestible energy (DE) (Fonnesbeck *et al.*, 1984) and metabolisable energy (ME) (Gonzalez and Everitt, 1982) was determined by using following formulae:

$$\text{RFQ} = \text{DMI} \times \text{TDN} \times 0.813$$

$$\text{DE (Mcal/kg)} = 0.27 + [0.0428 \times \text{DMD(\%)}]$$

$$\text{DE (MJ/kg)} = \text{DE (Mcal/kg)} \times 4.184$$

$$\text{ME (MJ/kg)} = \text{DE (MJ/kg)} \times 0.821$$

The experimental data were tabulated and analyzed using standard statistical methods (Gomez and Gomez, 1984). Significance of the treatments were tested using F test with 5% level of significance ( $P < 0.05$ ) and means were compared using the least significant difference (LSD) test. Correlation was studied with Statistical Package for Social Sciences (SPSS) software.

## RESULTS AND DISCUSSION

### Dry matter and organic matter content

Organic matter (OM) represents carbohydrate, lipids, proteins, nucleic acids, organic acids and vitamins *etc.* in

the plant cell. DM and OM content was significantly differed with varieties, whereas only OM could be able to make significant variation due to zinc application (Table 1). Amongst the varieties, C-152 recorded significantly higher DM and OM content which was at par with MFC-09-1. This could be due to plants exhibited varying degree of nutrient absorption and distribution which ultimately determine dry matter content in plant.

Basal application of 20 kg ZnSO<sub>4</sub> (Zn<sub>3</sub>) and foliar spray of 0.5% ZnSO<sub>4</sub> at 20 and 40 DAS (Zn<sub>5</sub>) showed significantly lower organic matter content. The organic matter of biomass constitutes three main structural biopolymers, *i.e.*, cellulose, hemicellulose and lignin content; hence, any variation in these parameters is highly correlated with organic matter and mineral matter. These results are in line with earlier findings of Kumar *et al.* (2017).

### Crude protein content

Crude protein determines not only the nitrogen from sources other than protein but also other sources such as free amino acids, amines and nucleic acids. Among the cultivars, C-152 (17.31%) recorded significantly highest crude protein content than MFC-08-14 and MFC-09-1 (Table 1). Variation amongst varieties for crude protein might be due to genetic and environment factors. Singh *et al.* (2018) also observed variation in crude protein content owing to heritability.

Crude protein content significantly enhanced with the zinc application and significantly higher crude protein content was admeasured under 20 kg/ha ZnSO<sub>4</sub> over control. Since, zinc is essential component of ribosome, required for their development and protein production in plants. Amino acid accumulation and therefore, protein production decreases due to zinc deficiency. These results are in line with the findings of Ganesh *et al.* (2015) on cowpea who reported significantly higher protein content with increased level of zinc.

### Ether extract content

Ether extract contains lipid, organic acids, alcohol and pigments. Cultivar MFC-08-14 (3.02%) recorded significantly highest ether extract content than other varieties. Antwi *et al.* (2007) endorsed significant variation among cowpea varieties with respect to ether extract content.

Ether extract (EE) content increased due to zinc application. Significantly higher ether extract content was recorded with soil application of 20 kg ZnSO<sub>4</sub>/ha. The decrease in ether extract content with Zn deficiency was due to reduction in total fatty acid content. These results are in consonance with findings of Rathore *et al.* (2015) who reported significant reduction in ether extract content due to zinc deficiency.

### Ash content

Total ash represents the inorganic constituent of the feed, *i.e.*, mineral content and acid insoluble ash represents silica content in the plant biomass which is responsible for structural stability to plant. Amongst varieties, C-152

**Table 1:** Effect of cultivars and zinc fertilization on quality parameters of cowpea.

Treatments	DM (%)	OM (%)	CP (%)	EE (%)	TA (%)	AIA (%)	DMD (%)	TDN (%)	NEI (MJ/kg)	RFQ (%)	DE (MJ/kg)	ME (MJ/kg)
<b>Varieties</b>												
V <sub>1</sub> : C-152	17.02	89.11	17.31	2.82	10.89	4.27	64.04	60.15	6.13	135.17	12.60	10.34
V <sub>2</sub> : MFC-08-14	16.30	89.55	16.85	3.02	10.45	4.45	63.86	59.86	6.10	132.81	12.57	10.32
V <sub>3</sub> : MFC-09-1	16.56	89.61	16.94	2.75	10.39	4.34	63.44	59.16	6.04	128.91	12.49	10.26
SED±	0.26	0.20	0.18	0.05	0.20	0.07	0.36	0.59	0.05	2.45	0.06	0.05
LSD (P=0.05)	0.53	0.42	0.38	0.09	0.42	0.14	NS	NS	NS	NS	NS	NS
<b>Zinc management practices</b>												
Zn <sub>1</sub> : Control	16.26	89.84	16.22	2.78	10.16	4.23	62.84	58.16	5.96	122.57	12.38	10.17
Zn <sub>2</sub> : 10 kg ZnSO <sub>4</sub> as basal	16.52	89.49	17.06	2.82	10.51	4.37	63.42	59.13	6.04	132.03	12.49	10.25
Zn <sub>3</sub> : 20 kg ZnSO <sub>4</sub> as basal	17.03	89.08	17.48	2.96	10.92	4.44	64.59	61.06	6.20	138.19	12.70	10.42
Zn <sub>4</sub> : 0.5% ZnSO <sub>4</sub> as foliar spray at 20 DAS	16.39	89.55	16.89	2.82	10.45	4.31	63.48	59.23	6.05	131.14	12.50	10.26
Zn <sub>5</sub> : 0.5% ZnSO <sub>4</sub> as foliar spray at 20 and 40 DAS	16.93	89.17	17.50	2.94	10.83	4.40	64.58	61.04	6.20	137.55	12.69	10.42
SED±	0.34	0.26	0.24	0.06	0.26	0.09	0.46	0.76	0.06	3.16	0.08	0.07
LSD (P=0.05)	NS	0.54	0.49	0.12	0.54	NS	0.94	1.56	0.13	6.48	0.17	0.14

**Note:** DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; TA: total ash; AIA: acid insoluble ash; DMD: dry matter digestibility; TDN: total digestible nutrients; NEI: net energy of lactation; RFQ: relative feed quality; DE: digestible energy; ME: metabolizable energy.

**Table 2:** Effect of cultivars and zinc fertilization on fibre content of cowpea.

Treatment	NDF	ADF	ADL	NDICP		ADICP		Hemi-cellulose	Cellulose	T-CHO
				DM	CP	DM	CP			
Cultivars										
V <sub>1</sub> : C-152	43.47	31.91	8.41	7.84	45.36	2.57	14.85	11.56	23.50	68.98
V <sub>2</sub> : MFC-08-14	44.09	32.14	9.04	7.75	46.11	2.65	15.74	11.95	23.10	69.69
V <sub>3</sub> : MFC-09-1	44.87	32.68	8.98	7.93	46.88	2.63	15.59	12.19	23.70	69.92
SED±	0.43	0.46	0.16	0.14	0.94	0.07	0.38	0.27	0.50	0.27
LSD (P=0.05)	0.89	NS	0.32	NS	NS	NS	NS	NS	NS	0.55
Zinc fertilization										
Zn <sub>1</sub> : Control	46.39	33.45	9.19	8.03	49.51	2.66	16.39	12.94	24.26	70.83
Zn <sub>2</sub> : 10 kg ZnSO <sub>4</sub> as basal	43.73	32.70	8.88	7.77	45.56	2.64	15.47	11.03	23.82	69.61
Zn <sub>3</sub> : 20 kg ZnSO <sub>4</sub> as basal	43.13	31.21	8.55	7.85	44.95	2.57	14.68	11.92	22.66	68.63
Zn <sub>4</sub> : 0.5% ZnSO <sub>4</sub> as foliar spray at 20 DAS	44.13	32.63	8.82	7.73	45.77	2.65	15.67	11.50	23.81	69.83
Zn <sub>5</sub> : 0.5% ZnSO <sub>4</sub> as foliar spray at 20 and 40 DAS	43.35	31.22	8.61	7.83	44.80	2.58	14.74	12.13	22.61	68.73
SED±	0.56	0.59	0.20	0.18	1.22	0.09	0.49	0.35	0.65	0.35
LSD (P=0.05)	1.14	1.21	0.42	NS	2.50	NS	1.01	0.71	NS	0.71

**Note:** NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; T-CHO: total carbohydrate; DM: dry matter basis; CP: crude protein basis.

produced significantly highest ash content and lower acid insoluble ash (AIA) content than rest of the varieties. Results are congruent with the study of Antwi *et al.* (2007) who reported that chemical composition (ash content) of cowpea depends upon genetic makeup and heritable traits and the silica distribution in plant cell wall is highly dependent on plant species which represents acid insoluble ash.

20 kg ZnSO<sub>4</sub> recorded significantly higher total ash content than control, but other treatments of zinc fertilization were found to be statistically at par with each other. Acid insoluble ash was ranged between 4.23 to 4.44% in case of zinc management practices but remained non-significant with control. Since, Zn interacts positively with potassium and enhances absorption of Cu and Mn in plant (Prasad *et al.*, 2016); therefore, increase in total ash content in plant which might be explained through increased minerals content in plant with zinc application.

#### Yields of DM, CP, EE and TA

C-152 recorded significantly higher DM yield (5.15 t/ha), CP yield (891.9 kg/ha), EE yield (150.1 kg/ha), TA yield (562 kg/

ha) than rest of the varieties (Fig 1,2). Variable DM, CP, EE and TA yields were attributed to the content and yield of the respective parameter. These results corroborated with earlier work of Makarana *et al.* (2017) and Manisha *et al.* (2021).

Significantly higher DM (5.07 t/ha), CP (886.1 kg/ha), EE (150.1 kg/ha) and TA yields (554.5 kg/ha) were recorded with 20 Kg ZnSO<sub>4</sub> as basal application. Application of micronutrients increases availability of other nutrients in soil, which in turn enhances the absorption of other nutrients also and consequently better root growth. Higher dry matter accumulation might be more translocation of photosynthates resulting from increased supply of nutrient. Significant improvement in dry matter yield due to zinc nutrition was also reported by Kumar *et al.* (2016a) in maize and Kumar *et al.* (2016b) in cowpea.

#### Fibre fraction

Neutral detergent fibre (NDF) represents the whole fibre content, whereas acid detergent fibre (ADF) indicates moderately indigestible portion of fodder plant (Newman *et al.*, 2009). Lignin becomes inaccessible to enzymatic

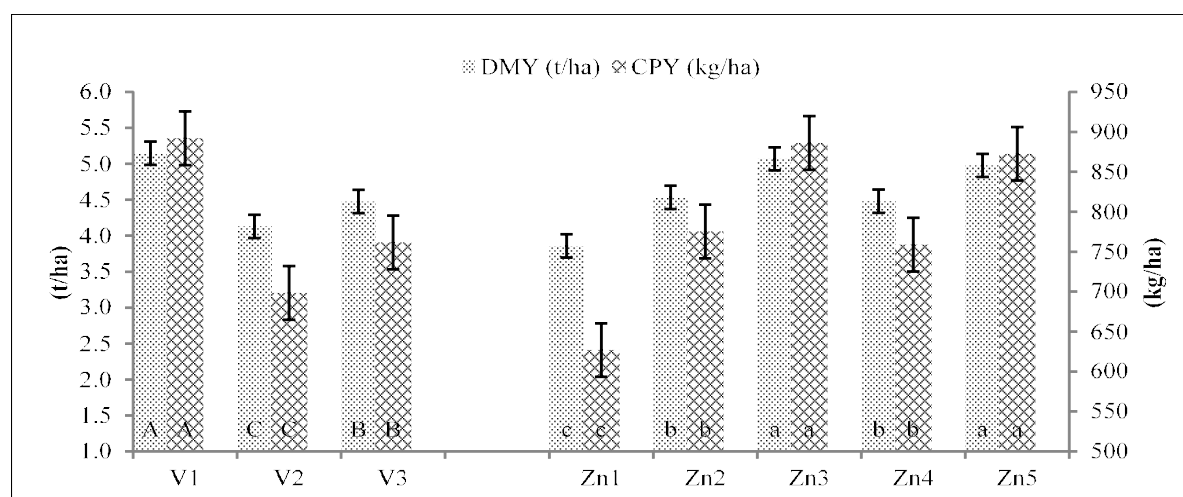


Fig 1: Effect of cowpea cultivars and zinc management practices on dry matter yield (DMY) and crude protein yield (CPY).

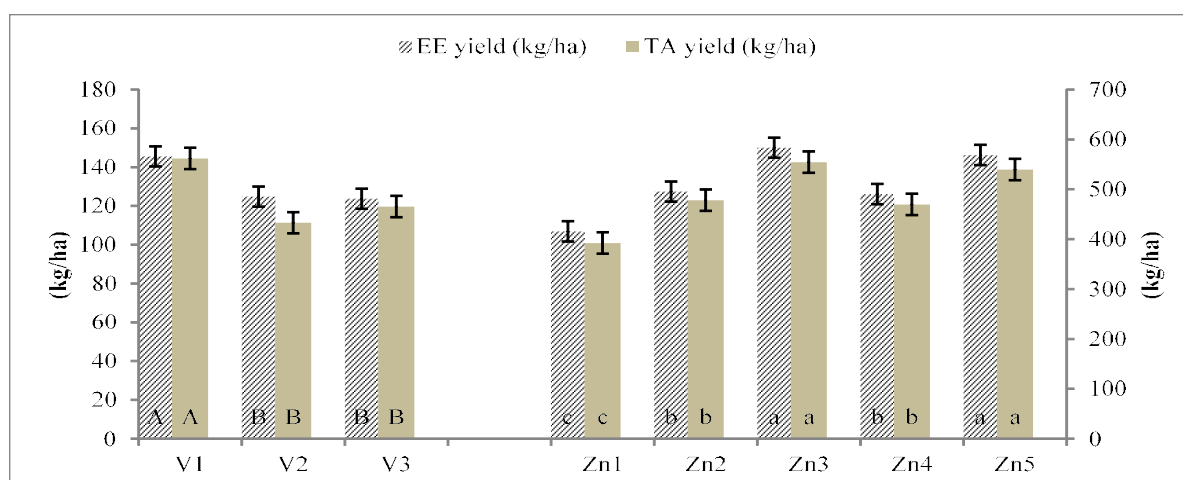


Fig 2: Effect of cowpea cultivars and zinc management practices on ether extract (EE) yield and total ash (TA) yield.

degradation because of strong bond exist among lignin, polysaccharides and cell wall protein. Acid detergent insoluble crude protein (ADICP) represents the portion of feed protein that is not available to ruminants. A perusal of data (Table 2) revealed that fibre fraction parameters found to be non-significant for varieties except neutral detergent fibre (NDF), acid detergent lignin (ADL) and total carbohydrate (T-CHO) content of fodder cowpea. C-152 (43.47%, 8.41% and 68.98%) recorded lower NDF, ADL and T-CHO content as compared to MFC-09-1 and MFC-08-14. These results are corroborated with Singh *et al.* (2018).

Zinc fertilization exhibited significant variation in fibre content of fodder cowpea except neutral detergent insoluble crude protein [NDICP (% DM basis)], acid detergent insoluble crude protein [ADICP (% DM basis)] and cellulose content. All zinc treatments significantly reduced NDF, ADF, ADL, NDICP (% CP basis), ADICP (% CP basis) and hemicellulose with respect to control and basal application of 20 kg ZnSO<sub>4</sub> recorded lowest fibre fractions. At cellular level, Cakmak (2000) explained the role of zinc in lignification of cell walls. The plant produces reactive oxygen species and is an important characteristic of all lignifying cells. Production of these species is catalysed by NADPH oxidase enzyme and zinc deficiency in the plant is highly correlated with enhanced activity of NADPH oxidase.

#### Nutritive values/energy indices

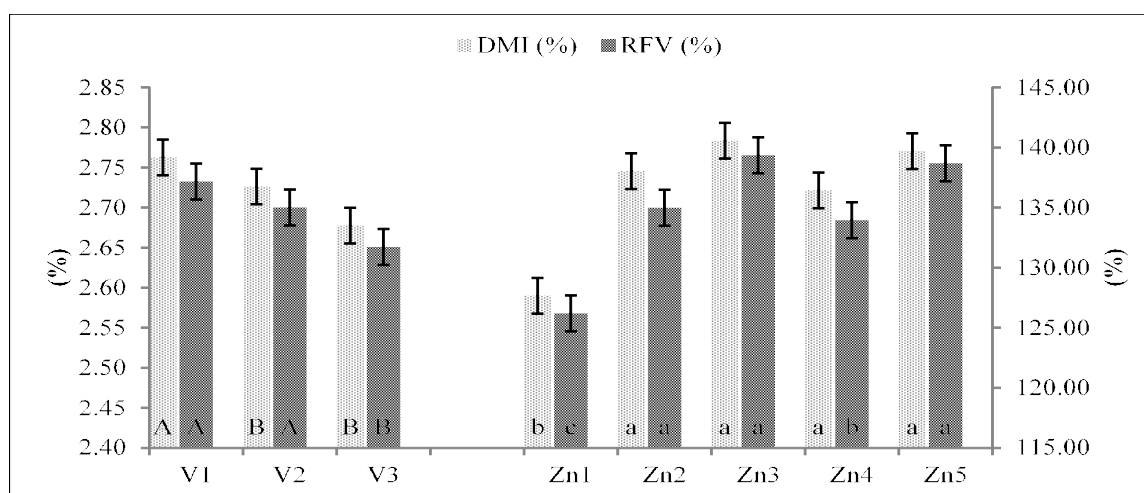
Data presented in Table 1 revealed that varieties could not differentiate the dry matter digestibility (DMD), total digestible nutrients (TDN), net energy of lactation (NEL), relative feed quality (RFQ), digestible energy (DE) and metabolizable energy (ME) but hold significant variation for dry matter intake (DMI) and relative feed value (RFV) as depicted in Fig 3. Varietal comparison showed (Table 1) that C-152

(2.76%, 137.15) exhibited significantly higher DMI and RFV among the varieties. Newman *et al.* (2009), revealed that NDF is an indicator of dry matter intake and RFV index is based on intake potential and digestible dry matter content of the feed. Therefore, the variations in values of RFV in cowpea varieties under zinc application are correlated with the NDF and ADF content of feed.

The secondary parameters viz., DMI, DMD, TDN, NEL, RFV, RFQ, DE and ME as shown in Table-1 and Fig.-3 increased significantly with respect to zinc treatments. Basal application of 20 kg ZnSO<sub>4</sub> as basal (64.59% and 2.76 %) considerably enhanced the DMD and DMI over rest of the treatments. Similar trend was also noted for TDN with highest content in 20 kg ZnSO<sub>4</sub>/ha treatment (61.06%). Zn<sub>3</sub> treatment remained significantly superior over control in terms of increasing NEL, RFV, RFQ, DE and ME. Dry matter digestibility is positively related with crude protein content, but negatively with ADF, NDF and lignin content in the plant as reported by Antwi *et al.* (2007) in cowpea. The variable values of RFV in cowpea varieties under zinc application are correlated with the NDF and ADF content of feed, since it is based on DMI and DMI content of the feed (Newman *et al.*, 2009). ME values are found to be low in treatments exhibited high fibre and low protein content.

#### Correlation matrix

Correlation studies (Table 3) indicated that dry fodder yield was strongly positive and significant ( $P<0.01$ ) correlated with CP ( $r=0.865$ ), TA ( $r=0.828$ ), DMI ( $r=0.716$ ), TDN ( $r=0.740$ ) and RFQ ( $r=0.763$ ). However, the relationship between DFY vs. NDF ( $r=0.723$ ), ADF ( $r=0.740$ ) and ADF ( $r=0.826$ ) was strong negative and significant ( $P<0.01$ ). The quality enhancing parameters viz., CP and TA content had strong negative and significant ( $P<0.01$ ) relationship with fibre



**Fig 3:** Effect of cowpea cultivars and zinc management practices on dry matter intake (DMI) and relative feed value (RFV).

**Note:** V<sub>1</sub>: C-152; V<sub>2</sub>: MFC-08-14; V<sub>3</sub>: MFC-09-1; Zn<sub>1</sub>: control; Zn<sub>2</sub>: 10 kg ZnSO<sub>4</sub> as basal; Zn<sub>3</sub>: 20 kg ZnSO<sub>4</sub> as basal; Zn<sub>4</sub>: 0.5% ZnSO<sub>4</sub> as foliar spray at 20 DAS; Zn<sub>5</sub>: 0.5% ZnSO<sub>4</sub> as foliar spray at 20 and 40 DAS; Vertical bars labelled with different upper and lower-case letters shows the significant variations among varieties and zinc management practices, respectively using LSD ( $P=0.05$ ); Capped lines indicate the standard error of mean.



**Table 3:** Correlation matrix of dry fodder yield vs. quality parameters.

	DFY	CP	EE	TA	NDF	ADF	ADL	DMI	TDN	RFQ
DFY	1									
CP	0.865**	1								
EE	0.032	0.162	1							
TA	0.828**	0.855**	0.221	1						
NDF	-0.723**	-0.871**	-0.356	-0.848**	1					
ADF	-0.740**	-0.860**	-0.454	-0.737**	0.796**	1				
ADL	-0.826**	-0.770**	0.019	-0.940**	0.781**	0.608*	1			
DMI	0.716**	0.868**	0.365	0.855**	-0.999**	-0.796**	-0.783**	1		
TDN	0.740**	0.860**	0.454	0.737**	-0.796**	-1.000**	-0.608*	0.796**	1	
RFQ	0.763**	0.910**	0.427	0.851**	-0.961**	-0.931**	-0.748**	0.962**	.931**	1

**Note:** DFY: Dry fodder yield; CP: Crude protein; EE: Ether extract; TA: Total ash; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; DMI: Dry matter intake; TDN: Total digestible nutrients; RFQ: Relative feed quality \* $P < 0.05$ , \*\* $P < 0.01$  were significant levels for Pearson correlations (two tailed).

fractions. Nutritive values/energy indices (DMI, TDN and RFQ) were also negatively correlated with fibre fractions.

## CONCLUSION

On the basis of experimental results, it was concluded that cowpea cultivar C-152 along with application of either 20 kg ZnSO<sub>4</sub> as basal or foliar application of 0.5% ZnSO<sub>4</sub> at 20 and 40 DAS was found to be most effective approach for obtaining quality fodder.

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