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Study on effect of elevated atmospheric temperature and carbon dioxide levels on *in vitro* dry matter digestibility and *in vitro* gas production of groundnut haulms

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Abstract
A study was conducted to assess the impact of elevated carbon dioxide and temperature levels on the quality of groundnut haulms used as livestock feed. Groundnut crop was grown with recommended package of practices in carbon dioxide and temperature gradient chambers (CTGC) at four different environmental conditions, *viz*: chamber with ambient temperature and carbon dioxide with 27 ± 0.5 °C temp and 380 ± 25 ppm CO_2 (T_1; control), chamber with elevated temperature of 5 ± 0.5 °C higher than control (T_2; eTemp), chamber with elevated CO_2 concentration of 550 ± 50 ppm (T_3; eCO_2) and chamber with elevated CO_2 concentration of 550 ± 50 ppm with elevated temperature of 5 ± 0.5 °C, over control (T_4; eCO_2+eTemp). At harvest stage, representative groundnut plants from each chamber were collected, haulms were separated and screened for further laboratory analysis. *In vitro* dry matter digestibility (IVDMD) of haulms of groundnut crop grown under different CTGC chambers differed significantly ($p<0.01$). Highest IVDMD in groundnut haulms was found in the groundnut crop grown in ambient (T_1; control), whereas lowest under elevated temperature (T_2; eTemp) environmental conditions. Digestible energy (DE; MJ/kg) content in the haulms of groundnut crop grown under different CTGC chambers differed significantly ($p<0.01$). Highest DE was found in the haulms of groundnut crop grown in ambient (T_1; control) whereas lowest was found in elevated temperature (T_2; eTemp) conditions. Metabolizable energy (ME; MJ/kg) content varied significantly ($p<0.01$) and highest was found in the haulms of groundnut crop grown in ambient (T_1; control) whereas lowest under elevated temperature (T_2; eTemp) conditions. The cumulative *in vitro* gas production (IVGP; ml/g^{-1}DM) from groundnut haulms grown in different CTGC chambers also varied significantly ($p<0.01$). The highest cumulative IVGP was found in haulms of groundnut crop grown in elevated carbon dioxide (T_3; eCO_2), while the lowest was found in elevated temperature (T_2; eTemp) conditions. The study concludes that the *in vitro* dry matter digestibility of groundnut crop haulms grown in CTGC chambers indicated that elevated temperature could impact the dry matter digestibility compared to elevated carbon dioxide. Under elevated temperature conditions, the digestibility and energy content of groundnut haulms decreased due to increased lignification. The highest cumulative gas production was found in haulms of groundnut crop grown in elevated carbon dioxide, while elevated temperature had no significant effect on cumulative gas production, though higher temperatures were maintained in both the eTemp and eCO_2+eTemp chambers.

Keywords: Carbon dioxide, environment, groundnut haulms, *in vitro* dry matter digestibility, *in vitro* gas production, temperature

1. Introduction
Climate change is one of the most critical threats facing the world today, with predicted increases in global mean temperature, length and severity of drought events and atmospheric carbon dioxide (CO_2) concentration due to human activities. Greenhouse gases are the primary cause of rising temperatures in the atmosphere. The CO_2 level has risen at a pace of 1.9 ppm per year over the last twelve years and is expected to exceed 570 ppm by the middle of this century and as a result, the global surface temperature is expected to rise by 3-4.5 °C (IPCC, 2014) [6]. Many plants respond to elevated atmospheric carbon dioxide (eCO_2) concentrations by increased growth, biomass and productivity, with C_3 plants generally benefitting more than C_4 plants (Santos *et al*., 2014) [5]. There is a growing evidence suggesting that many crops, notably C_3 crops, may respond positively to increased atmospheric CO_2 in the absence of other stressful conditions (Long *et al*., 2004) [9]. The dry matter production of C_3 and C_4 plants is likely to be increased by 30% and 10% respectively because of doubling effects of CO_2 (Newton, 1991) [11].

In C_3 plants like groundnut, the carbon from CO_2 is fixed into stable organic products in the mesophyll cells of leaves where, ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCo) enzyme is capable of catalysing two distinct reactions: one leading to the formation of two molecules of phosphoglycerate when CO_2 is the substrate; the other resulting in one molecule each of phosphoglycerate and phosphoglycolate (2C molecule) when oxygen (O_2) is the substrate (Long *et al*., 2004) [9]. In general, C_3 plants are more responsive to elevated CO_2, which leads to greater main shoot length, elongation of branches, individual leaf area per plant, and dry mass. It is understood that the accumulation of sugars and starch in the leaves of elevated CO_2 grown plants reflects higher photosynthetic carbon assimilation (Cure and Acock, 1986) [2].

There is limited information available on the influence of rising CO_2 levels and temperatures on the quality of groundnut haulms. Hence, the present study was planned to determine the effects of elevated CO_2 and temperature on the digestibility, energy content, and gas production of groundnut haulms.

2. Materials and Methods

The study was conducted at Animal Science Laboratory, ICAR-CRIDA, Santoshnagar and Hayathnagar Research Farm, ICAR-CRIDA, Hayathnagar, Hyderabad. The groundnut (K6 variety) crop was grown with recommended package of practices in carbon dioxide and temperature gradient chambers (CTGC) at four differently set environmental conditions, *viz*: Chamber with ambient temperature and carbon dioxide with 27 ± 0.5ºC temp and 380 ± 25 ppm CO_2 (T_1; control), Chamber with elevated temperature of 5 ± 0.5 °C higher than control (T_2; eTemp), Chamber with elevated CO_2 concentration of 550 ± 50 ppm (T_3; eCO_2) and Chamber with elevated CO_2 concentration of 550 ± 50 ppm with elevated temperature of 5 ± 0.5 °C, over control (T_4; eCO_2+eTemp).

At harvest stage, a bunch of plants from 4 corners and also from centre (5 samples (G1, G2, G3, G4 and G5) with 2 replicates each, totalling 10 samples) from each gradient chamber (T_1, T_2, T_3 and T_4) were collected and haulms were separated. Haulms were initially air dried and then oven dried at 60 ± 5 °C. Dried samples were ground to pass a 2 mm sieve in a Wiley mill. Further analysis of samples for *in vitro* dry matter digestibility (IVDMD), digestible energy (DE), metabolizable energy (ME) and *in vitro* gas production were estimated in the animal nutrition laboratory at ICAR-CRIDA, Santoshnagar, Hyderabad.

2.1 *In vitro* Dry Matter Digestibility

In vitro DM digestibility of samples was determined by Tilley and Terry (1963) [17] method using the instrument *Daisy incubator* by Ankom Technology.

2.2 Digestible Energy

DM digestibility values are used to estimate digestible energy (DE, MJ kg^{-1}) using the regression equation suggested by Fonnesbeck *et al*. (1984) [3].

2.3 Metabolizable Energy

DE values are converted to ME using the formula reported by Khalil *et al*. (1986) [8].

2.4 *In vitro* Gas Production

In vitro gas samples were analysed using gas chromatograph (450-GC, BRUKER Daltonics, Bremen, Germany) with a stainless-steel column (80/100 mesh Porapak Q column) and a C-R3A integrator.

2.5 Statistical Analysis

Statistical analysis of the data was carried out using SPSS (Statistical Package for Social Sciences) Version 15. Least square analysis of variance was used to test the significance between various gradients according to the procedure described by Wilkinson *et al*. (1996) [16].

3. Results and Discussion

3.1 *In vitro* Dry Matter Digestibility (IVDMD)

The results of the present study pertaining to *in vitro* dry matter digestibility are presented in Table 1. The statistical analysis of the data revealing that the *in vitro* dry matter digestibility (IVDMD) of haulms of groundnut crop grown under carbon dioxide and temperature gradient chambers (CTGC) differed significantly (p<0.01). Highest IVDMD in groundnut haulms was found in the groundnut crop grown in ambient (T_1; control), whereas lowest under elevated temperature (T_2; eTemp) environmental conditions. Significantly (p<0.01) higher IVDMD content was found in haulms of groundnut crop grown under ambient and eCO_2+eTemp chamber conditions, which could be due to decrease in structural carbohydrate as well as decreased lignification. Temperature generally increases the concentration of structural carbohydrates. Temperature is the major factor that influences the nutritional value of forages (Buxton and Fales, 1994) [1]. Temperature increases NDF, ADF, and lignin content while decreasing digestibility and energy. The result of the present study corroborating Moore *et al*. (2001) [10] who reported decreasing ADL content increases digestibility because lignin inhibits digestion. Picon-Cochard *et al*. (2004) [12] also found similar results, indicating that a decrease in NDF was associated with an increase in *in vitro* DM digestibility.

Table 1. *In vitro* dry matter digestibility in groundnut haulms grown in CTGC under different environmental conditions

Treatments	IVDMD (%) in groundnut haulms samples#					
	G1	G2	G3	G4	G5	Overall Mean
T_1 (Control)	72.48	71.87	72.35	72.48	72.37	72.31d
T_2 (eTemp)	69.60	69.42	68.60	68.39	68.19	68.84a
T_3 (eCO_2)	69.20	69.75	69.81	70.31	70.39	69.89b
T_4 (eCO_2+eTemp)	69.75	69.86	70.25	70.26	70.35	70.09c
Overall Mean	70.25	70.22	70.25	70.36	70.32	70.28
N	8	8	8	8	8	40
CV	0.020	0.015	0.021	0.022	0.023	0.224
LSD	0.367	0.340	0.304	0.346	0.393	0.165

[abc]Means in rows with different superscripts differ significantly (p<0.01)

[#]Each value is an average of duplicate analysis, N - Number of samples,

CV - Coefficient of variation, LSD - Least significance difference

3.1.1 Energy content of groundnut haulms (DE and ME)

Digestible energy (DE) and metabolizable energy (ME) content of groundnut haulms varied significantly (p<0.01) across CTGC chambers. The digestible energy (MJ/kg) content recorded in haulms of groundnut crop grown in

carbon dioxide and temperature gradient chambers (CTGC) is presented in Table 2. Among different chambers, highest digestible energy was found in the haulms of groundnut crop grown in ambient (T_1; control) and lowest in elevated temperature (T_2; eTemp) climatic conditions.

A similar trend was observed in metabolizable energy (ME) content of groundnut haulms. The metabolizable energy (MJ/kg) content recorded in haulms of groundnut crop grown in carbon dioxide and temperature gradient chambers (CTGC) is presented in Table 3. Highest ME in groundnut haulms was found in the groundnut crop grown in ambient (T_1; control) whereas lowest under elevated temperature (T_2; eTemp) climatic conditions.

Significantly (p<0.01) higher DE and ME content was found in haulms of groundnut crop grown under ambient and eCO_2+eTemp chamber conditions, which could be due to decrease in structural carbohydrate as well as decreased lignification. Temperature is the major factor that influences the nutritional value of forages (Buxton and Fales, 1994) [1]. Temperature generally increases the concentration of structural carbohydrates. Temperature increases NDF, ADF, and lignin content while decreasing digestibility and energy. The results of the present study are in agreement with the findings of Katyaayani *et al*. (2022) [7], who observed higher DE and ME (p<0.05) content in leaf and stem portion of maize fodder grown under ambient and eCO_2 conditions.

Table 2: Digestible energy (MJ/kg) in groundnut haulms grown in CTGC under different environmental conditions

Treatments	Digestible energy (MJ/kg) in groundnut haulms samples#					
	G1	G2	G3	G4	G5	Overall Mean
T_1 (Control)	13.25	13.14	13.23	13.25	13.23	13.22d
T_2 (eTemp)	12.73	12.70	12.56	12.52	12.48	12.60a
T_3 (eCO_2)	12.67	12.76	12.77	12.86	12.88	12.79b
T_4 (eCO_2+eTemp)	12.76	12.78	12.85	12.85	12.87	12.82c
Overall Mean	12.85	12.85	12.85	12.87	12.86	12.86
N	8	8	8	8	8	40
CV	0.019	0.014	0.020	0.022	0.022	0.212
LSD	0.062	0.062	0.062	0.062	0.062	0.028

[abc]Means in rows with different superscripts differ significantly (p<0.01)

[#]Each value is an average of duplicate analysis, N - Number of samples,

CV - Coefficient of variation, LSD - Least significance difference

Table 3: Metabolizable energy (MJ/kg) in groundnut haulms grown in CTGC under different environmental conditions

Treatments	Metabolizable energy (MJ/kg) in groundnut haulms samples#					
	G1	G2	G3	G4	G5	Overall Mean
T_1 (Control)	10.88	10.79	10.86	10.88	10.86	10.85d
T_2 (eTemp)	10.46	10.43	10.31	10.28	10.25	10.34a
T_3 (eCO_2)	10.40	10.48	10.49	10.56	10.57	10.50b
T_4 (eCO_2+eTemp)	10.48	10.50	10.55	10.56	10.57	10.53c
Overall Mean	10.55	10.55	10.55	10.57	10.56	10.56
N	8	8	8	8	8	40
CV	0.019	0.014	0.020	0.022	0.022	0.215
LSD	0.062	0.062	0.062	0.062	0.062	0.023

[abc]Means in rows with different superscripts differ significantly (p<0.01)

[#]Each value is an average of duplicate analysis, N - Number of samples,

CV - Coefficient of variation, LSD - Least significance difference

3.2 *In vitro* Gas Production (IVGP)

The cumulative *in vitro* gas production (ml/g^{-1}DM) recorded in haulms of groundnut crop grown in carbon dioxide and temperature gradient chamber (CTGC) under different climate conditioned chambers (T_1, T_2, T_3 and T_4) is presented in Table 4.

The cumulative *in vitro* gas production (ml/g^{-1}DM) from groundnut haulms grown in carbon dioxide and temperature gradient chambers (CTGC) varied significantly (p<0.01) between ambient (control), elevated temperature gradient (eTemp), elevated carbon dioxide (eCO_2) and elevated carbon dioxide and temperature gradient (eCO_2+eTemp) chambers. The highest cumulative gas production (ml/g-1 DM) was found in haulms of groundnut crop grown in elevated carbon dioxide (T_3; eCO_2), while the lowest was found in haulms grown in elevated temperature (T_2; eTemp) chambers. Temperature had no significant effect on cumulative gas production (ml/g^{-1}DM) in groundnut haulms from G1 to G5 gradients though the temperature increased in both eTemp and eCO2+eTemp CTGC chambers. Significantly (p<0.01) lower IVGP from haulms of groundnut crop grown under T_2 (eTemp) and T_4 (eCO2+eTemp) chamber conditions was observed, which might be due to increased structural carbohydrates as well as increased lignification. This result is in accordance with the reports of Moore *et al*. (2001) [10], who observed a decreasing ADL content increases digestibility as lignin inhibits digestion. Similar results were reported by He *et al*. (2015), who found that the wheat straw grown in eCO_2+eTemp chamber produced significantly less gas and informed that the IVGP is positively associated with crude protein. In contrast to this, Getachew *et al*. (2004) [4] reported a negative correlation between the CP content of feeds and IVGP at 24 and 48 hours. Katyaayani *et al*. (2022) [7] observed higher IVGP (p<0.05) from the leaf portion of maize fodder grown under ambient conditions.

Table 4: Cumulative gas production (ml/g^{-1}DM) from groundnut haulms grown in CTGC under different environmental conditions

Treatments	Cumulative gas production (ml/g-1DM) in groundnut haulms samples#					
	G1	G2	G3	G4	G5	Overall Mean
T_1 (Control)	28.75	28.81	28.73	28.78	28.75	28.76b
T_2 (eTemp)	28.35	28.35	28.40	28.36	28.36	28.36a
T_3 (eCO_2)	29.03	29.06	29.09	29.05	29.08	29.06c
T_4 (eCO_2+eTemp)	28.73	28.76	28.79	28.72	28.81	28.76b
Overall Mean	28.71	28.74	28.75	28.72	28.75	28.74
N	8	8	8	8	8	8
CV	0.009	0.010	0.009	0.009	0.010	0.172
LSD	0.062	0.088	0.088	0.108	0.124	0.051

[abc]Means in rows with different superscripts differ significantly (p<0.01)

[#]Each value is an average of duplicate analysis, N - Number of samples,

CV - Coefficient of variation, LSD - Least significance difference

5. Conclusions

In vitro dry matter digestibility of haulms of groundnut crop grown in CTGC chambers indicated that elevated temperature could impact the dry matter digestibility of haulms compared to elevated carbon dioxide. Under elevated temperature conditions, the digestibility and energy content of groundnut haulms decreased due to increased lignification. The highest cumulative gas production was found in haulms of groundnut crop grown in elevated carbon dioxide. Temperature had no significant effect on cumulative gas production, though higher

temperatures were maintained in both the eTemp and eCO_2+eTemp environment conditioned chambers.

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