Trends in
Agricultural
Sciences

OPEN ACCESS

# Effect of Source of Water and Days to Harvest on Volatile Fatty Acids of Maize (*Zea mays*) and Wheat (*Triticum aestivum*) Fodder Under the Hydroponic Condition

*Adetayo Bamikole Adekeye*
*World Bank African Centre of Excellence, Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State, NIgeria*

## ABSTRACT

**Background and Objective:** Volatile Fatty Acids (VFAs) are Short-Chain Fatty Acids (SCFAs) and energy sources for ruminants. This study was conducted in Ibadan, Nigeria, to quantify the Volatile Fatty Acids (VFAs) content and composition of fodders produced under hydroponic conditions with varying sources of irrigation water and days to harvest. **Materials and Methods:** Two forage species, maize (*Zea mays*) and wheat (*Triticum aestivum*) were evaluated. Two water sources-nutrient solution and borehole water and 3 days to harvest 8, 10 and 12 days were combined in a factorial arrangement to form six treatments in a Complete Randomized Block Design with five replicates. Seeds of each experimental crop were sown in an aluminum tray after being subjected to pre-sowing treatments for hydroponic fodder production. At harvests, fodder samples were randomly taken, weighed, oven-dried at 70°C for 48 hrs and milled for onward VFAs determination using high-performance liquid chromatography. **Results:** Results showed that the chief VFAs in both fodders was acetic acid. Largely, VFAs in fodders from both species were higher ($p<0.05$) when irrigated with a nutrient solution than borehole water, while values of VFAs increased with age in wheat, no definite pattern was recorded for maize fodder. Interacting source of water and days to harvest showed that both acetic acid, ethanol, iso-valeric and N-butyric were highest ($p<0.05$) when harvesting was delayed till 12 days after sowing using nutrient solution than in other treatments. This study recorded that days to harvest and sources of water are important tools to influence the VFAs contents and composition within a forage species under hydroponic conditions. **Conclusion:** Therefore, concluded that for a quantifying amount of VFAs harvesting is best done 8-10 DAS for maize-fodder and up till 12 DAS using a nutrient solution for wheat under hydroponic conditions.

## KEYWORDS

Volatile fatty acids, hydroponic fodder, maize-fodder, wheat-fodder, nutrient solution, borehole water



## INTRODUCTION

Volatile Fatty Acids (VFAs) are also known as Short-Chain Fatty Acids (SCFAs) and they are principal energy sources for ruminants as they account for up to 80% of the total metabolizable energy, they are the end products of anaerobic microbial fermentation of carbohydrates in the ruminant gastrointestinal tract. They

ISSN: 2957-9449 (Online)
ISSN: 2957-9430 (Print)
https://doi.org/10.17311/tas.2022.45.50

Received: 08 Dec. 2021
Accepted: 17 Aug. 2022
Published: 01 Oct. 2022

represent the most predominant anions in the ruminant for stomach and large intestine[1]. Diets that support fermentation and greater production of VFAs also promote greater levels of productivity than less fermentable diets. Acetic acid (vinegar), propionic acid and butyric acid are the major VFAs and they make up 95% of the acids produced in the rumen. In addition to their importance in ruminant diets, a considerable amount of propionate can cause milk fat depression but when acetate supply exceeds the energy needs it is stored as fat. Even though the nutritive value is indicated by forage chemical composition, digestion by the rumen microflora affects the composition of nutrients absorbed by the host. Microbial activity is controlled to some extent by the host animal and is affected by substrate availability, especially dry matter discharge from ruptured plant cells and particulate surface area available for bacterial colonization[2,3].

Forage contributes to the rumen VFAs, however, their content declines with plant maturity, due to a decrease in leaf/stem ratio and initiation of flowering and leaf senescence[4]. In the tropics, forages generally grow and mature faster and reach the age of senescence quicker as compared to temperate conditions[5]. Research is flimsy on VFAs content and composition of forages and even if ever available it was limited to grass species grown conventionally. Therefore, the objective of this trial was to evaluate the VFAs content and composition of hydroponic fodders from maize and wheat under varying water sources and days to harvest.

## MATERIALS AND METHODS

**Experimental site:** The experiment was conducted between August, 2018 in the Hydroponic Fodder Unit of Azemor Agribiz Ltd., Ibadan, Nigeria.

**Hydroponic system:** The hydroponic fodder system used for this study is composed of a room constructed from planks covered with a poultry net and mosquito net. The roof is made of white translucent polyethylene materials. The house consists of metal shelves coated with aluminium. The experiment was conducted under an average room temperature of 25.27±0.78°C absolute natural climatic conditions.

**Plant materials:** Seeds of maize (*Z. mays*) and wheat (*Triticum aestivum*) used for this trial which were freed from any chemical treatment were sourced from a reputable agro-allied store. The seeds were subjected to viability germination before the commencement.

**Irrigation water:** Two sources of irrigation water were used in this trial, which was the Nutrient Solution (NS) and Borehole Water (BW). The NS was constituted using BIC concept hydroponic liquid solution A and B at 1 and 2.5 mL $L^{-1}$, respectively while borehole water was collected from the main source of water used on the farm (the borehole water is safe for drinking).

**Pre-sowing seeds treatment:** Experimental seeds were cleaned from debris and other dirty materials and sterilized by soaking in a 20% sodium hypochlorite solution for 30 min to control the formation of mould. The trays were also cleaned and disinfected. Thereafter, the seeds were thoroughly washed from residues of the sterilized solution and soaked overnight in borehole water (for 10 hrs) before sowing.

**Seed sowing and irrigation:** The seeds were sown in trays which were lined with aluminium sheets at 2.15 kg/tray and labelled accordingly and stacked on the shelves. Treatments meant for the same irrigation water were randomly stacked together on the same shelf and irrigated manually twice daily as sufficient to keep the seeds/seedlings moist.

**Experimental design and procedure:** This experiment involved maize (*Z. mays*) and wheat (*Triticum aestivum*) irrigated with nutrient solution and borehole water and harvested at 8, 10 and 12 Days After Sowing (DAS). The experiment was therefore a 2×3 factorial in a randomized complete block design, each treatment had five replicates.

**Data collection:** At harvest, the entire mat of the fodder from each species (comprising the root and the green leaves) was lifted and removed from the tray, while a representative fresh fodder sub-samples (300 g) from each tray was taken, oven-dried, milled for onward VFAs analysis.

**Volatile fatty acid determination:** This was determined using high-performance liquid chromatography at the Analytical Laboratory of Sciantec Analytical Services, Stockbridge Technology, United Kingdom.

**Statistical analysis:** All data were subjected to Two-way Analysis of Variance (ANOVA) in a Randomized Complete Block Design (RCBD) using a statistical package[6] while significant means were separated and compared using Duncan's Multiple Range Test[3] at a 5 % level of significance.

## RESULTS

Significant differences were recorded for the effects of sources of water and days to harvest on the Volatile Fatty Acids (VFAs) of the maize and wheat fodders produced under hydroponic conditions. The proportions of acetic acid and Iso-valeric were significantly higher (999.67 mg kg$^{-1}$) in maize fodder irrigated with NS than with borehole water (642.05 mg kg$^{-1}$), whereas, maize fodder irrigated with borehole water produced ($p<0.05$) more N-butyric (34.07 mg kg$^{-1}$) than those with nutrient solution (25.13 mg kg$^{-1}$). Acetic acid and N-butyric were observed to reduce with extending harvest, on the contrary, the contents of both Iso-butyric and Iso-valeric increased as harvesting was delayed from 8-12 days in Table 1. The Volatile Fatty Acids (VFAs) contents namely lactic acid, ethanol, heptanoic, hexanoic, n-valeric, propan-1-ol, propane-1, 2-diol and propionic were not affected by sources of water and days to harvest ($p>0.05$).

The proportion of acetic acid was observed to be at the peak of maize fodder harvested on the 8 DAS (1261.46 mg kg$^{-1}$) under NS irrigation in Table 2. A reduction in acetic acid was observed as the days to harvest increased using NS but the reverse was observed using borehole water. Maize fodder harvested 12 DAS with both NS and borehole water produced the highest Iso-butyric. Iso-butyric was observed to increase in value with delay in the harvesting under both irrigation water sources. In addition, the value

Table 1: Main effects of sources of irrigation water and days to harvest on volatile fatty acid of maize fodder under the hydroponic condition

| | Irrigation water days to harvest | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | NS | BW | SEM | 8 | 10 | 12 | SEM |
| Lactic acid (%) | 0.07 | 0.08 | 0.00 | 0.08 | 0.08 | 0.07 | 0.00 |
| Acetic acid (mg kg$^{-1}$) | 999.67$^{a}$ | 642.05$^{b}$ | 69.93 | 881.41$^{a}$ | 744.10$^{c}$ | 788.00$^{b}$ | 68.03 |
| Ethanol (mg kg$^{-1}$) | 25.66 | 25.30 | 1.52 | 25.50 | 25.59 | 25.37 | 1.12 |
| Heptanoic (mg kg$^{-1}$) | 25.47 | 25.75 | 2.02 | 25.60 | 25.87 | 25.61 | 3.52 |
| Hexanoic (mg kg$^{-1}$) | 25.27 | 25.20 | 2.04 | 25.13 | 25.67 | 25.73 | 2.13 |
| Iso-butyric (mg kg$^{-1}$) | 28.90 | 27.73 | 2.37 | 26.47$^{b}$ | 25.93$^{b}$ | 29.80$^{a}$ | 2.24 |
| Iso-valeric (mg kg$^{-1}$) | 44.20$^{a}$ | 40.00$^{b}$ | 2.43 | 37.47$^{b}$ | 39.40$^{a}$ | 40.247$^{a}$ | 2.50 |
| N-butyric (mg kg$^{-1}$) | 25.13$^{b}$ | 34.07$^{a}$ | 1.10 | 25.33$^{b}$ | 36.20$^{a}$ | 25.20$^{b}$ | 1.60 |
| N-valeric (mg kg$^{-1}$) | 25.60 | 25.93 | 2.01 | 26.31 | 25.64 | 25.51 | 2.22 |
| Propan-1-ol (mg kg$^{-1}$) | 25.13 | 25.13 | 2.23 | 25.33 | 25.27 | 25.47 | 2.21 |
| Propane-1, 2-diol (mg kg$^{-1}$) | 25.13 | 25.33 | 1.97 | 25.51 | 25.32 | 25.58 | 1.55 |
| Propionic (mg kg$^{-1}$) | 25.13 | 25.27 | 2.58 | 25.35 | 24.77 | 25.78 | 2.03 |

$^{a,b,c}$Means on the same row with different superscripts are significantly varied ($p<0.05$), NS: Nutrient solution, BW: Borehole water, and SEM: Standard error of mean of Iso-valeric was highest (45.90 mg $kg^{-1}$) in maize fodder harvested 10 DAS irrigated with NS. N-butyric was highest (52.14 mg $kg^{-1}$) in maize fodder produced from borehole water and harvested 10 DAS. There was no effect of irrigation water source and days to harvest for the following VFAs: Lactic acid, ethanol, heptanoic, hexanoic, N-valeric, propan-1-ol, propane-1, 2-diol and propionic ($p>0.05$).

The proportions of acetic acid, ethanol, Iso-butyric, Iso-valeric, n-butyric hexanoic and propionic were higher ($p<0.05$) in wheat fodder irrigated with NS than BW. Except for the value of Iso-butyric acid which declined with extended days to harvest, there was an increase ($p<0.05$) in the proportions of other VFAs as harvest was extended from 8-12 DAS in Table 3.

Values for both acetic (1374.38 mg $kg^{-1}$), ethanol (91.86 mg $kg^{-1}$), Iso-valeric (76.52 mg $kg^{-1}$) and N-butyric (96.60 mg $kg^{-1}$) were highest in wheat fodder harvested 12 DAS using NS. The outcome of this trial showed no differences resulting from the interaction between sources of water and days to harvest for the following VFAs: Lactic acid, ethanol, heptanoic, hexanoic, N-valeric, propan-1-ol and propane-1, 2-diol. The content of propionic acid in wheat fodder irrigated with NS was higher (88.31 mg $kg^{-1}$, $p<0.05$) when harvested at 10 DAS in Table 4.

Table 2: Interaction effects of sources of irrigation water and days to harvest on volatile fatty acid of maize fodder under the hydroponic condition

| | Nutrient solution | | | Borehole water | | | |
|---|---|---|---|---|---|---|---|
| Parameters | 8 | 10 | 12 | 8 | 10 | 12 | SEM |
| Lactic acid (%) | 0.08 | 0.08 | 0.07 | 0.08 | 0.07 | 0.08 | 0.00 |
| Acetic acid (mg $kg^{-1}$) | $1261.46^{a}$ | $898.72^{b}$ | $838.86^{c}$ | $587.42^{g}$ | $603.90^{f}$ | $734.84^{e}$ | 66.03 |
| Ethanol (mg $kg^{-1}$) | 25.58 | 25.56 | 25.84 | 25.62 | 25.26 | 25.04 | 1.54 |
| Heptanoic (mg $kg^{-1}$) | 25.40 | 25.38 | 26.02 | 26.22 | 26.24 | 25.08 | 2.42 |
| Hexanoic (mg $kg^{-1}$) | 25.42 | 25.12 | 25.42 | 24.66 | 25.22 | 25.82 | 2.43 |
| Iso-butyric (mg $kg^{-1}$) | $28.30^{b}$ | $25.46^{c}$ | $32.94^{a}$ | $25.02^{c}$ | $26.10^{c}$ | $32.10^{a}$ | 2.47 |
| Iso-valeric (mg $kg^{-1}$) | $44.54^{ab}$ | $45.90^{a}$ | $42.14^{c}$ | $42.46^{bc}$ | $41.46^{c}$ | $36.18^{d}$ | 2.57 |
| N-butyric (mg $kg^{-1}$) | $25.58^{c}$ | $24.98^{c}$ | $25.40^{c}$ | $24.88^{c}$ | $52.14^{a}$ | $25.24^{c}$ | 1.60 |
| N-valeric (mg $kg^{-1}$) | 26.68 | 25.22 | 25.28 | 26.02 | 26.22 | 25.60 | 2.25 |
| Propan-1-ol (mg $kg^{-1}$) | 25.38 | 25.28 | 25.12 | 25.04 | 24.98 | 25.42 | 2.71 |
| Propane-1, 2-diol (mg $kg^{-1}$) | 25.16 | 25.60 | 25.72 | 25.26 | 25.48 | 24.82 | 2.00 |
| Propionic (mg $kg^{-1}$) | 25.56 | 24.46 | 26.10 | 25.32 | 25.34 | 25.16 | 2.93 |

[a,b,c]Means on the same row with different superscripts are significantly varied ($p<0.05$) and SEM: Standard error of the mean

Table 3: Main effects of sources of irrigation water and days to harvest on volatile fatty acid of wheat fodder under the hydroponic condition

| | | | | Days to harvest | | | |
|---|---|---|---|---|---|---|---|
| Parameters | NS | BW | SEM | 8 | 10 | 12 | SEM |
| Lactic acid (%) | 0.07 | 0.08 | 0.00 | 0.08 | 0.07 | 0.07 | 0.00 |
| Acetic acid (mg $kg^{-1}$) | $1152.87^{a}$ | $883.85^{b}$ | 53.99 | $907.67^{c}$ | $1125.80^{b}$ | $1219.12^{a}$ | 51.99 |
| Ethanol (mg $kg^{-1}$) | $65.87^{a}$ | $32.34^{b}$ | 1.09 | $30.40^{c}$ | $47.80^{b}$ | $61.68^{a}$ | 1.69 |
| Heptanoic (mg $kg^{-1}$) | 25.99 | 25.62 | 1.47 | 25.79 | 25.48 | 25.92 | 1.24 |
| Hexanoic (mg $kg^{-1}$) | 25.59 | 25.28 | 1.78 | $25.41^{c}$ | $26.97^{b}$ | $28.67^{a}$ | 1.90 |
| Iso-butyric (mg $kg^{-1}$) | $56.37^{a}$ | $40.13^{b}$ | 1.87 | $52.05^{a}$ | $48.35^{b}$ | $46.87^{c}$ | 1.17 |
| Iso-valeric (mg $kg^{-1}$) | $52.07^{a}$ | $45.61^{b}$ | 1.05 | $38.20^{c}$ | $44.50^{b}$ | $76.23^{a}$ | 1.65 |
| N-butyric (mg $kg^{-1}$) | $66.57^{a}$ | $48.51^{b}$ | 1.35 | $54.27^{b}$ | $31.17^{c}$ | $77.80^{a}$ | 1.42 |
| N-valeric (mg $kg^{-1}$) | 25.37 | 25.55 | 1.46 | 25.00 | 25.57 | 25.53 | 1.19 |
| Propan-1-ol (mg $kg^{-1}$) | 25.23 | 25.42 | 1.08 | 25.41 | 25.53 | 25.51 | 1.68 |
| Propane-1, 2-diol (mg $kg^{-1}$) | 25.26 | 25.36 | 1.15 | 25.82 | 25.42 | 25.05 | 1.25 |
| Propionic (mg $kg^{-1}$) | $62.80^{a}$ | $53.27^{b}$ | 1.31 | $33.93^{c}$ | $77.08^{a}$ | $62.18^{b}$ | 1.29 |

[a,b,c]Means on the same row with different superscripts are significantly varied ($p<0.05$), NS: Nutrient solution, BW: Borehole water and SEM: Standard error of mean

Table 4: Interaction effects of sources of irrigation water and days to harvest on volatile fatty acid of wheat fodder under the hydroponic condition

| | Nutrient solution | | | Borehole water | | | |
|---|---|---|---|---|---|---|---|
| Parameters | 8 | 10 | 12 | 8 | 10 | 12 | SEM |
| Lactic acid (%) | 0.08 | 0.08 | 0.07 | 0.08 | 0.07 | 0.08 | 0.00 |
| Acetic acid (mg kg$^{-1}$) | 964.80$^{c}$ | 1175.12$^{b}$ | 1374.38$^{a}$ | 849.24$^{f}$ | 889.12$^{e}$ | 913.18$^{d}$ | 51.99 |
| Ethanol (mg kg$^{-1}$) | 25.40$^{e}$ | 80.34$^{b}$ | 91.86$^{a}$ | 40.76$^{c}$ | 30.50$^{d}$ | 25.78$^{e}$ | 1.69 |
| Heptanoic (mg kg$^{-1}$) | 25.40 | 25.98 | 26.30 | 26.42 | 25.08 | 25.36 | 1.25 |
| Hexanoic (mg kg$^{-1}$) | 26.12 | 25.54 | 25.10 | 25.16 | 25.78 | 24.90 | 1.90 |
| Iso-butyric (mg kg$^{-1}$) | 63.20$^{a}$ | 60.18$^{bc}$ | 45.72$^{d}$ | 61.94$^{ab}$ | 25.72$^{f}$ | 32.70$^{e}$ | 1.27 |
| Iso-valeric (mg kg$^{-1}$) | 40.40$^{d}$ | 39.28$^{d}$ | 76.52$^{a}$ | 30.18$^{e}$ | 50.88$^{c}$ | 55.76$^{b}$ | 1.65 |
| N-butyric (mg kg$^{-1}$) | 77.76$^{b}$ | 25.60$^{d}$ | 96.60$^{a}$ | 59.68$^{c}$ | 25.36$^{d}$ | 60.50$^{c}$ | 1.32 |
| N-valeric (mg kg$^{-1}$) | 25.74 | 25.60 | 25.62 | 25.56 | 25.63 | 25.54 | 1.20 |
| Propan-1-ol (mg kg$^{-1}$) | 25.58 | 26.14 | 25.54 | 25.55 | 25.94 | 25.44 | 1.66 |
| Propane-1, 2-diol (mg kg$^{-1}$) | 25.10 | 25.70 | 24.80 | 26.06 | 25.10 | 25.10 | 1.24 |
| Propionic (mg kg$^{-1}$) | 25.22$^{e}$ | 88.31$^{a}$ | 75.06$^{b}$ | 53.0$^{d}$ | 57.94$^{c}$ | 51.70$^{d}$ | 1.39 |

$^{a,b,c}$Means on the same row with different superscripts are significantly varied ($p<0.05$) and SEM: Standard error of the mean

## DISCUSSION

The outcome of this trial further strengthens green fodder as a source of Volatile Fatty Acids (VFAs)[3] which complement VFAs that are produced during the anaerobic microbial fermentation of complex carbohydrates in the fore-stomach and large intestine[6] of ruminants. Though the trend in maize fodder was not definite, however, results recorded from wheat fodder largely showed an increment in the contents of VFAs with advanced maturity, these results were contrary to the report of the earlier researcher including[3] where fatty acid was reported to consistently reduced with the advance in maturity in tropical forages grown conventionally, the variation in the reports might result from the medium of growing. This further strengthening strengthens the reports that fatty acid concentrations in forages depend on many factors, including species and senescence[5] and growth stage[7], conservation method, as well as wilting, shading and silage additives.

Results recorded from maize and wheat fodders produced under hydroponic conditions showed that the chief VFA in the fodders was acetic acid. This agreed with the finding[1] that Acetate, propionate, butyrate are the predominant Short-Chain Fatty Acids (SCFAs) and are readily absorbed and assimilated as a nutrient source by the ruminant. This study showed that hydroponic fodder will be a good source of VFAs supplement to the ruminants since they depend on SCFAs for up to 80% of their maintenance energy requirements[1] and they are produced in large amounts through ruminal fermentation and are of paramount importance in that they provide greater metabolizable energy for the ruminant. In addition, the presence of acetic acid in the fodders both from maize and wheat is a strong indication that the fodders will be a complementary building regime for milk production since acetate is an essential component in the formation of milk fat, while propionate is used for glucose production, which is needed for the synthesis of milk sugar (lactose)[6]. Another study by Khan *et al.*[3] reported that in ruminants, acetate, propionate and butyrate are the end products of anaerobic microbial fermentation of carbohydrates in the ruminant gastrointestinal tract. They represent the most predominant anions in the ruminant forestomach and large intestine and they are the major substrate of hepatic gluconeogenesis and are readily absorbed into the bloodstream and transported to body tissues. Largely the microorganisms for reproduction and growth use them, with the excess production being used by the ruminant itself. This then suggests that fodders with higher values of VFAs are better sources of energy to the livestock than other sources.

Higher contents of VFAs in maize and wheat fodders irrigated with nutrient solution corroborates earlier report that the application of N fertilization increases the FA content in forage plants and delays the decline in FA content with advancing herbage maturity[8] and that there is considerable variation in the FA content and composition of forages due to species, maturity and environmental conditions.

## CONCLUSION

This current study has been able to establish the potential of hydroponically produced fodder as a viable source of volatile fatty acids and hence, a good energy supplement for ruminants since they are readily absorbed into the bloodstream and transported to body tissues where they are used for hepatic gluconeogenesis, lipogenesis in peripheral tissues and milk synthesis. However, for a considerable volatile fatty acids content of fodder under hydroponic conditions, harvesting is best done 8-10 and up till 12 days after sowing using the nutrient solution for maize and wheat, respectively.

## SIGNIFICANCE STATEMENT

This study discovered that hydroponic fodder can be a source of energy in ruminants' nutrition and as a non-conventional method of fodder production, it could help stakeholders in ruminant industries to make energy for their animals regularly.


## ACKNOWLEDGMENT

I acknowledge the funding support of The World Bank African Centre of Excellence in Agricultural Development and Sustainable Environment (CEADESE), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria and my supervisory committee led by Prof. Femi Onifade.


## REFERENCES


1. Bezabih, M., W.F. Pellikaan, A. Tolera, N.A. Khan and W.H. Hendriks, 2014. Chemical composition and *in vitro* total gas and methane production of forage species from the mid rift valley grasslands of Ethiopia. Grass Forage Sci., 69: 635-643.
2. Capstaff, N.M. and A.J. Miller, 2018. Improving the yield and nutritional quality of forage crops. Front. Plant Sci., Vol. 9. 10.3389/fpls.2018.00535.
3. Khan, N.A., M.W. Farooq, M. Ali, M. Suleman and N. Ahmad *et al*., 2015. Effect of species and harvest maturity on the fatty acids profile of tropical forages. J. Anim. Plant Sci., 25: 739-746.
4. Aluwong, T., P.I. Kobo and A. Abdullahi, 2010. Volatile fatty acids production in ruminants and the role of monocarboxylate transporters: A review. Afr. J. Biotechnol., 9: 6229-6232.
5. de Beni Arrigoni, M., C.L. Martins and M.A. Factori, 2016. Lipid Metabolism in the Rumen. In: Rumenology, Millen, D.D., M. de Beni Arrigoni and R.D.L. Pacheco (Eds.), Springer, Cham, Switzerland, ISBN: 978-3-319-30531-8, pp: 103-126.
6. SAS Institute, 2012. SAS/STAT User's Guide. 10th Edn., SAS Institute Inc., Cary, North Carolina, USA, Pages: 772.
7. Witkowska, I.M., C. Wever, G. Gort and A. Elgersma, 2008. Effects of nitrogen rate and regrowth interval on perennial ryegrass fatty acid content during the growing season. Agron. J., 100: 1371-1379.
8. Clapham, W.M., J.G. Foster, J.P.S. Neel and J.M. Fedders, 2005. Fatty acid composition of traditional and novel forages. J. Agric. Food Chem., 53: 10068-10073.