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Research Paper

In vitro digestibility of selected forages in Sargodha district, Pakistan.

Arif M, Hayat Z, Saeed M, Asif Arain M, Ali Shah Q, Ali Siyal F, Faizi Z, Soomro RN, Abbasi IHR, Rehman A, Abbas Raza SH, Hayat F.

Online J. Anim. Feed Res., 6(3): 62-72, 2016; pii: S222877011600009-6

Abstract

The present study conducted to evaluate the digestibility values of four tree fodder species i.e. Mulberry (*Morus alba*), Kikar, (*Acacia nilotica*), Ber (*Zizphus jujube*) and Shirin (*Albezia procera*); three grasses i.e. Bermuda grass (*Cynodon dactylon*), Mott grass (*Penisetum perpureum)* and Rhode grass (*Chloris gayana*) and two fodder crops i.e. Sorghum (*Sorghum bicolor*) and Alfalfa (*Medicago sativa*) were selected as treatment having three replicates. Duplicate sample of each treatment was collected from seven sub districts of Sargodha. The results showed that dry matter content varied from 17.50% in *Penisetum perpureum* to 44.23% in *Albezia procera*. Crude protein contents were highest in *Morus alba* (22.56 %) and lowest in *Sorghum bicolour* (5.60 %). Acid detergent insoluble fiber (ADF) and neutral detergent insoluble fiber (NDF) values were highest for *Penisetum perpureum* (ADF 45.43%and NDF 74.56%) and lowest for *Acacia nilotica* and *Zizphus jujube* (ADF 14.46% and NDF 31.56%), respectively. The ash contents were highest in *Penisetum perpureum* (11.50%) and lowest in *Cynodon dactylon* (5.46%). *In vitro* DM digestibility was determined at different time intervals (6, 12, 24 and 36 hours) and found highest P<0.05; 78.26% in *Morus alba* and lowest 54.20% in *Chloris gayana*. In conclusion, results recommended that the *Morus alba forage* use as alternative cheap source of ruminants due to high nutritive and IVDMD (*In vitro* dry matter digestibility) values.

Key Words: Forages, Digestibility Evaluation, Ruminants

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Research Paper

Interactive effects of stocking density and feed type on growth, survival and cannibalism among African catfish (*C. gariepinus* Burchell 1822).

Shourbela RM, El-Hawarry WN, and Abd El-Rahman SH.

Online J. Anim. Feed Res., 6(3): 73-82, 2016; pii: S222877011600010-6

Abstract

Food type and stocking density are two major factors influencing aquaculture production. To evaluate their effects on growth, survival rate, and cannibalistic activities among African catfish (*C. gariepinus*) larvae, a 3×3 factorial design was used. Three feed types (*Artemia* nauplii, Zooplankton, and dry feed) and three different stocking densities (10, 20 and 40 larvae l^{-1}) were performed throughout a 21 days rearing period (each treatment was triplicated). T_1 Catfish larvae (Artemia nauplii and 10 larvae l^{-1}) and T9 (Dry feed and 40 larvae l^{-1}) showed the highest growth performance parameters and significantly lower growth performance parameters as expressed by final body weight (T_1; 165.03 mg, T_9; 34.36 mg), specific growth rate (T_1; 22.83% day^{-1}, T9; 14.83% day^{-1}). Meanwhile, the survival rate percentage was the lowest (29.37%) and the highest (82.37%); in T4 (Zooplankton and 10 larvae l^{-1}) and T_9 (Dry feed and 40 larvae l^{-1}) respectively. Additionally, higher stocking densities of catfish larvae had expressed higher rates of cannibalism when compared to the lower stocking densities. The lowest cannibalism rate (3.46%) was recorded for T_1 (Artemia nauplii and 10 larvae l^{-1}) by the end of the experiment. Despite the absence of significant interaction effect between stocking density and feed on rearing performance of *C. gariepinus larvae,* results of the current study indicated successful rearing and well performance of catfish larvae concerning growth performance, cannibalism and survival rates at lower stocking density. The density of 10 larvae l^{-1} was the maximum threshold capacity for *C. gariepinus* larval best growth when fed on either *Artemia* or zooplankton. However, further investigations are required to explore the effect of using other dry feed types in the rearing phase of African catfish larvae.

Key Words: African catfish (*C. gariepinus*), Larval Rearing, Food Type, Stocking Density, Cannibalism, Survival Rate

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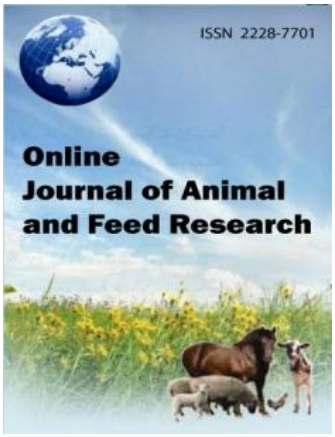

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IN VITRO DIGESTIBILITY OF SELECTED FORAGES IN SARGODHA DISTRICT, PAKISTAN

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ABSTRACT: The present study conducted to evaluate the digestibility values of four tree fodder species i.e. Mulberry (*Morus alba*), Kikar, (*Acacia nilotica*), Ber (*Zizphus jujube*) and Shirin (*Albezia procera*); three grasses i.e. Bermuda grass (*Cynodon dactylon*), Mott grass (*Penisetum perpureum)* and Rhode grass (*Chloris gayana*) and two fodder crops i.e. Sorghum (*Sorghum bicolor*) and Alfalfa (*Medicago sativa*) were selected as treatment having three replicates. Duplicate sample of each treatment was collected from seven sub districts of Sargodha. The results showed that dry matter content varied from 17.50% in *Penisetum perpureum* to 44.23% in *Albezia procera*. Crude protein contents were highest in *Morus alba* (22.56 %) and lowest in *Sorghum bicolour* (5.60 %). Acid detergent insoluble fiber (ADF) and neutral detergent insoluble fiber (NDF) values were highest for *Penisetum perpureum* (ADF 45.43%and NDF 74.56%) and lowest for *Acacia nilotica* and *Zizphus jujube* (ADF 14.46% and NDF 31.56%), respectively. The ash contents were highest in *Penisetum perpureum* (11.50%) and lowest in *Cynodon dactylon* (5.46%). *In vitro* DM digestibility was determined at different time intervals (6, 12, 24 and 36 hours) and found highest P<0.05; 78.26% in *Morus alba* and lowest 54.20% in *Chloris gayana*. In conclusion, results recommended that the *Morus alba forage* use as alternative cheap source of ruminants due to high nutritive and IVDMD (*In vitro* dry matter digestibility) values.

Key Words: Forages, Digestibility Evaluation, Ruminants

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INTRODUCTION

Livestock contributes approximately 55.9 percent value addition in agriculture and 11.8 percent of national GDP in Pakistan (Economic survey of Pakistan, 2013-14). Livestock addition to the gross value has increased from Rs.756.3 billion (2012-13) to Rs.776.5 billion (2013-14). That shows the progress of 2.7 percent as compared to previous year. There are 39.7 million cattle, 34.6 million buffalo, 29.1 million sheep, 66.6 million goats, 1.0 million camels, 0.4 million horses, 4.9 million asses and 0.2 million mules in Pakistan (Economic survey of Pakistan, 2014) out of which Sargodha has about 696 thousand cattle,799 thousand buffalo,143 thousand sheep and 675 thousand goats. Production per animal is very poor as compared to others countries and factor behind this situation is shortage of nutrients (Sarwar et al., 2002). Livestock require 13.5 and 10.3 million tons of CP and TDN, respectively (Economic survey of Pakistan, 2006). Present feed resources fulfil only 62% CP and 75% TDN requirement of livestock. Tree leaves and grasses could be used as a feed complement and they provide upper limit dietary profit (Bhatta et al., 2005). Fodder crops cover only 14% total area cultivated in the country and are decreasing with the passage of time as 2% every 10 years due to high demands of cash crop production (Gill, 1998). Forages are main component of feed for dairy cows on the grounds that they give coarse fiber to improve rumen capacity and they must be supplemented with other ingredients. Forages have been generally examined for CP and fiber fixations because of contribution in the formulation of feed (Masahito and Mike, 2005). Grasses, foliage of trees, shrubs and water plants are major components of ruminants feed (Wanapat et al., 2008). There are several alternatives for improving the performance of ruminants by providing low quality basal diets. One of the alternatives is to use trees and shrubs which are high in protein content but have moderate to high digestibility (Egan, 1997). Recently, in many tropical countries and regions, there has been a focus on identifying and using locally available shrubs and tree leaves as a source of feeds or feed ingredients for ruminants because of their high nutritive value and positive effects on rumen function (Omar et al., 1999; Yao *et al*., 2000). However, due to its high protein content with which quality is comparable or even superior to that of soybean meal (Nguyen Xuan Ba *et al*., 2005).

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In Pakistan green leaves of trees and some grasses are fed to the large and small ruminants in extreme climate during fodder scarcity. In those areas where limited source of fodder is available for the animals, tree leaves are provided to the animals for their nutrition (Reddy, 2006). In small ruminant protein and energy sources are obtained from tree leaves (Singh et al., 1989). Diets supplemented with these two mulberry products in an isocaloric and isonitrogenous manner have similar effects to corn grain and cotton seed meals on steer performance, blood biochemical parameters and carcass characteristics (Zhou et al 2014). Fodder tree leaves are important components of sheep and goat feed these are also provided during scarcity period because they contain proteins, vitamins and minerals (Kamalak et al., 2004). Plants can be easy and cheap source of energy for ruminants as they maintain higher total sugar content that improves the growth animals (Areghore and Hunter., 1999 and Ahmad et al., 2008).

Nutritional deficiencies in ruminants mostly occur by anti-nutritional factors such as tannins and other secondary compounds. Fodder tree legumes and grasses have high protein level, so deficiencies occur in ruminants due to anti nutritional factors such as tannins and other secondary compound that can be minimized by using as feed supplement in ruminants (EL-Waziry, 2007). Plants having anti-nutritional factor as tannins or phenol compounds can be utilized in rumen of goat by certain bacteria such as *Streptococcus caprinus*, *Selenomonas ruminantium*, *Prevotella ruminicola*, *Butyrivibrio* spp., *Lactobacillus* spp. and *Enterobacteriacae* spp. These bacteria can utilize both condensed and utilizable tannin (Pell et al., 2001). There is an imperative need for the development of rapid screening technique and proper methods to study digestibility of different forages. Keeping in view, the situation of livestock, nutrient availability and importance of different forages, in Sargodha district.

Present study was planned for the evaluation of *in vitro* digestibility estimation of different nine fodder species available in Sargodha district of Pakistan and to explore their nutritive value. The information gained would be useful in the estimation of nutritive value and digestibility of different forages in this area.

MATERIAL AND METHODS

Sampling of forage species

Based on data taken from the farmers through the questionnaire, sample of commonly available forages were taken and identified on the basis of their botanical names Table 1. One kg sample of each species was collected from two different sites of each tehsil. Composite samples were prepared and packed as sooner in polythene bag. These composite samples were taken in the laboratory of University College of agriculture, University of Sargodha. The schedule of sample collection is shown in Table 2. The samples of forages were analyzed for proximate analysis.

Chemical analysis

Chemical analysis of selected forages was noted by taking randomly, 250g sample from each bag and then it was dried in hot air oven at 65-70 ^{o}C and was stored for further analysis. The oven dried samples were ground through 1mm sieve and were analyzed for DM, CP, NDF, ADF and ASH (AOAC 1990; Van Soest et al., 1991).

Moisture and Dry matter estimation

Moisture in fecal samples was determined by drying the known quantity (W_1) of sample at 105^{o}C in a hot air oven for 24 hours. After 24 hours, samples were transferred directly from oven to desiccators for 5-10 minutes to cool them down to and then weighed again W_2. Moisture contents in samples were calculated using following formula:

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where

W_1 indicates weight of sample before drying and W_2 stands for weight of sample after drying. Dry matter was determined by using the formula: DM (%) = 100 – Moisture contents

Crude protein determination

Total nitrogen in a sample was determined by Kjeldhal method. A known amount of the oven dried sample (W_1) was taken in along Kjaldhal flask. Five grams of a catalyst mixture $CuSO_4$ (9:1) and 25 ml of concentrated H_2SO_4 was added the sample and boiled in a digestion rack initially at low temperature and then at vigorous boiling till the content became clear. After cooling the contents of the flask, distilled water was added in a 250 ml volumetric flask for dilution. A 10 ml of this solution was transferred to micro Kjaldhal distillation apparatus and distilled in a presence of 50 mg of zinc dust and 40 % NaOH solution. The ammonia so produced was collected in beaker containing 2 % boric acid solution having two drop of methyl red as an indicator. The distillate was titrated

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against standard 0.1 NH_2SO_4 solution to light pink indicate the percentage of nitrogen was calculated according to the following formula.

% CP = {(Vol. of 0.1N H_2SO_4 used × Dilution of sample solution × 0.0014 × 6.25) / (Weight of sample × sample solution used)} × 100.

Ash determination

A known amount of samples (W_1) was taken in a crucible. It was heated on an oxidizing flame till no smoke was given out. The crucible was then placed in muffle furnace at about 600 ^oC till all the oxidized matter was recorded. The percentage of ash was calculated by the following formula.

$$\text{Ash \%} = \frac{\text{weight of ash } (W_1)}{\text{weight of sample } (W_2)}$$

W_1= Wt. of crucible including sample weight.

W_2= Wt. of sample after burning.

Neutral detergent in soluble fiber determination

One gram of dry, well-ground homogenized feed sample was taken and passed through 1mm mesh in 500 ml capacity flask and added 0.5 gm of sodium sulphite. The solution of 100 ml ND solution was added across the wall of cylinder for the prevention of soap forming. Rapidly it was brought to boiling temperature and boiled gently for an hour and watch glass was placed on the flask for condensing purpose. Then the flask was removed, cooled and filtered through suction assembly. Residue was washed with hot water (85-95 ^oC) to remove ND solution and then washed with acetone (20 ml) and shifted the residue in crucible and constant weight was taken by putting at 105 ^oC repeatedly.

NDF = (weight of crucible + cell wall contents) - weight of crucible × 100/weight of dry sample.

Acid detergent fiber determination

Approximately 1 gram of sample was weighed into a beaker or container suitable for reflux by difference. Acid detergent solution (room temperature) of 100 ml cold and 2ml deca-hydro-nephthlene were added and heated for boiling in 5 to 10 minutes. Then heat was reduced to avoid foaming as boiling had begun. Reflux 60 minutes from onset of boiling and adjusting boiling to as low even level. It was filtered on a previously weighed crucible, that was set on the filtering apparatus using light suction and broke up the filter material with a rod and washed twice with hot water (90-100^oC) crucible in the same manner. It was washed with acetone and repeated until that showed no more colour and then was broke up all the lamps so that come into contacts with all particles of fiber. Then hexane was washed and it was added while crucible still contained some acetone (hexane 8 was omitted because of lumping problem). Acid detergent fiber free of hexane was sucked and dried at 105^oCovernight. Then it was cooled in desiccators at room temperature and weighed.

$$\text{ADF} = \frac{\text{(Wt. of crucible+ fiber- empty wt. of crucible)}}{\text{Wt of sample on dry matter bases}}$$

In-Vitro Dry matter Digestibility

The forage samples were analyzed for *in-vitro* DM digestibility according to the method as described by Tilley and Terry (1963). The fresh rumen contents were brought from local slaughter house in insulated bottles and transported immediately to the experimental site. The rumen contents were squeezed through four layer of cheese cloth kept in water bath having temperature 39 ^oC until incubation take place. Representative samples of the mixtures (2.5g DM) was taken in a separate bottle having 0.05 liters rumen liquor 0.2 liters buffer solution (Buffer solution: KCl 0.57 g/L, $MgSO_4$. $7H_2O$ 0.12 g/L, NaCl 0.47 g/L, $CaCl_2$ 0.04 g/L, $Na_2HPO_4.12H_2O$ 9.30 g/L, $NaHCO_3$ 9.80 g/L, Cysteine 0.25 g/L (Elmenofy *et al*., 2012, Tilley and Terry, 1963). The bottles were kept in water bath having fix temperature 39^oC degree. The samples were run for *in-vitro* DM digestibility at 6, 12, 24 and 36 hours of incubation. The in *in-vitro* DM digestibility was determined by using following formula.

$$\text{In-vitro DM digestibility \%} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}}$$

Statistical analysis

The data recorded was subjected to statistical analysis using analysis of variance under CRD. The difference among treatments was studied as described by Steel et al. (1997).

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Table 1 - Botanical names of different experimental forages.

Sr No	Forage names	*Botanical names*
1	Bermuda grass	*Cynodon dactylon*
2	Mott grass	*Penisetum perpureum*
3	Rhode grass	*Chloris gayana*
4	Kikar	*Acacia nilotica*
5	Mulberry	*Morus alba*
6	Ber	*Ziziphus jujube*
7	Shirin	*Albezia procera*
8	Sorghum	*Sorghum bicolor*
9	Alfalfa	*Medicago sativa*

Table 2 - Sample collection schedule from different tehsils of Sargodha district.

Sr No	Location	Date	Type
1	Sahiwal	15-03-2014	Forages
2	Kotmomin	17-03-2014	Forages
3	Bhera	19-03-2014	Forages
4	Bhalwal	21-03-2014	Forages
5	Silanwali	23-03-2014	Forages
6	Sargodha	25-03-2014	Forages
7	Shahpur	27-03-2014	Forages

RESULTS AND DISCUSSION

Dry matter

Results of present study showed that DM content in Albezia procera was the highest (P<0.05; 44.23%) and lowest for Penisetum perpureum (17.50%; Table 3). Results of this study are in line with the finding of (Datt et al., 2008) who reported that higher DM contents of Albezia procera as compared to other forage species ranging from 43.14 to 47%. The highest DM content of Albezia procera might be attributed to higher level of cell wall component and lower level of moisture contents (Weinberg and Muck, 1996) Results of present study are not in line with the finding of Borreani et al. (2007) who found higher (19.35%) DM contents in Cynodon dactylon and lower (17.02%) in Cenchrus ciliaris from spring to summer. This might be attributed to unhealthy leaves and absence of flowers in this season.

Results of the current study indicated that CP value of the *Morus alba* was the highest (P<0.05; 22.56%) while that of *Sorghum bicolor* was the lowest (5.60%; Table 3). Results of this study are same with the finding of Cheema et al. (2011); Kandylis et al. (2009) and Shayo. (1997) who reported that CP value of *Morus alba* were more as compared to others forages ranging from 18.6 to 23%. Higher CP level of *Morus alba* than other forages might be due to more accumulation of protein content in them during growth. Yulistiani et al. (2015) reported that Supplementation of mulberry to TRS-based diet at 1.2% BW or at 32% of total diet had similar effect to urea rice bran supplementation on the DMI, nutrient digestibility and N utilization that create efficient of rumen ecosystem and microbial protein supply It is reported that *Morus alba* leaves have an appreciable potential as protein source in small ruminant feeding and concluded that leaves of *Morus alba* can be used as main feed in small ruminants (Yao et al., 2000). However, present study differs with Omar et al. (1999) who reported that CP content in mulberry leaves was 15.9%. Hirano (1982) and Mandal (1997) found that CP value in mulberry leaves was lower; it might be attributed to differences in localities. Alam and Djajanigra (1994) reported that rumen degradation is affected if the level of CP in feed is less than 10 %.

Neutral detergent fiber and Acid detergent fiber

Results of the this study indicated that NDF and ADF contents of the *Penisetum perpureum* g rass were 74.56 and 45.43%, respectively while NDF contents was the lowest (31.56%) in *Ziziphus jujube* and ADF contents was minimum (26.33%) in *Sorghum bicolor.* Results of current study are in line with the finding of Sarwar and Nisa, (1999) and Touqir et al. (2009) who reported that NDF and ADF value of *Penisetum perpureum* were 70.6 and 62.0% and 40.8 and 32.4%, respectively. The result might be due to increase in amount of fiber component because of other cell content having carbon skeleton is converted into fiber component and lignin content increase (Ruiz et al., 1992; Smith et al*.,* 1972). Higher in NDF concentration have negative effect on the performance of animals that can reduce intake of energy (Zinn and Ware, 2007). Lower values of ADF in these forages showed higher potential ruminant feed (Bakshi & Wadhwa, 2007). Results of this study differ with the finding of Liu *et al.* (2002) who reported that leaves contain high protein and low fiber as compared to stem and trunk.

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Table 3 - Chemical composition of different forages

Items (%)	*Cynodon dactylon*[1]	*Penisetum perpureum*[2]	*Chloris gayana*[3]	*Acacia nilotica*[4]	*Morus alba*[5]	*Ziziphus jujube*[6]	*Albezia procera*[1]	*Sorghum bicolor*[8]	*Medicago sativa*[9]	S.E.M[2]
Dry matter	30.10^{d}	17.50^{f}	40.04^{b}	33.50^{c}	28.84^{b}	33.50^{a}	44.23^{a}	17.61^{f}	20.77^{e}	0.32
Crude protein	12.43^{de}	11.46^{e}	9.63^{f}	11.40^{e}	22.56^{a}	13.76^{c}	13.26^{cd}	5.60^{g}	16.00^{b}	0.21
Neutral detergent fiber	65.60^{b}	74.56^{a}	59.26^{c}	23.13^{f}	23.66^{f}	31.56^{e}	45.63^{d}	44.53^{d}	32.43^{e}	0.28
Acid detergent fiber	29.03^{c}	45.43^{a}	38.53^{b}	14.46^{e}	14.53^{e}	15.43^{e}	30.50^{c}	26.33^{d}	27.13^{d}	0.29
Ash	5.46^{fg}	11.50^{a}	8.70^{b}	5.80^{ef}	8.43^{bc}	7.56^{cd}	7.33^{d}	4.56^{g}	6.70^{de}	0.22

^{abcdefg} Means on the same rows with different superscripts are significantly different($p<0.05$) [1]BermudaGrass,[2]Mott grass, [3]Rhode grass, [4]Kikar[5]Mulberry, [6]Ber, [7] Sirin,[8]Sorghum,[9]alfalfa; SEM= stand for Standard error mean

Table 4 - *In vitro* Dry matter digestibility of different forages

Items (%)	*Cynodon dactylon*[1]	*Penisetum perpureum*[2]	*Chloris gayana*[3]	*Acacia nilotica*[4]	*Morus alba*[5]	*Ziziphus jujube*[6]	*Albezia procera*[1]	*Sorghum bicolor*[8]	*Medicago sativa*[9]	S.E.M
After 6 (h)	23.33e	20.10^{f}	25.43^{d}	31.46^{b}	35.00^{a}	23.40^{e}	25.63^{d}	29.53^{c}	29.80^{c}	0.14
After 12 (h)	32.53$_{cd}$	31.60$_{de}$	28.80^{f}	34.10$_{bc}$	43.40^{a}	30.63^{e}	34.70^{b}	31.56$_{de}$	34.20$_{bc}$	0.48
After 24 (h)	41.70^{e}	50.53^{b}	32.30^{h}	41.50^{e}	68.29^{a}	33.76^{g}	48.53^{c}	38.20^{f}	42.50^{d}	0.11
After 36 (h)	53.13^{c}	53.16^{c}	39.46^{d}	53.36^{c}	78.26^{a}	59.80^{b}	51.96^{c}	52.36$_{ab}$	51.60^{c}	0.55

^{abcdefg} Means on the same rows with different superscripts are significantly different($p<0.05$) [1]BermudaGrass,[2]Mott grass, [3]Rhode grass, [4]Kikar[5]Mulberry,[6]Ber.[7] Sirin,[8]Sorghum,[9]alfalfa; SEM= stand for Standard error mean; h: hour

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Ash

Results of the current study indicated that ash content of *Penisetum perpureum* was the highest (P<0.05; 11.50%) and the lowest for *Sorghum bicolor* (4.56%; Table 3). Results of this study are same with the finding of Bilal (2008); Touqir et al. (2007). Arshadullah et al. (2009) who reported higher ash contents of *Penisetum perpureum* as compared to other forages and were in range from 9.60% to 12.40%. The ash content in forage may be derived from two sources i.e. internal plant source and external source such as soil. Total ash content of fodder trees, shrubs and climbers was high. The average result (8.03 %) of current study is not similar with the finding of Manzoor, (2013) who reported that average ash content was 13.70%.

In vitro dry matter digestibility

Results of the current study indicate that IVDMD at 36 hours of incubation was the highest (P<0.05; 78.26%) for *Morus alba* while that of *Chloris gayana* was the lowest (39.46% Table 4). Results of this study are similar with the finding of Bakshi and Wadhwa, (2007) Omar et al. (1998); who reported that IVDMD of *Morus alba* leaves was higher than other forage species. Shayo (1997) reported IVDMD of *Morus alba*leaves was 82.1%. This might be attributed to high CP level and increased concentration of ammonia nitrogen in rumen (Hristov et al., 2004). Higher ammonia nitrogen in rumen improves microbial activity and growth of fibrolytic bacteria resulting in more DM digestibility Griswold et al. (2003). In-*vivo* digestibility and in-*vitro* of mulberry leaves was 78.4-80.8% and was 80.2-95.0% respectively (Sanchez, 2000). Maximum DM digestibility of *Morus alba* was noted due to more in CP level and less in NDF level Wiedmeier et al. (1983). Results of present study are not in line with the finding of Cheema et al. (2011) who found higher (92%) digestibility of fresh *Mous alba* leaves. This might be due to *in-situ* digestion trial on buffalo bull with fresh mulberry leaves. Dry *Morus alba* leaves had less CP value which may affect DM digestibility (Playne, 1978; Kawashima et al. 2007).

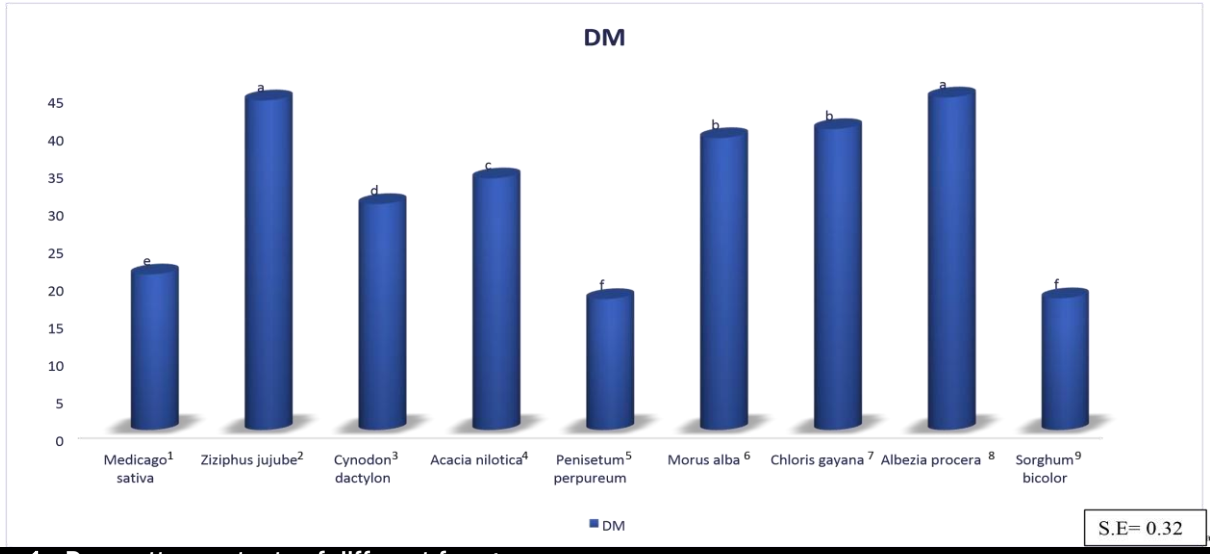

Figure 1 - Dry matter contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

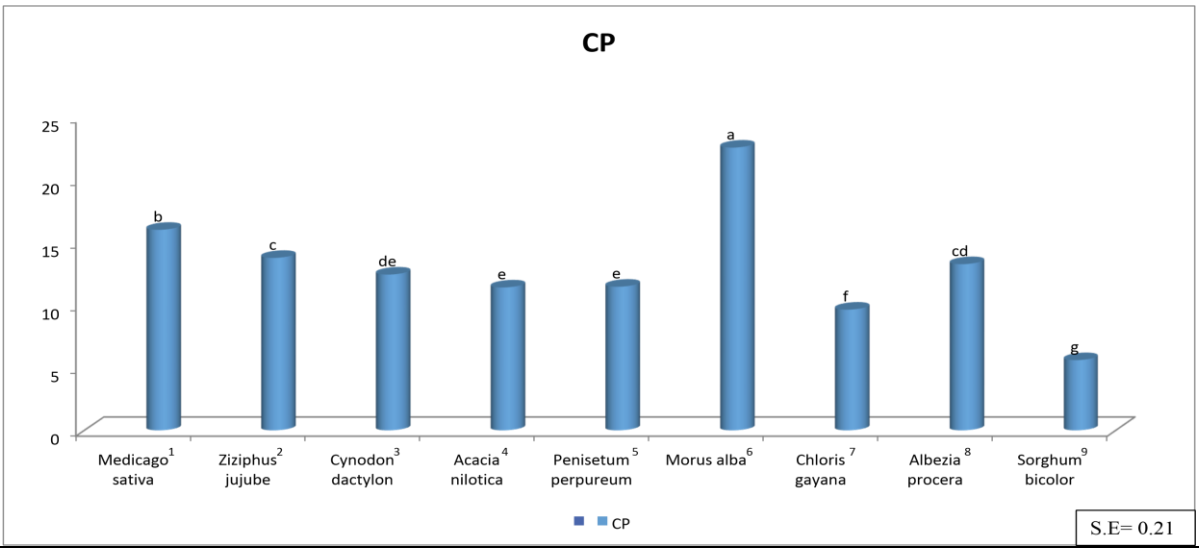

Figure 2 - Crude protein contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

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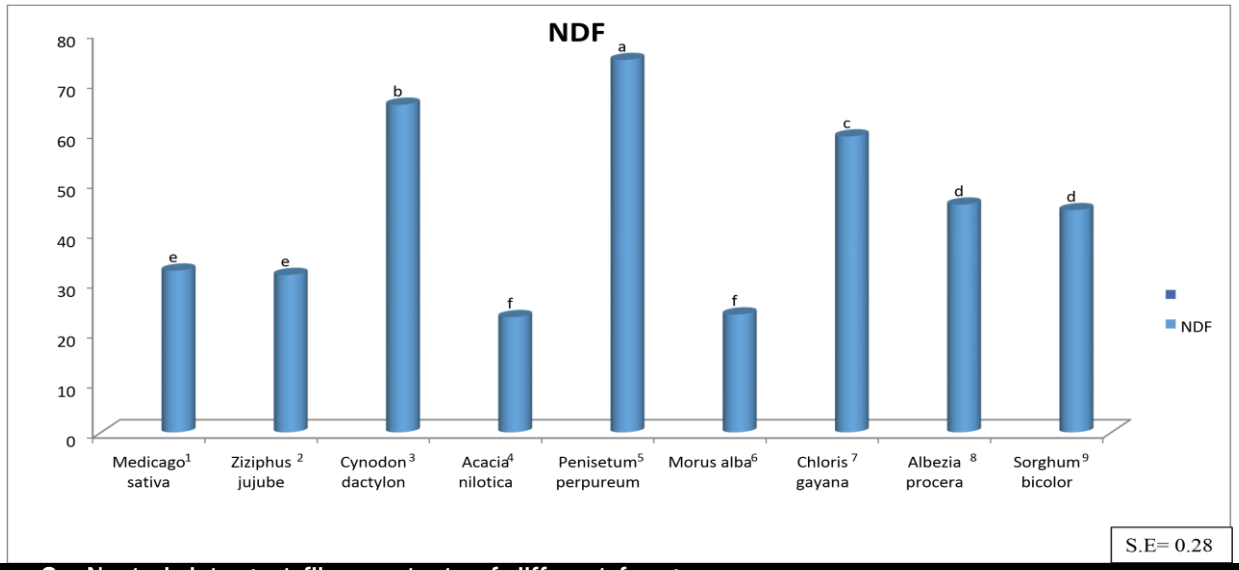

Figure 3 - Neutral detergent fiber contents of different forages (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

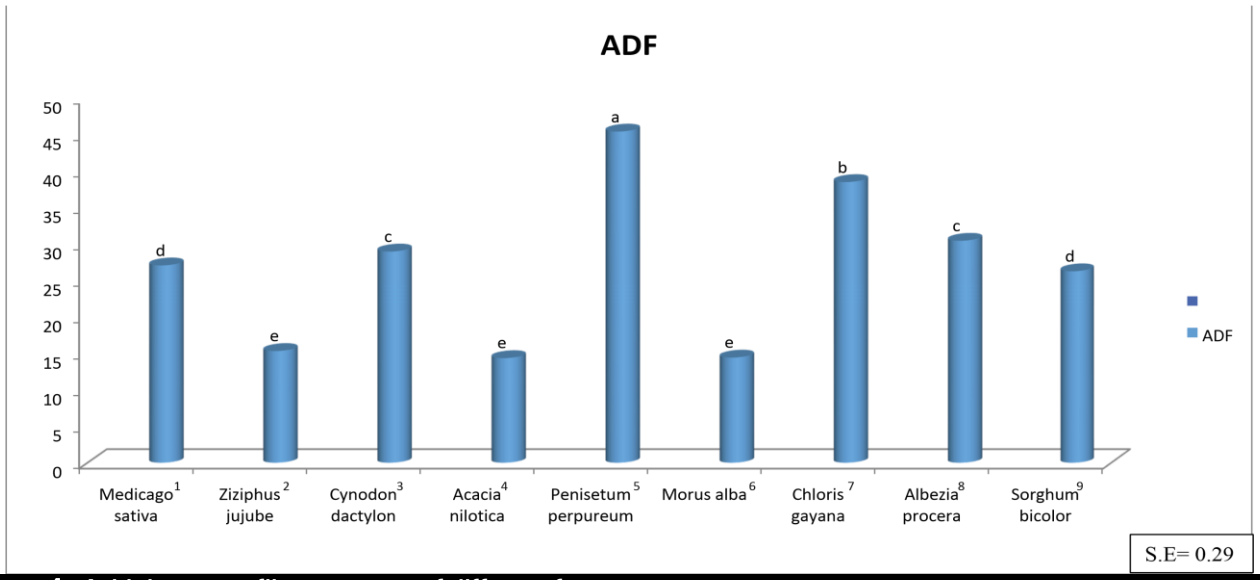

Figure 4 - Acid detergent fiber contents of different forages (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

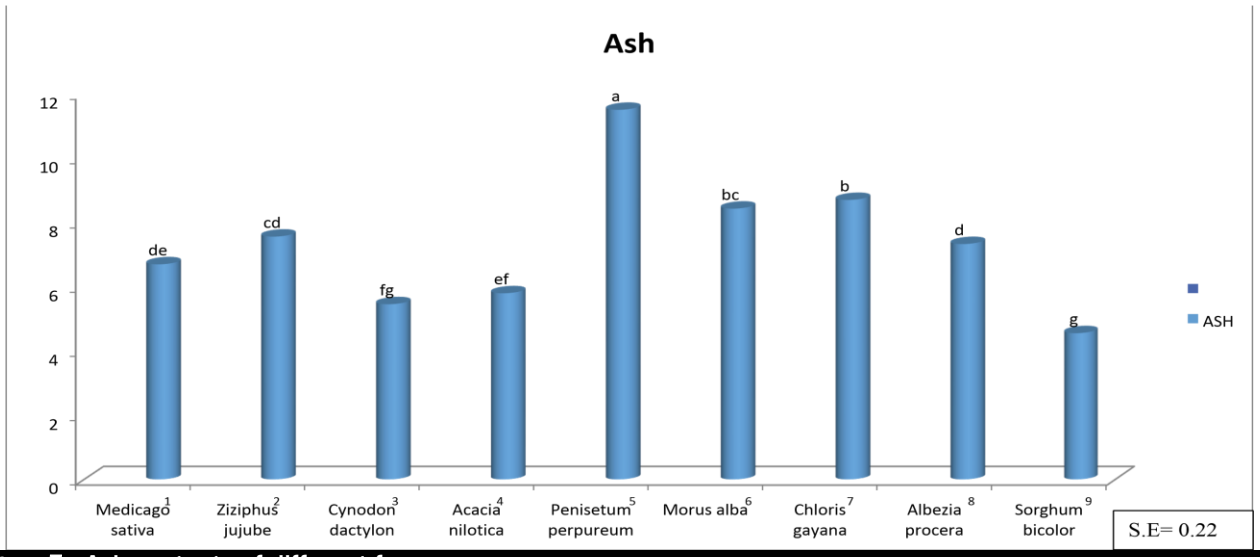

Figure 5 - Ash contents of different forages (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

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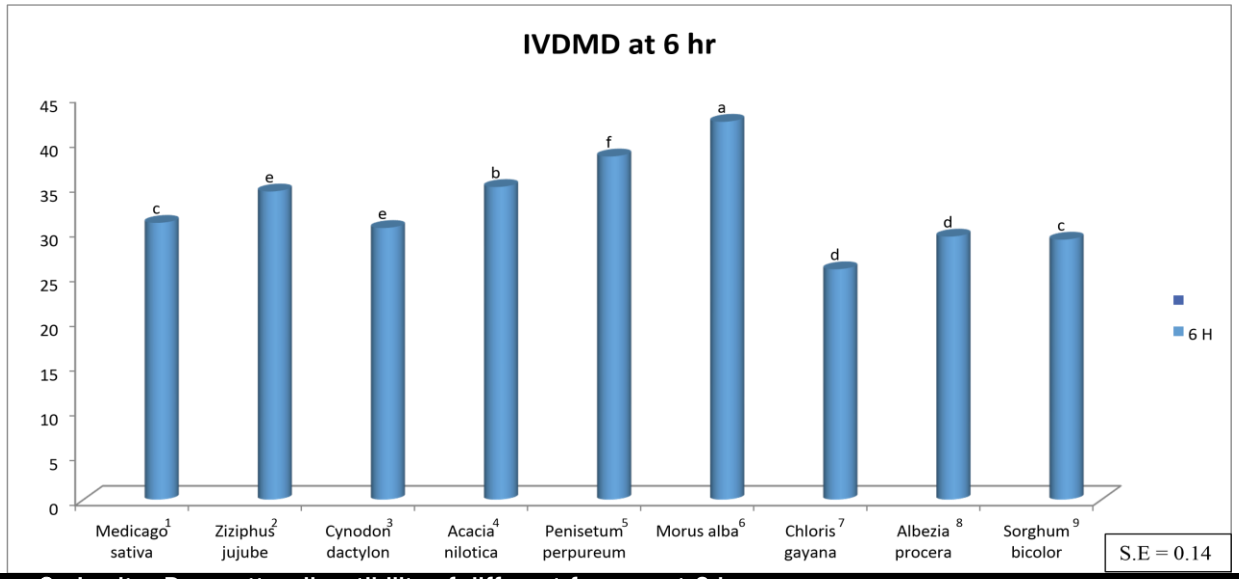

Figure 6 - In vitro Dry matter digestibility of different forages at 6 hour (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

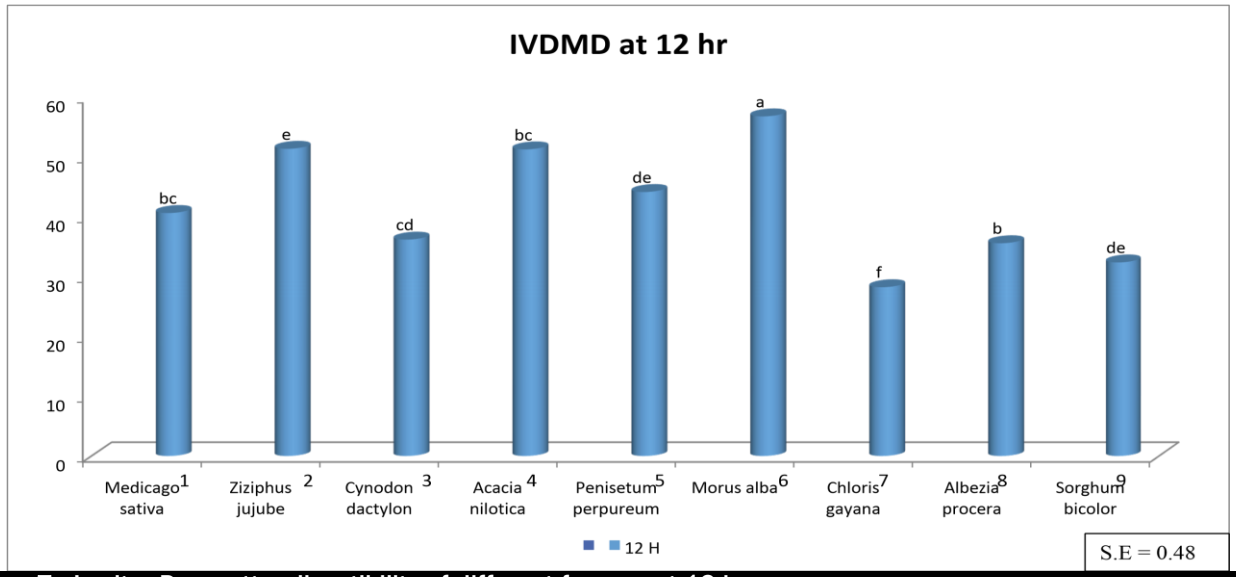

Figure 7 - In vitro Dry matter digestibility of different forages at 12 hour (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

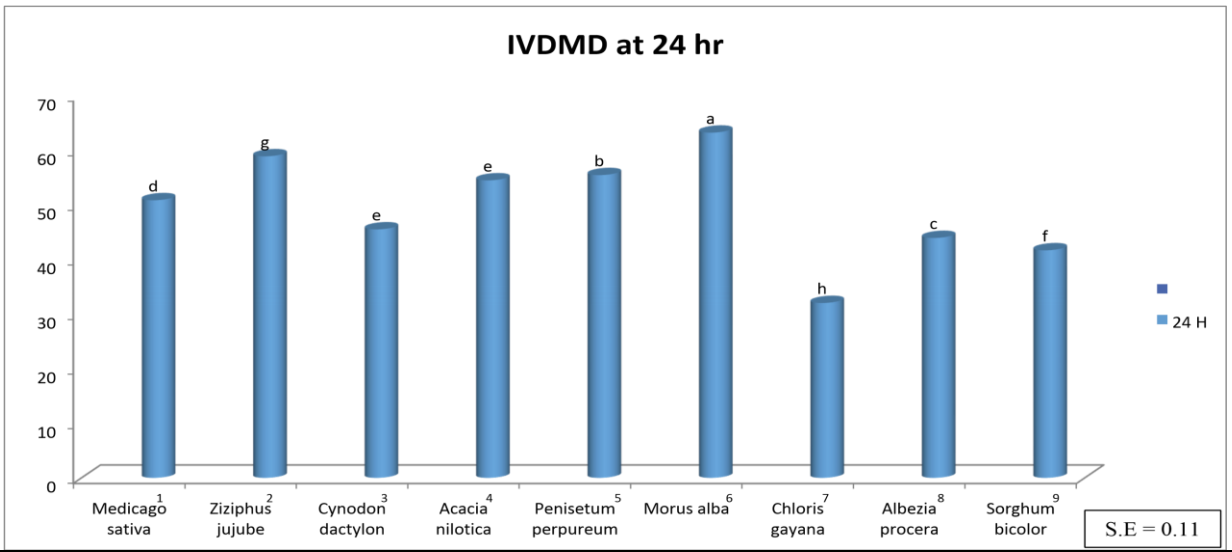

Figure 8 - In vitro Dry matter digestibility of different forages at 24 hour (abcdeg Means on the column with different superscripts are significantly different ($p<0.05$); [1]alfalfa. [2]Ber, [3]Bermuda Grass, [4]Kikar, [5]Mott grass, [6]Mulberry, [7]Rhode grass,[8]Sirin,[9]Sorghum; S.E. stand for Standard error mean; h: hour).

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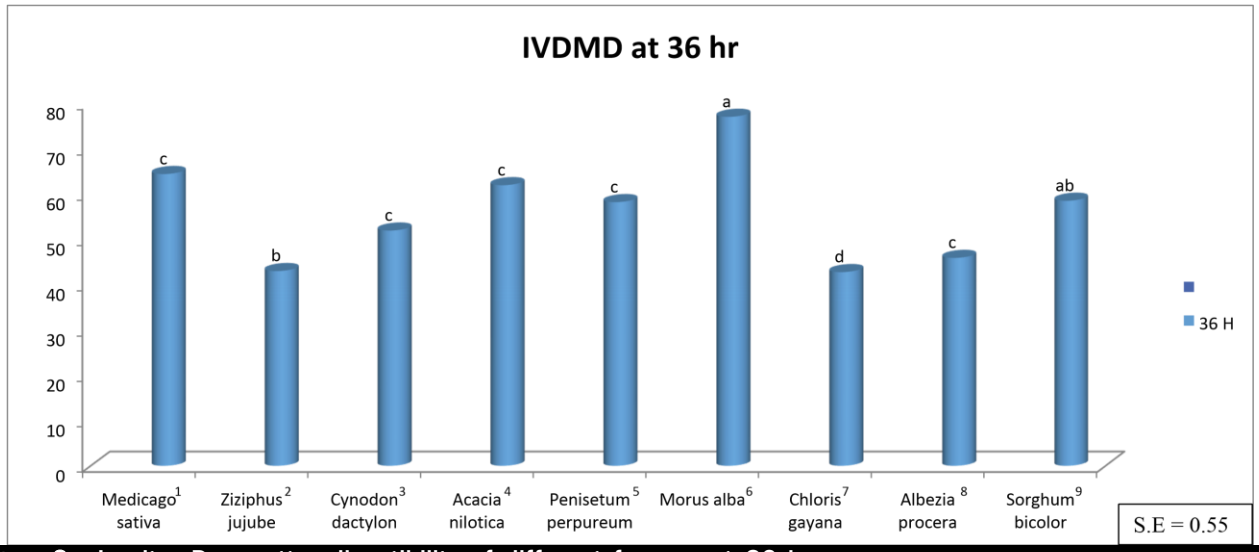

Figure 9 - In vitro Dry matter digestibility of different forages at 36 hour (abcdeg Means on the same rows with different superscripts are significantly different ($p<0.05$); [1]Bermuda Grass, [2]Mott grass, [3]Rhode grass, [4]Kikar, [5]Mulberry, [6]Ber, [7]Sirin, [8]Sorghum, [9]alfalfa; S.E. stand for Standard error mean).

CONCLUSION AND RECOMMENDATIONS

In conclusion, the results of our study recommended that the above mentioned *forage* use as alternative cheap source of nutrient so could be supplemented in ruminant feed due to high nutritive and IVDMD (*In vitro* dry matter digestibility) values.

Competing interests

The authors declare that they have no competing interests exist.

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INTERACTIVE EFFECTS OF STOCKING DENSITY AND FEED TYPE ON GROWTH, SURVIVAL AND CANNIBALISM AMONG AFRICAN CATFISH (*Clarias gariepinus* Burchell 1822)

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ABSTRACT: Food type and stocking density are two major factors influencing aquaculture production. To evaluate their effects on growth, survival rate, and cannibalistic activities among African catfish (*C. gariepinus*) larvae, a 3×3 factorial design was used. Three feed types (*Artemia* nauplii, Zooplankton, and dry feed) and three different stocking densities (10, 20 and 40 larvae l^{-1}) were performed throughout a 21 days rearing period (each treatment was triplicated). T_1 Catfish larvae (Artemia nauplii and 10 larvae l^{-1}) and T9 (Dry feed and 40 larvae l^{-1}) showed the highest growth performance parameters and significantly lower growth performance parameters as expressed by final body weight (T_1; 165.03 mg, T_9; 34.36 mg), specific growth rate (T_1; 22.83% day^{-1}, T9; 14.83% day^{-1}). Meanwhile, the survival rate percentage was the lowest (29.37%) and the highest (82.37%); in T4 (Zooplankton and 10 larvae l^{-1}) and T_9 (Dry feed and 40 larvae l^{-1}) respectively. Additionally, higher stocking densities of catfish larvae had expressed higher rates of cannibalism when compared to the lower stocking densities. The lowest cannibalism rate (3.46%) was recorded for T_1 (Artemia nauplii and 10 larvae l^{-1}) by the end of the experiment. Despite the absence of significant interaction effect between stocking density and feed on rearing performance of *C. gariepinus larvae,* results of the current study indicated successful rearing and well performance of catfish larvae concerning growth performance, cannibalism and survival rates at lower stocking density. The density of 10 larvae l^{-1} was the maximum threshold capacity for *C. gariepinus* larval best growth when fed on either *Artemia* or zooplankton. However, further investigations are required to explore the effect of using other dry feed types in the rearing phase of African catfish larvae.

Key Words: African catfish (*C. gariepinus*), Larval Rearing, Food Type, Stocking Density, Cannibalism, Survival Rate

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INTRODUCTION

Ration and stocking densities are two major elements in aquaculture affecting growth, welfare, and health (Saether and Jobling, 1999; Ellis et al., 2005). Restricted rations are routinely associated with reduced growth rates (Saether and Jobling, 1999; Verbeeten et al., 1999). Meanwhile, fish farmers focus on finding species of fish of high growth rate, adaptive with the environmental conditions, easy to be produced, with suitable prices. Owing to its high environmental tolerance and easily controllable breeding habits, African catfish (*Clarias gariepinus*, Burchell, 1822) was selected as a promising candidate for aquaculture production (FAO, 2015).

However, the Intensive larval production under optimum hatchery managements is still, hampered by egg and larvae qualities and the essential requirements of live food during the post-larvae stage. The changeover from endogenous to exogenous feeding is a critical time for fish larvae so that, availability of food of a suitable size is, therefore, essential for commercial rearing (Gulbrandsen, 1993; Jähnichen and Kohlmann, 1999).

At the onset of exogenous feeding, African catfish larvae require live feeds such as *Artemia* nauplii/cyst, yeast, unicellular algae, rotifers, copepods, cladocerans as the most appropriate starter feeds (Kolkvoski, 2001; Ajepe et al., 2014; Adewumi, 2015). *Artemia* is the most preferred and reliable live food organisms not only in rearing fish and crustacean larvae but also for newly hatched catfish larvae that survive and grow best when raised on a diet of live food notably *Artemia* nauplii (Olurin and Oluwo, 2010). The relationship between food type and density of fish larvae has received attention in recent years as a possible factor influencing growth and survival of larvae both in nature and hatchery managements (Atsi et al., 2009; d'Orbcastel et al., 2009 and Musa et al., 2012). Moreover, cannibalism is a serious problem during larval and early juvenile rearing, particularly under hatchery conditions (Hecht and Appelbaum, 1988). During larval and juvenile rearing of *C. gariepinus* a problematic cannibalism due to a number of environmental factors have been identified; food availability appears to be the

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major factor (Al-Hafedh and Ali, 2004). Another major constraint for successful rearing of this species is the high mortality rate due to cannibalism because of insufficient larval food and an improper feeding regime (Hecht and Appelbaum, 1988; Legendre, 1992; Otéméet., 1996).

Therefore, the negative consequences of feed type, stocking density and the need for profitable production dictate the valuation of optimum feed type and density limits for African catfish (*Clarias gariepinus* larvae).

MATERIALS AND METHODS

Apparently healthy African catfish larvae were sourced from the experimental broodstock that was previously induced to spawn by HCG in the fish breeding and production laboratory belongs to the Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University. Two days after hatching, larvae from two parents were pooled and removed with a tablespoon (Petkam and Moodie, 2001). Larvae were placed in cylindrical plastic bowls (each; 40 cm diameter and 25 cm depth), (Hossain et al., 1998). These bowls were filled with a previously stored tap water in an open tank for at least 24 hrs before being used. Water height in each plastic bowl was similar and fixed at 15 cm. The stagnant water in each bowl was gently aerated with a single air stone. Water quality was maintained by replacing the water in the plastic bowls once per day. Feeding was started 48 hrs post hatch when the yolk sac was completely absorbed.

Experimental design

A completely randomized design (CRD) was employed in this experiment. Catfish larvae were randomly allocated into 10 L of water in the plastic tank. A 3×3 experimental design using three feed types (Artemia nauplii, Zooplankton, and dry feed) and three different stocking densities (10, 20 and 40 larvae l^{-1}) was performed throughout 21 days rearing period where each treatment was triplicated. The larvae in three treatments were fed *Artemia* nauplii in excess twice per day for 21 days. Zooplankton was served and following density superior to 3000 rotifers, cop, clad, $larvae^{-1}$ day^{-1}, supplied to three treatments. Dry feeds based on the liver of beef and yeast dry diet was used in the other three treatments. Larvae were fed twice times a day. Dry diet distributed at 20% of larvae biomass and adjusted every four days after weighting.

Preparation of Experimental Foods

***Artemia* nauplii:** Artemia nauplii cysts (*Artemia salina)* were incubated and hatched under optimal condition according to the manufacturer protocol in 30 ppt saline water for 24 hrs at 30°. Newly hatched Artemia nauplii were separated from the hatching debris by interrupting the air supply in the hatchery vessels. The nauplii were then siphoned out to a fine mesh (100 µm) harvesting box. Newly hatched *Artemia* were then kept in aerated saline water and used within 12 hrs. *Artemia* was washed with tap water and provided to the larvae. Larvae feed in excess (controlling distribution of live food 30-40 minute after distribution).

Zooplankton production: Natural zooplankton (Mainly; rotifer, *Brachions spp.*, copepods *Termocyclops spp.*, cladocerans, *Daphnia spp.*, *Monia sp*., and *Ceriodaphnia spp.*) was produced in a rectangular tank (2x2x1 m^3) with sac fertilization methods. Chicken droppings were used to fertilize water and each tank was inoculated with 25 L water obtained from a pond using plankton nets (50 µm). Natural zooplankton was harvested from the concert tank with sieve net 50-100 µm after 5-7 days of culture and thoroughly washed with tap water prior to feeding. Zooplankton constituted for rotifer 65 % copepods (28 %) and cladoceran (water flea) 7% collected daily and treated with formalin 50 ppm for 1-2 minutes to eliminate any pathogen and still alive. The zooplankton was served following a density superior of 3000 (rotifer, copepods, cladoceran) $larvae^{-1}$ day^{-1}, this number of zooplankton was daily assessed after fixating in 0.5 ml of formalin 4 % in 5 ml filtrate observation microscope and counting in one ml filtrate feed granule particle size 200 to 300 µm were fed.

Dry feeds: Dry feed based on beef liver and yeast was used in order to improve the survival and growth rates (Hung et al., 2002). The proximate analysis of dry feeds is summarized in Table 1. Larvae were fed twice per day. Dry diet distributed at 20 % of larvae biomass and adjusted every four days after weighting.

Water quality: The water quality within the plastic tanks was maintained as it was cleaned daily and water totally replaced prior to the first feeding schedule in the morning. The temperature was monitored twice daily with a centigrade thermometer at 08.00 and 15.00 hours (Table 3). Dissolved oxygen was measured every 4 days (Fermin and Bolivar, 1991) using a digital oxygen meter *(Oxyguard handy III, Oxyguard international, Birkerod, Denmark),* pH using a hanna pH meter. Ammonia nitrogen (TAN), nitrate-nitrogen, nitrite-nitrogen, organic matter and total hardness were analyzed using analytical kits *(HACH Company, Loveland, co 80539 USA).*

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Table 1 - Ingredients composition of the dry feed.

Ingredients	Percentage	Proximate on dry basis	Percentage
Yeast powdered (protibel)	50	Moister	7.8
Beef liver	35	Crude protein	35.7
Soybean oil	5	Crude lipids	10.9
Vitamin mix	5	Ash	11.7
Mineral mix	5	Fiber	6.8

Growth measurements

Body weight developments (BW, mg larva $^{-1}$): The mean of initial weights of the stock in each plastic bowl was measured using Mettler electronic top-loading balance of four digits. At the end of the experiment, feeding was stopped; larvae were starved for 24 hrs before they were removed for weighing and measurement (Petkam and Moodie, 2001). Ten larvae were randomly sampled from each plastic bowl. They were placed on paper towel to absorb water and weighted for each replicate as a patch to the nearest 0.1 mg.

Specific growth rate (SGR, % day^{-1}):

$$SGR\ \% = \frac{\text{Ln wf} - \text{Ln wi}}{\text{time days}} \times 100$$

Where;
w_i= initial weight (mg) of larvae
w_f = final weight (mg) larvae
Ln = natural logarithmic
W= weight of larvae (mg).

Body length (BL, mm larva $^{-1}$): The mean of initial and final total lengths of the stock in each plastic bowl was measured using a stereo microscope. Ten larvae from each plastic bowl were preserved in 5% formalin to reduce the shrinkage of total length 24h after preservation (Petkam and Moodie, 2001).

Survival rate percentage (SR, %): Survival rates during the experiment were estimated from daily mortality. Dead larvae were removed twice daily at 8 am and 8 pm hrs. Final survival rates were determined by the number of alive at the end of each treatment.

$$SR\ \% = \frac{Ni}{No} * 100$$

Where;
Ni= number of larvae alive at the end of the experiment.
No= number of larvae stocked at the beginning of the experiment
SR % = percentage of survival.

Cannibalism rate percentage (CR, %): CR % = 100 – {survival rate (%) + observed mortality (%)}

Statistical analysis

Differences in growth rate due to stocking density and food type were determined by GLM according to **SAS (2004)**, taking into account the normality of the data distribution and the homogeneity of variances. Significance was established at $P < 0.05$. Further, the effect of significant interaction between replicate and treatment was performed and non-significant interaction was recorded.

Statistical model: $X_{ij} = \mu + t_i + r_j + ti*r_j + e_{ij}$
X_{ij} : Growth parameters and production parameters.
μ: Population mean.
t_i: Treatment effect of feed types and stocking density.
r_j: Replicate effects.
e_{ij}: experimental error.

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RESULTS

Body weight (BW, mg larvae $^{-1}$)

Table 2 depicts body weight (BW) for each feed type (*Artemia* nauplii, Zooplankton, and dry feed) and density (10, 20 and 40 larvae l^{-1}). Average body weight at the start of the experiment did not differ significantly ($P > 0.05$) and ranged from 1.64 to 1.75 mg. By the end of the experiment; the body weight developments revealed significant ($P < 0.05$) differences among various treatments. The highest body weight (165.03 mg) was observed in the *Artemia* nauplii fed larvae maintained under low stocking density (T_1) followed by T4 (121.85 mg) zooplankton fed larvae. Meanwhile, the body weight of the larvae fed dry diet was not significantly different density (10, 20 and 40 larvae l^{-1}) treated groups at the end of the experiment.

Body weight of the larvae under high stocking density of T_3 (94.36 mg) and T6 (94.27 mg) were significantly ($P < 0.05$) lower than those of other treatments fed on the same type of food. The lowest body weight (34.36 mg) was recorded for T9 (Dry feed and 40 larvae l^{-1}). Regarding feed type; significant ($P < 0.05$) differences were observed among different groups with different diets. Zooplankton fed larvae showed significant ($P < 0.05$) lower body weight than *Artemia* feed larvae. Meanwhile, groups fed on live food showed significant ($P < 0.05$) higher body weight than dry diets fed larvae.

Specific growth rate (SGR % day^{-1})

As shown in Table 2 the average specific growth rate (SGR) of African catfish *(C. gariepinus)* larvae demonstrated a significant difference ($p < 0.05$) among different experimental groups. The low density results in the highest SGR (22.83% day^{-1}) among larvae of African catfish namely *Artemia* fed larvae T_1 (*Artemia* nauplii and 10 larvae l^{-1}). Whereas, the lowest SGRs (16.38% day^{-1}), (14.73% day^{-1}) and (14.83% day^{-1}) were observed in the dry diet fed catfish larvae; T_7 (Dry feed and 10 larvae l^{-1}), T_8 (Dry feed and 20 larvae l^{-1}) and T_9 (Dry feed and 40 larvae l^{-1}) respectively.

Body length (BL mm)

Average body length of larvae at the start of the experiment ranged from 5.63 to 5.81 mm Table 2. By the end of the experiment, the body length of T_1 (*Artemia* nauplii and 10 larvae l^{-1}) was significantly ($p < 0.05$) larger (24.54 mm) than the other treated larvae groups. The averaged body length of the dry fed larvae was significantly ($p < 0.05$) lower than live food fed larvae.

Survival rate

Table 2 illustrates the effects of stocking density and food types on survival rates of *C. gariepinus* larvae. Statistical analysis of the present data indicated non-significant differences among the different groups at the start of the experiment. By the end of the experimental period; the survival rates observed were significantly ($P < 0.05$) different among respective treatments. The dry diet fed larvae kept under high stocking density (T_9: 40 larvae^{-1}) had the lowest survival rate (29.37 %). In the same trend, *Artemia* and zooplankton fed larvae maintained under high stocking density (T3 and T6 respectively) showed lower survival rates when compared to those fed on the same food type at low and medium density. On the contrary, low density resulted in high survival rates (82.37 %) and 81.79 % in zooplankton (T4: 10 larvae^{-1}) and *Artemia* (T_1: 10 larvae^{-1}) fed larvae respectively.

Cannibalism rate (%)

Results for the effects of stocking density and food types on cannibalism of *C. gariepinus* larvae are shown in Table 3 and Figure 1. Cannibalism rates were very low in the first five days of the experiment. By larval aging, the rate of cannibalism increased among all treated groups. Additionally, cannibalism was significantly high ($P < 0.05$) at high stocking density in all treatments (33.45 %, 27.45 % and 13.26 % for T6, T9, and T3; respectively). Meanwhile, cannibalism was significantly low ($P < 0.05$) in all *Artemia* fed treated larvae together with a significant increase in the rate of cannibalism in zooplankton and dry food fed larvae.

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Table 2 - LSM ± SD of Body weight, Specific growth rate, Body length , and Survival rate of African catfish *(C. gariepinus)* larvae (% day $^{-1}$) during 21 days culture as influenced by different stocking densities and feed types (T_1 to T_9).

Treatment	Stoking Density (Laravae l^{-1})	Food Type	Initial Body Weight (mg larva^{-1})	Final Body Weight (mg larva^{-1})	Specific Growth Rate (% day^{-1})	Initial Body Length (mm larva^{-1})	Final Body Length (mm larva^{-1})	Survival Rate %
T_1	10	Artemia	1.74^{a}±0.34	165.03^{a}±23.50	22.83^{a}±0.76	5.81^{a}±0.48	24.54^{a}±0.84	81.79^{a}±3.53
T_2	20		1.71^{a}±0.35	127.16^{b}±27.74	21.54^{b}±0.68	5.76^{a}±0.54	21.91^{bc}±1.49	74.97^{ab}±0.27
T_3	40		1.64^{a}±0.39	94.36^{c}±27.06	20.18^{d}±0.44	5.80^{a}±0.47	20.50^{c}±2.05	52.66^{c}±0.52
T_4	10	Zooplankton	1.71^{a}±0.35	121.85^{b}±28.77	21.30^{bc}±0.53	5.64^{a}±0.42	22.8^{b}±1.40	82.37^{a}±2.40
T_5	20		1.75^{a}±0.34	108.75^{bc}±29.95	20.51^{cd}±1.06	5.78^{a}±0.51	21.95^{bc}±2.11	72.31^{ab}±1.41
T_6	40		1.7^{a}±0.33	94.27^{c}±41.48	19.75^{d}±1.25	5.77^{a}±0.50	21.45^{bc}±2.53	38.74^{de}±15.06
T_7	10	Dry feed	1.71^{a}±0.35	46.4^{d}±14.46	16.38^{e}±0.77	5.63^{a}±0.45	18.03^{d}±2.44	68.05^{b}±0.99
T_8	20		1.71^{a}±0.35	34.1^{d}±12.43	14.73^{f}±1.47	5.69^{a}±0.51	15.68^{e}±1.85	48.93^{cd}±1.24
T_9	40		1.69^{a}±0.33	34.36^{d}±14.24	14.81^{f}±1.22	5.76^{a}±0.54	14.63^{e}±1.46	29.37^{e}±2.60

LSM=Least square means; SD= Standard deviation; Means within a column with different superscripts differ significantly (P<0.05).

Table 3 - LSM ± SD of cannibalism rate (%) of African catfish *(C. gariepinus)* larvae during culture periods 0, 5, 9, 13, 17 and 21 days as influenced by different feed types (T_1 to T_9).

Treatment	Stocking Density (Larvae l^{-1})	Food Type	Rearing period (days)						
			0	5	9	13	17	21	Total
T_1	10	Artemia	0	0	0	1.11^{c}±1.57	1.09^{c}±1.53	1.26^{d}±0.34	3.46^{c}±0.37
T_2	20		0	0	0	2.18^{bc}±0.04	2.90^{c}±0.78	3.39^{cd}±0.21	8.47^{bc}±0.53
T_3	40		0	0	2.04^{b}±0.04	3.33^{bc}±1.15	2.85^{c}±0.92	5.04^{bc}±1.13	13.26^{b}±3.16
T_4	10	Zooplankton	0	0	0	2.08^{bc}±0	3.29^{c}±1.51	1.76^{cd}±0.78	7.13^{bc}±0.71
T_5	20		0	0	0	1.59^{bc}±0.76	6.18^{b}±0.70	2.51^{cd}±2.13	10.27^{bc}±0.51
T_6	40		0	0	3.31^{b}±1.82	7.28^{a}±2.88	13.59^{a}±1.71	9.27^{a}±0.38	33.45^{a}±6.79
T_7	10	Dry feed	0	0	0	1.68^{bc}±0.42	2.12^{c}±2.29	1.76^{cd}±0.79	5.56^{c}±0.08
T_8	20		0	0.50^{a}±0.71	3.61^{b}±2.18	1.90^{bc}±0.72	1.47^{c}±0.03	2.63^{cd}±0.54	10.10^{bc}±1.60
T_9	40		0	1.95^{a}±2.75	7.69^{a}±4.31	4.16^{b}±0.15	6.14^{b}±0.66	7.85^{ab}±3.89	27.78^{a}±2.86

LSM= Least square means; SD= Standard deviation; Means within a column with different superscripts differ significantly (P<0.05).

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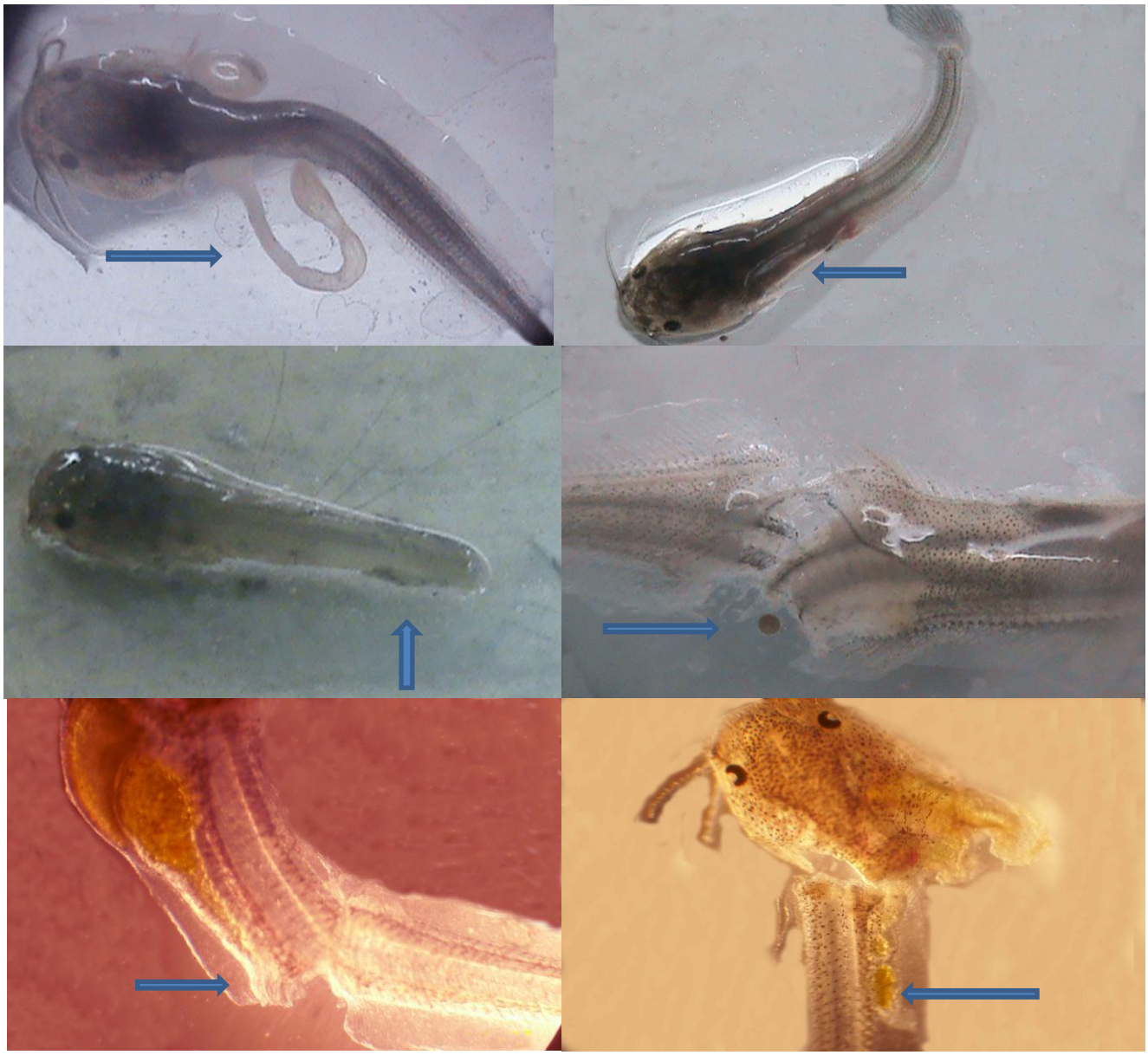

Figure 1 - Cannibalistic behavior among African catfish *(C. gariepinus)* larvae during culture periods (arrows refer to area of cannibalism)

DISCUSSION

Results of the current study indicated density dependent growth of African catfish *Clarias gariepinus* larvae as expressed by the higher growth performance (body weight development, specific growth rate) of the low density treated groups fed different food types. The growth of *C. gariepinus* larvae at 10 larvae l^{-1} density was significantly different to that of *C. gariepinus* larvae stocked at 20, 40 larvae l^{-1}, indicating that a density of 10 larvae l^{-1} was the maximum threshold capacity for *C. gariepinus* larval best growth. Several studies on African catfish larvae have shown negative effects of increasing stocking density, demonstrated by decreased growth performance (Adewumi, 2015; Atse et al., 2009; Haylor, 1991; Hossain et al., 1998; Josiah et al., 2012; Musa et al., 2012).

Individual growth and population density are closely interrelated. Inter-individual contacts, competition for food and stress that are more important in high densities could adversely affect on growth performances (Haylor, 1992 and Barcellos et al., 2004). According to Ruane et al. (2002), high density of larvae in combination with the high food availability might result in a stressful situation, not only from the build- up of metabolites but also from a competitive interaction. Such stressful situation may have adverse impacts on the physiological, health and/or behavioural status of the individual fish involved resulting in reduced energy intake and increased energy utilization, so prolonged activation of the HPI axis is likely to indirectly reduce growth through a negative effect on energy balance (Ellis et al., 2002; Huntingford et al., 2006). Additionally, suppression of growth hormone secretion in

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stressed fish is another expected cause of declined growth (Pickering et al., 1991 and Farbridge and Leatherland, 1992).

It is evident from the present study that; live food organisms were more effectively utilized by *C. gariepinus* larvae than dry artificial diet. Meanwhile, feeding *Artemia* nauplii had significantly improved the growth performance in all *Artemia* fed treated groups. The same conclusion was reported in *C. gariepinus* (Atse et al., 2009; Ngupula et al., 2014; Olurin and Oluwo, 2010; Yakubu et al. 2015), H. longifilis (Kerdchuen and Legendre, 1994 and Atse et al., 2009) and in *Clarias macrocephalus* (Fermin and Bolivar, 1991). The better growth observed in *Artemia* nauplii fed groups may not be attributed to the nutritive and digestibility values of live *Artemia* nauplii only, but also, to the fact that *C. gariepinus* larvae display an innate predatory nature to capture motile live food particles as observed during this experiment. Most of the aforementioned studies related this phenomenon to the presence of enzymes in natural feeds that hasten the digestive processes together with the lack of functional stomach and particularly the absence of pepsin digestion during the first days of exogenous feeding (Cahu and Zambonino, 2001; Verreth et al., 1993).

On the contrary, Adeyemo et al. (1994) reported opposite results for two other catfish, *H. bidorsalis*, and *C. gariepinus*. Larvae fed on M. dubia had higher growth rates than those fed on *Artemia* nauplii. Similarly, Evangelista et al. (2005) found that the growth performance of Catfish larvae fed *Artemia*, Moina and Chironomus did not differ significantly but, those larvae fed on Brachionus and artificial diet had the lowest growth among all treatments. Differences in research findings may due to the nutritional quality of the Moina, which varies according to culture conditions (Watanabe et al., 1983).

Averaged body length of larvae in T_1 (*Artemia* nauplii and 10 larvae l^{-1}) (24.54 mm) was significantly larger compared to the other treatments (Table 4). These results may be attributed to the higher growth rate of larvae in T_1 (*Artemia* nauplii and 10 larvae l^{-1}) than other treatments. Similarly, Evangelista et al. (2005) stated that the total length of the catfish *C. macrocephalus* fed on *Artemia* was higher than other treatments fed on dry diets, Moina, Brachionus, and Chironomus. Also, Oyero et al. (2009) reported higher length in the group fed on *Artemia* than fed on liquid larvae. Data demonstrated a non-significant difference between T_2 (*Artemia* and 20 larvae l^{-1}) (21.91 mm), T_4 (22.8 mm), T5 (21.95 mm) and T_6 (21.45 mm). These results may be attributed to the same body weight development of larvae of these groups. On the other hand, the lower body lengths of T_7 (18.03 mm), T_8 (15.68 mm) and T_9 (14.63 mm) recorded in the current study might be attributed to the low body weight development of larvae of these groups. The length of catfish is also density dependent, thus, the high stocking density resulted in low length. Similarly, Bombeo et al. (2002) reported a declining length of *C. macrocephalus* larvae at high stocking density than low stocking density.

In this study, increasing stocking density decreased the survival rate of the larvae; consequently, mortality was lower in the lower density treatments. Growth and survival of African catfish *Clarias gariepinus* are known to be strongly influenced by stocking density (Hecht, 1982; Hecht and Appelbaum, 1988; Appelbaum and Van Damme, 1988; Haylor, 1991, 1992). Hecht and Appelbaum (1987) observed that lower stocking densities always gave the higher growth rate in an experiment with 25-day old *C. gariepinus* fingerlings density range, 5–20 fish l^{-1}. Meanwhile, the decreased survival rates of larvae fed on dry diets are comparable with the findings recorded in other catfish. *H. longifilis* larvae fed on trout-starter feed, (Kerdchuen and Legendre, 1994) and *C. gariepinus* larvae (Hogendoorn, 1980) had low survival rates; 32 % and 12 %; respectively. This might be related in part to the feed quality and the digestibility or to the ill-developed digestive systems at first feeding. Moreover, rapid degradation of the excess feed with a subsequent increase in ammonia in the water (Sharma and Chakrabarti, 1999) and enhanced growth of pathogenic microbes following excess feed might be another reason for decreased survival rates (Charlon and Bergot, 1984).

Results of cannibalism in the present study clearly indicated a density dependent cannibalism rate in *C. gariepinus* larvae. Cannibalistic behavior in the present experiment was observed primarily in five-day-old larvae. The rate of cannibalism in the first five days was very low probably because of size heterogeneity which was initially low and did not permit cannibals to find prey that was small enough to be swallowed as a whole. By aging, the larger larvae cannibalized the smaller and weaker larvae, so there was less variation in the size of the larvae and, subsequently, resulted in a declined cannibalism rate. Hence; a density threshold appeared to exist at ~20 fish L^{-1}, below which a direct relationship between density and cannibalism, occurred, and above which the rate of cannibalism, increased. Size variation has already been demonstrated to be a major cause of cannibalism (Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993). Additionally, social interactions for food and space might be another possible cause for the reduction in growth at high densities allowing few large larvae to dominate the feeding area, consuming most part of the food, growing faster and becoming cannibals (Toko et al., 2008).

In regard to the food type; the present study revealed a low cannibalism rate in the entire group larvae fed on *Artemia*. Whereas, the rate of cannibalism had increased significantly in the zooplankton and dry feeds fed larvae; especially at high stocking density. Similarly, Fermin and Bolivar (1991) observed a high cannibalism rate in dry

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diet fed *C. macrocephalus* larvae than those fed on *Artemia*. Larvae fed trout-starter diet had a higher cannibalism rate than those fed live food (Piennar, 1990; Hung et al., 2002). The known balanced nutrient composition of *Artemia* nauplii might be a direct cause of the low size variation observed among live food fed larvae in this study, when compared to those fed dry diet. As size heterogeneity is an important component in cannibalistic dynamics; If a cannibal does not gain growth advantages over non-cannibalistic siblings, as observed in the larvae of Asian catfish *Pangasius djambal* (Baras et al., 2010), cannibalism should decrease during the nursery period since cannibalistic larvae would consume most of the potential prey from the population. However, if cannibals do possess growth advantage over their siblings ingesting formulated diet, cannibals will have higher growth rates than the non-cannibalistic congeners, leading to greater size variation among the population and consequently high cannibalistic rate (Baras, 2013).

CONCLUSION

Despite the absence of significant interaction effect between stocking density and feed on rearing performance of *C. gariepinus larvae,* results of the current study indicated successful rearing of catfish larvae from the standpoint of growth performance, survival rate and cannibalism rate at lower stocking density. The density of 10 larvae l^{-1} was the maximum threshold capacity for *C. gariepinus* larval best growth when fed on either *Artemia* or zooplankton. However, further investigations are required to explore the effect of using other dry feed types in the rearing phase of African catfish larvae.

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Competing interests

The authors declare that they have no competing interests exist.

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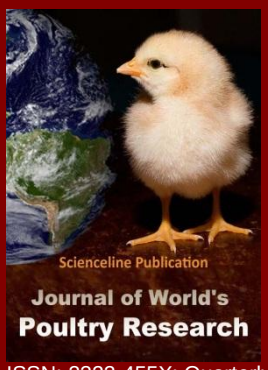

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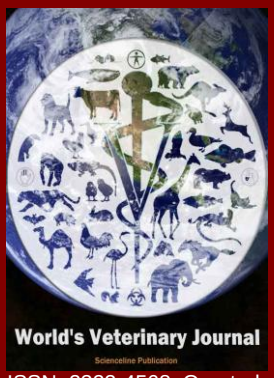

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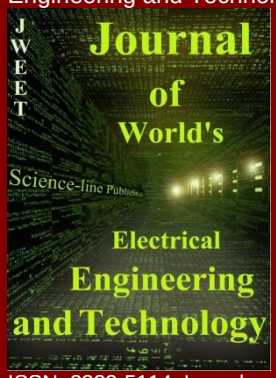

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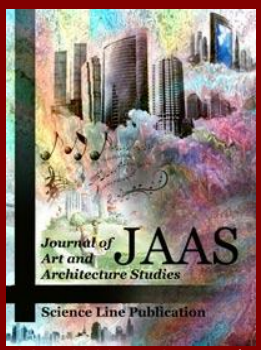

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