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Full Length Research Paper

Effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight of Nigerian indigenous chickens

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A total of 101 randomly selected birds from a population of 601 birds (obtained from a 3×3 diallel mating system involving normal, frizzle and naked neck Nigerian indigenous chickens) were used to evaluate the effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight. Blood samples for DNA analysis were collected at 20 weeks of age. IGF-I genotypes were identified using polymerase chain reaction (PCR)-RFLP method. Data obtained were analysed for frequencies and impact on body weight (BWT). Obtained results showed three polymorphic variants (designated AA, AB and BB) of the IGF-I gene. The overall frequencies for genotypes AA, AB and BB were 55.45, 37.62 and 6.93%, while alleles A and B were 74.26 and 25.74%, respectively. The analysis of the chicken IGF-I gene revealed that the chicken population was in Hardy Weinberg equilibrium, thus, samples were obtained from a large random mating population. IGF-I gene significantly ($P < 0.05$) influenced BWT with the AA genotype having higher BWT than AB and BB at all ages (4-20 weeks) except at hatch. Therefore, the effect of IGF-I gene on BWT indicated that it could be appropriate as a candidate gene in selection for BWT in Nigerian indigenous chickens.

Key words: Insulin-like growth factor I, polymorphism, bodyweight, indigenous chicken, PCR-RFLP.

INTRODUCTION

Nigerian indigenous chickens have been described as small bodied, slow growing, poor feed converters and poor meat animals (Ajayi, 2010). Despite these

drawbacks, they play vital role in socio-economic life of the rural and semi urban populace. Therefore, the need to genetically improve the performance of Nigerian

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indigenous chickens through selection and breeding cannot be overemphasized.

Much genetic research is now directed towards determining the relationship between physiological, biochemical and metabolic product/markers on the productive efficiency of farm animals (Isidahomen et al., 2011). Insulin-like growth factor (IGF) consists of a family of polypeptide hormones structurally associated with insulin with multiple metabolic functions (Li et al., 2003). The IGF are important regulators of growth, protein synthesis and cell proliferation and differentiation in a variety of cell types (Scanen et al., 1999).

Since candidate gene approach has become a powerful technique for genetic improvement (Zhu and Zhao, 2007), IGF-I gene has been reported by several authors to influence growth rate, carcass traits and feed efficiency in poultry (Amills et al., 2003; Zhou et al., 2005; Promwatee and Duangjinda, 2010). Although numerous population studies have focused on exotic and pure bred chickens, the information on the specific effects of the IGF-I gene on performance traits of Nigerian indigenous chicken genotypes and their crosses have not been reported or it is scanty. Consequently, there is need to investigate the possibility of using the IGF-I gene as a candidate gene in Nigerian indigenous chicken. The current work therefore, aimed at determining the effect of IGF-I gene polymorphism on body weight of pure and crossbred Nigerian indigenous chicken.

MATERIALS AND METHODS

Experimental location

This study was conducted at the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The farm was located at latitude 11° 09' 06" N and longitude 7° 38' 55" E, at an altitude of 706 m above sea level (Ovimaps, 2012).

Source of experimental birds and management

A total of 601 birds were generated from a 3x3 diallel mating design (involving normal, frizzle and naked neck Nigerian indigenous chickens) as described in an earlier study (Musa et al., 2015). They were fed starter (2652.00 kg/kg DM and 21.00% CP), grower (2400.00 kg/kg DM and 16.08% CP) and breeder (2520.08 kg/kg DM and 18.05% CP) diets at starter, grower and breeder phases, respectively. The birds were tagged at day old and raised on deep litter system. Feed and water were offered *ad libitum*.

Data collection

Data was collected on body weight at day old and on monthly basis until the end of the study (20 weeks). 101 birds were randomly selected and bled from the population of 601 birds. 2 ml of blood was collected from each bird using a 2 ml syringe (needle gauge, 23G x 11/4) into ethylene diamine tetra acetic acid (EDTA) sample bottles. The blood samples were immediately taken for DNA analysis at Centre for Biotechnology Research and Training

(CBRT), Ahmadu Bello University, Zaria.

DNA extraction

Genomic DNA was isolated by using Thermo Scientific GeneJET Genomic DNA Purification kit. The protocol used is as described for DNA purification from nucleated blood. A spectrophotometer was used to investigate the quality and quantity of DNA. The purity and concentration of DNA samples was estimated using UV-visible range spectrophotometer. DNA was also examined by loading samples on 0.75% agarose gel and visualizing the band under gel documentation system.

Polymerase chain reaction (PCR)

The IGF-I gene primer was selected from a previous publication (Nie et al., 2005) for use in amplifying the Nigerian indigenous chicken ortholog. According to Nie et al. (2005), the sequences of the candidate gene of the somatotrophic axis is from Genbank (<http://www.ncbi.nlm.nih.org>). The primers had been designed using the GENETOOL program (<http://www.biologysoft.com>). The primer were synthesised through a commercial service (BioNEER Corp., USA). Information on the primer is given in the Table 1. The PCR was performed in a total volume of 50 μ L in each PCR tube, containing 25 μ L of 2 x PCR master mix, 1 μ L each of the forward and reverse primers, 1 μ L of genomic DNA and 22 μ L of nuclease free water. The PCR tube was put in thermocycler (FTGENE5D by TECHNE Cambridge) and the PCR condition was set at 94°C for 5 min for initial denaturing, followed by 35 cycles at 94°C for 30 s for denaturing, 52°C for 45 s for annealing, and 72°C for 90 seconds extension, and a final extension step at 72°C for 5 min.

Restriction digest and restricted fragment length polymorphism

Restriction digest was done using 1 μ L of Fast Digest enzyme (EcoRI) according to the manufacturer's (Thermo Scientific) recommendation and at an incubation temperature of 37°C for 20 min. The enzyme was subsequently deactivated by heating for 5 min at 80°C. The digested products were loaded on a gel. Then, the electrophoresis tank was set up and connected to electrical source to run for 20 min at 75-100 V. The gel was removed and viewed under UV light to observe the bands. The restriction patterns were visualized by 0.75% agarose gel electrophoresis; gels were stained with GR Green DNA stainer. Gels were visualized and photographed using a gel documentation system (Uvipro Silver by Uvitec).

Statistical analysis

General linear model procedure of Statistical Analysis System program (SAS, 2002) was used to test the effects of the genetic groups and sex. Significant means were separated using Tukey-Kramer HSD (honestly significant difference) multiple comparison test (Tukey, 1953; Kramer, 1956). The following model was used to investigate effect of IGF-I genotypes on body weight:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

Where: Y_{ijk} = body weight of bird of the j^{th} sex in the i^{th} IGF-I genotype; μ = overall population mean; G_i = effect of the i^{th} IGF-I genotype; S_j = effect of the j^{th} sex; e_{ijk} = random error.

The gene frequencies were calculated according to Hardy-

Table 1. Details of primer used for amplifying IGF-I gene in Nigerian indigenous chicken.

Primer	Gene	Sequence of oligonucleotide primers (Forward primer 5' – 3'/ Reverse Primer 5' – 3')	Sequence ID ¹	Length (bp)	Annealing temperature for PCR amplification (°C) ²
309	IGF-I	AGCTGTTTCCAATGATGGTGTTCCT / GCCCCAGCATTCTCTTCCT	AY253744	583	56.4 58.2

Source: Nie et al. (2005); ¹Sequence accession numbers used for primer designing. ²Annealing temperature as specified by the manufacturer (BioNEER Corporation, USA).

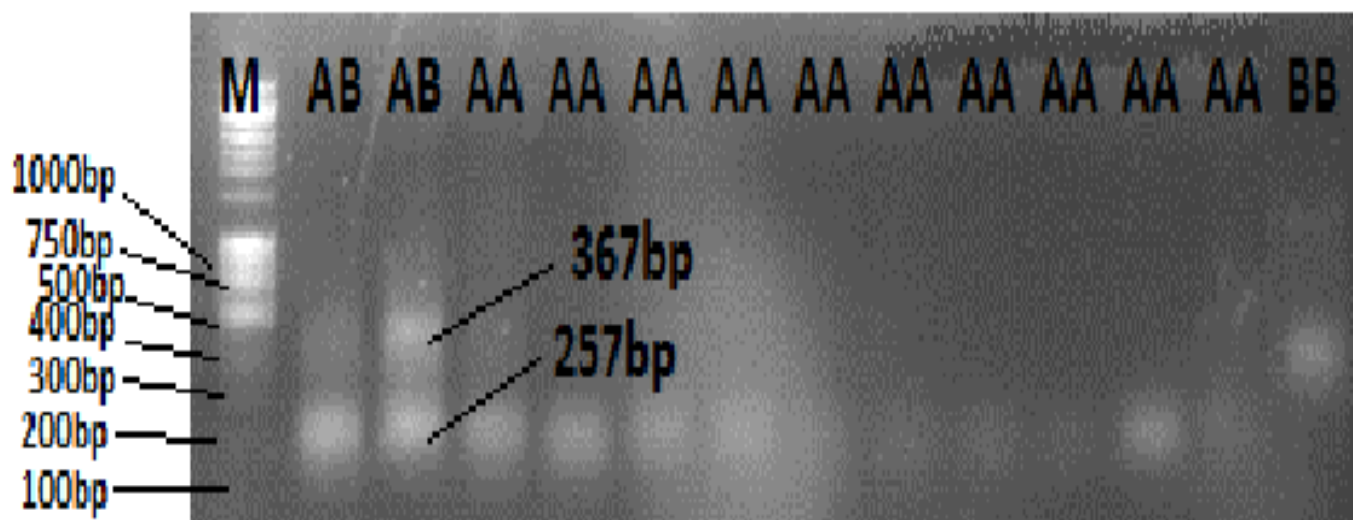


Plate 1. Electrophoregram showing genotyping profiles of IGF-I gene in Nigerian indigenous chickens detected by PCR-RFLP method.

Weinberg’s equation as follows:

$$p = \frac{2(AA) + (AB)}{2N}$$

$$q = \frac{2(BB) + (AB)}{2N}$$

Where: p = the gene frequency of allele A; q = the gene frequency of allele B and; N = the total number of birds tested.

A Chi-squared test for goodness-of-fit was performed to verify if genotype frequencies agree with Hardy-Weinberg’s equilibrium (HWE) expectations with the following formula:

$$\chi^2 = \frac{\sum (O - E)^2}{E}$$

Where: O= observed frequency; E= expected frequency.

RESULTS AND DISCUSSION

Genotyping of insulin-like growth factor I (IGF-I)

The IGF-I gene was successfully amplified using genomic DNA samples. The restriction digest analysis of the PCR products using EcoR1 indicated the presence of two restriction patterns. Fragment sizes of 257 and 367bp were observed in the first and second restriction patterns and were assigned AA and BB genotypes, respectively while those with both were assigned AB genotype (Plate 1).

Effects of IGF-I genotypes on body weight at various ages

The effects IGF-I genotypes on body weight at various ages is presented in Table 2. Birds with the AA genotype had significantly (P<0.05) higher body weights than birds of AB and BB at all ages except at hatch. The results of higher body weights in AA genotype was consistent with those of Promwatee et al. (2013) who reported higher

Table 2. Effects of IGF-I genotypes on body weight (g) of Nigerian indigenous chickens at various ages.

IGF-I	N (101)	Day old	Week 4	Week 8	Week 12	Week 16	Week 20
AA	56	23.32 ^b ±0.57	107.69 ^a ±2.28	354.61 ^a ±7.46	667.80 ^a ±13.47	856.31 ^a ±17.82	1206.89 ^a ±22.56
AB	38	26.13 ^{ab} ±0.57	93.61 ^b ±2.27	314.91 ^b ±7.40	597.32 ^b ±13.37	765.49 ^b ±17.69	1058.44 ^b ±22.39
BB	7	26.37 ^a ±1.45	93.34 ^b ±5.80	285.19 ^b ±18.94	583.00 ^b ±34.21	743.42 ^b ±45.25	1051.36 ^b ±57.28
SEM		0.32	1.29	4.21	7.60	10.05	12.72
CV (%)		12.1	13.03	12.99	12.3	12.71	11.39
LOS		*	*	*	*	*	*

SEM= Standard error of means; CV= coefficient of variation; LOS= level of significance; * = (P<0.05); N= number of observation.

Table 3. Genotypic and allelic frequencies of the IGF-I gene in various genetic groups of Nigerian indigenous chickens.

Groups Sex	N	Allelic frequency (%)		Genotypic frequency (%)			χ^2
		A	B	AA	AB	BB	
Male	49	70.41	29.59	51.02	38.78	10.20	0.24
Female	52	77.88	22.12	59.62	36.54	3.85	0.19
Overall	101	74.26	25.74	55.45	37.62	6.93	0.03

N= number of observations.

body weights at 4, 8, 12 and 14 weeks of age in the AA genotype than in the BB genotype in the Khai Mook Esarn and Soi Pet population of chickens. However, the result of significantly higher body weights in the BB genotype at hatch differed with those of Promwatee et al. (2013), who observed higher body weight in AA genotype in the Soi Nin population. Furthermore, Amills et al. (2003) reported mutation of the IGF-I gene in two chicken strains of the Black Penedesenca breed and significant association of IGF-I SNP1 was found for body weight up to 107 days of age in one of the strains. According to the authors, IGF-I-SNP1 marker was located in the promoter region of the IGF-I gene, so the existence of suggestive associations among IGF-I-SNP1 and growth might be interpreted in the light of differences in the transcriptional rate of both alleles. In fact, the analysis of the promoter sequence revealed that the substitution A→C involved the suppression of one potential chicken homeobox containing gene (CdxA) transcription factor binding site (Amills et al., 2003). Thus, the chicken IGF-I gene is likely a potential marker for use in body weight selection programme.

Genotypic and allelic frequencies of the IGF-I gene in Nigerian indigenous chickens

The genotypic and allelic frequencies varied across sex (Table 3). The observed genotypic frequencies for all the males were 51.02, 38.78 and 10.20% for AA, AB and BB genotypes, while the allelic frequencies were 70.41 and 29.59% for A and B alleles, respectively. For the females,

the genotypic frequencies were 59.62, 36.54 and 3.85% for AA, AB and BB genotypes while the allelic frequencies were 77.88 and 22.12% for A and B alleles, respectively. However, the overall frequencies obtained were 55.45, 37.62 and 6.93% for AA, AB and BB genotypes, 74.26 and 25.74% for A and B alleles, respectively. The frequency of allele A was however, higher than allele B in Nigerian indigenous chickens. The analysis of the chicken IGF-I gene revealed that the population was in Hardy Weinberg's equilibrium as evidenced by the insignificant Chi square values. The observed result in this study was not consistent with those of Li et al. (2010) and Shah et al. (2012) who reported that the population of chickens studied were not in Hardy Weinberg equilibrium. However, the observed results in this study agreed with those of Abbasi and Kazemi (2011) who reported 3 polymorphic forms of the gene and population being in Hardy-Weinberg equilibrium which implies that samples were obtained from a large random mating population.

Conclusions

The Nigerian indigenous chickens conformed to Hardy Weinberg's equilibrium based on IGF-I locus, thus, samples were obtained from a large random mating population. The IGF-I gene significantly influenced body weight at all ages with the AA genotype having heavier weight than AB and BB except at hatch. Thus, IGF-I gene may be appropriate as a candidate marker for selection for body weight in Nigerian indigenous chickens. A

breeding plan that incorporates individuals who possess the AA genotype of the IGF-I gene should be considered for possible improvement in body weight of the indigenous breeds of chicken in Nigeria. Further studies on the influence of the IGF-I gene on reproductive performance is recommended.

Conflict of interests

The authors have not declared any conflict of interestS.

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Full Length Research Paper

Acaricide resistance of *Rhipicephalus microplus* ticks in Benin

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After the introduction of the invasive cattle tick *Rhipicephalus microplus* in West Africa in the last decade, farmers encounter ticks resistance to the use of acaricides in different region in Benin. In order to evaluate the level of resistance, an *in vitro* study was performed on five samples of *R. (Boophilus) microplus* collected from five farms in four of the eight agro-ecological zone of Benin. The districts concerned with the study in the agro-ecological zone were Houeyogbe (Kpinnou), Zangnanado (Samiondji), Tchaourou (Okpara), Gogounou (Fana) and Bassila (Manigri). A toxicological test, the Larval Packet Test (LPT) was performed in the laboratory of Biotechnology Research Unit of the Animal Production and Health (URBPSA) at the Polytechnic School of University of Abomey-Calavi in Benin with the susceptible, *Rhipicephalus geigy* strain from Hounde in Burkina Faso. Three (3) acaricides commonly used by farmers in Benin to control ticks were evaluated: alpha-cypermethrin, deltamethrin and amitraz. The results showed that the resistance ratio at 50% (RR_{50} 95% CI) for the whole experiment varies from 1.96 to 338.5. Based on RR_{50} and RR_{90} values, only the population of Samiondji's state farm was susceptible to the alpha-cypermethrin with a resistance ratio $RR_{50} = 1.64$ (95% CI: 0.2 to 12.6), all the other resistance tests conducted on moderate or high resistance Bassila and Kpinnou appear to host the most resistant samples. Moreover, a certain high variability of dose response relationship has been noticed with amitraz on the base of the higher slope of the related curves.

Key words: Resistance ratio, distribution, *Rhipicephalus microplus*, acaricide, larval packet test (LPT), Benin.

INTRODUCTION

In Benin, recently conducted studies have revealed the presence of 10 ticks species in the northern part of the country and 11 species in central and southern part of the country (Farougou et al., 2007), including the recently introduced Asian cattle tick *Rhipicephalus microplus* (Madder et al., 2012). This tick species has a great ability for ecological adaptation and is suspected to be resistant to various conventional acaricides (Madder et al., 2012). However, the struggle against ticks and ticks borne pathogens transmitted not only depend on the effectiveness of acaricides used, but also and especially on the practice of the farmers.

A number of reports have described cases of resistance in *R. microplus* (also called blue ticks or cotton belt tick), because of a certain number of non-completely elucidated factors among which an inadequate posology of acaricide (Li et al., 2004; Ducornez et al., 2005). Organophosphoruses (OP) such as chlorpyrifos were the first acaricide molecules used in Argentina for which the resistance has been reported in the 1970s (Aguirre et al., 2000). Moreover, the first case of resistance to synthesis pyrethroids (SP) was reported in Argentina in 1996 (Caracostantogolo et al., 1996). Nearly 15 years later, the first resistance case to amitraz was identified (Cutullé et al., 2012).

The first cases of resistance of amitraz have been reported after ten and seven years of use respectively in Australia (Nolan, 1981) and Mexico (Soberanes et al., 2002; Andrew et al., 2004). In Mexico, *R. microplus* resistant ticks to organophosphate were documented in 1981. In 1993, the first cases of pyrethroid resistance were detected and in 2000 resistance to amitraz (Sanchez-Cespedes et al., 2002 Temeyer et al., 2004). Control of tick infestation for with acaricides began in the 1970s with diethylethion OP (ND Rhodiace). From 1992 to the end of 1997, deltamethrin (ND Butox) was used but the tick population was declared resistant and was replaced by amitraz (ND Taktic).

Several studies have reported the resistance of *R. microplus* to acaricide in South Africa (Taylor and Oberem, 1995), Brazil (Martins et al., 1995; Furlong, 1999) and Colombia (Benavides et al., 2000). In the West African sub-region, all toxicology studies have been made on other tick species then *Rhipicephalus geigy* and revealed no ticks resistance (Adakal et al., 2013b; Jongejan and Uilenberg, 2004; Kaljouw, 2008). Until now, no study has been conducted in Benin in order to determine the effectiveness of different acaricides used to control ticks, although *R. microplus* ticks are known to have developed resistance to many acaricides (Chevillon et al., 2007; Kumar et al., 2012; Lovis et al., 2013).

In view of this, there is a strong need to evaluate the level of resistance of *R. microplus* to "... commonly used acaricides by farmers in Benin where inefficiency of acaricides is reported (Madder et al., 2012). Infestations with this exotic tick constitute a serious threat to livestock production and health in West africa. The invasive nature of this tick and especially its ability displace of other native ticks species of the same genus *Rhipicephalus annulatus*, *R. geigy* or *Rhipicephalus decoloratus* has been documented recently (Madder et al., 2012). This study will rehabilitate programs against these parasites based on the data obtained. Indeed, it is recognized in the world that *Bos indicus* cattle type is more resistant to ectoparasites than *Bos taurus* cattle (Mattioli et al., 1993; Bianchin et al., 2007).

The present study aims to evaluate the resistance of the cattle tick *R. microplus* along a north-south Region of Benin.

MATERIALS AND METHODS

Study area and collection of ticks

R. microplus males and engorged females were collected from cattle at the state farms of Kpinnou (Houeyogbe), Opkara (Tchaourou), Samiondji (Zangnanado) and at two private farms in Fanan (Gogounou) and Manigri (Bassila) between October and December, 2013, a period of high tick abundance (Farougou et al., 2007). Ten cattle between 2 and 3 years of age and visibly infested with ticks, were selected on each of the farms. The animals received no acaricide treatment, at least three months prior to the day of collection. Ticks were collected on different species of cattle of Girolando (Kpinnou), Lagunaire (Samiondji), Borgou (Okpara, Bassila and Gogounou). The collection from each animal was kept in a separate labeled tube with perforated lid. The animal number, date of collection, name of farm, the village, the town and the county was recorded on the label. All samples were geo-referenced using a Garmin Xtrex.

Tick identification and rearing of larvae

The identification of ticks and resistance tests were performed in the Laboratory of Biotechnology Research Unit of the Animal Production and Health (the URBPSA) at the Polytechnic School of Abomey Calavi (PSAC) of University of Abomey in Benin. For the purposes of the acaricide resistance test, 50 engorged females ticks collect on at least ten animals (maximum eight of one animal) identified as *R. microplus* for each of the five farms were placed in empty vials perforated medium fine mesh and placed in an incubator at a temperature between 27 and 29°C with a relative humidity between 85 and 95% (Lovis et al., 2013).

After oviposition, the eggs were collected during 3 to 4 days post-laying, weighed and placed in tubes covered with fine mesh fabric at a rate of 0.5 g per tube. The larvae used in these resistance tests

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were aged from 14 to 21 days (Lovis et al., 2013).

Larval packet test pocket preparation

The Larval Packet Test (LPT) standard (FAO, 1984) was used to evaluate the acaricide resistance of ticks to acaricides. The filter paper Whatman used to manufacture packets for larvae was performed at CIRDES. Three acaricides at variable purity (Table 1), supplied by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) were tested. This is deltamethrin, alpha-cypermethrin and amitraz, all in powder form. A serial dilution of each acaricide was prepared using a solvent, composed of 1 volume of olive oil mixed with 2 volumes of trichlorethylene (Miller et al., 2002). Seven different concentrations were prepared for each acaricide test (Table 1) with two filter papers (8.5 x 7.5 cm) by concentration. The assays were performed in two replicates. A volume of 0.67 ml of each dilution was applied using a micropipette on each of two Whatman paper filters (Cat. No. 3001 917). The papers were then dried under a hood, with gas aspiration to outside for 2 h to allow the evaporation of trichlorethylene. The dried papers were then wrapped in aluminum foil by concentration and kept refrigerated at 4°C. For each test, two whatman filter papers impregnated with acaricide were used by concentration. The amount of M pure acaricide to be used for the preparation of the stock solution is obtained by the formula: $M = X / (\% \text{ of purity}) \times (20/3)$, where X = % of the highest concentration, final volume = 20 and 3 = a constant.

Reference tick strain

In the absence of a susceptible references strain of *R. microplus*, the level of resistance of the collected ticks was compared with a susceptible strain of *R. geigy*. This strain was collected on Baoule cattle breed, in Houde, a town in the province of Tuyin Hauts-Basins in south western of Burkina Faso. It was maintained at CIRDES for twelve generations (four years), fed on the same cattle breed and did not show any signs of resistance (Adakal et al., 2013a).

Execution of the larval packet test

The aluminum foils containing the impregnated papers were opened using forceps, each paper was folded in two. Each folded acaricide paper was retained by two plastic clasps to form an open pocket on one side. The pocket receives using a brush number N°4, approximately 100 tick larvae through the open side of the folded paper. This side was then closed with a third clasp. After doing this for all concentrations, all envelopes were put into a tray and placed in a heat chamber for 24 hours at a temperature of 28±1°C with a humidity of 90 ± 5% (Lovis et al., 2011). Each envelope was marked with pencil by the corresponding concentration, the name of the acaricide used and date of preparation. After 24 h of incubation, the individual packages are removed from the heat chamber to control larval mortality in each envelope.

The concentrations used in our tests ranged from 0.0156 to 4%, depending on each lethal concentration known of acaricide (Adakal et al., 2013a; Lovis et al., 2013).

Statistical analysis

Non-linear regression analyses of dose-mortality data were performed in R (version 2.15.3) (Ritz and Streibig, 2005) using the drc (Dose-Response Curves) package (version 2.3-96), specific for

modelling dose-response curves (Ritz and Streibig, 2005). As age of larvae considered was different from one acaricide to another, they were considered as covariance in the modelling as well. Three functions: four-Log-logistic model, four and three Weibull models were tested in order to choose the one giving the lowest residual deviance. To model the data using the Dose Regression Mortality (DRM) command, bottom and top values locked at 0 and 100, respectively. LC₅₀ and LC₉₉ values and their 95% confidence intervals (95% CI) were estimated using the effective dose (ED) command and the delta option for the interval parameter. The difference between LC₅₀ estimated was designated as significant if their 95% CI did not overlap. The resistance ratios (RR) of *R. microplus* at Gogounou, Kpinnou, Samiondji, Okpara and Bassila were computed relatively to the susceptible reference strain tick *R. geigy* Houde 2005. The plots of the model were performed to well evaluate trends that each model presented.

Resistance ratios and their CI at 95% were calculated, so were the slopes and intercepts of the regression line (Robertson and Preisler, 1992). Differences are significant when the number 1 is excluded from the CI of resistance ratio (Ducornez et al., 2005). According to Jonsson and Hope (2007), a tick population is said to be sensitive to an acaricide when RR < 4; moderately resistant if 4 < RR < 10 and super-strong when RR > 10.

RESULTS

General trend

Four-parameters Log-logistic is the best model for the present analysis (residual deviance=0.60). Lethal concentrations 50 and 90 (LC₅₀ and LC₉₀) and their 95% CI are reported in Tables 1, 2 and 3 for the three acaricides tested: deltamethrin, alpha-cypermethrin and amitraz respectively. The resistance ratio 50 and 90 (RR₅₀ and RR₉₀) and their related 95% CI are also reported in the same tables for the three acaricides tested. These values made it possible to carry out a geographic distribution card of the *R. microplus* resistance to usual acaricides in Benin (Figure 1). It appears that RR₅₀ CI are narrower than that of RR₉₀ for alpha-cypermethrin and deltamethrin; at the opposite, amitraz does not follow the same trend. The deltamethrin dose responsive curve of Gogounou and Samiondji samples show a greater slope than that of the reference, the same situation occurs with alpha-cypermethrin dose responsive curve for Gogounou and Okpara sample and also with amitraz dose responsive curve for Gogounou, Bassila and Okpara samples.

For each of the acaricides used, no mortality was recorded at the lowest concentrations in the susceptible reference sample. With the susceptible reference sample, 100% mortality was recorded at the highest concentration for each of the three acaricides used. The number 1 is excluded from the CI of RR₅₀ and RR₉₀ for all field samples, which lead to a significant difference between any of the field sample and the Houde strain (2005) at 95% CI.

Resistance to deltamethrin

Houde's responsive curve occupy a backward position

Table 1. Different sets of dilution applied to "pure" tested acaricides.

Acaricide (purity percentage)	Chemical family	Strains (Latitude, Longitude)	Dilution series (%)
Alpha-cypermethrin (95%)	Synthesis Pyrethroids	Kpinnou (N6.56828, E1.78623)	
Deltamethrin (99.5%)	Synthesis Pyrethroids	Samiondji (N7.41667, E2.36667) Gogounou (N10.73843, E2.92359)	0 - 0.0078 - 0.0156 - 0.0312 - 0.0625 - 0.125 - 0.25 - 0.5 - 1 - 2 - 4
Amitraz (98%)	Amidines	Okpara (N9.30501, E2.73148) Bassila (N8.94135, E1.77063)	
Alpha-cypermethrin (95%)	Synthesis Pyrethroids		0.0312 - 0.0625 - 0.125 - 0.25 - 0.5 - 1
Deltamethrin (99.5%)	Synthesis Pyrethroids	Hounde (N11.48333, W3.51667)	0.0281-0.0562- 0.112- 0.225 - 0.45 - 0.9
Amitraz (98%)	Amidines		0.0256-0.0512 - 0.102 - 0.205 - 0.41 - 0.82

Hounde: sensitive reference strain; Kpinnou, Samiondji, Gogounou, Okpara and Bassila: field strain; AI: active ingredient

Table 2. Lethal Concentration (LC₅₀ and LC₉₀) and Deltamethrin Resistances ratios (RR₅₀ and RR₉₀).

Sites	Deltamethrin				Slopes
	LC ₅₀ (CI)	LC ₉₀ (CI)	RR ₅₀	RR ₉₀	
Hounde	0.02 (0.016-0.023)	0.04 (0.029-0.054)	-	-	2.419±0.072
Gogounou	0.87 (0.80-0.94)	1.78 (1.44-2.12)	54.42 (51.76-56.89)	44.94 (38.79-50.39)	3.065±0.366
Okpara	0.85 (0.78-0.93)	1.70 (1.38-2.03)	53.59 (50.74-56.24)	43.01 (37.00-48.33)	1.794±0.057
Bassila	2.27 (1.86-2.68)	13.05 (6.94-19.17)	142.09 (120.32-162.42)	329.64 (186.61-406.20)	1.256±0.135
Samiondji	0.60 (0.55-0.66)	1.27 (0.97-1.56)	37.70 (35.67-39.88)	31.95 (26.19-37.05)	3.191±0.407
Kpinnou	2.29 (1.86-2.72)	13.41 (6.93-19.88)	143.34 (120.64-164.53)	338.50 (186.39-473.09)	1.243±0.136

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios

in comparison with all the field sample curves (Figure 2A). Gogounou and Okpara curves in one hand and Bassila and Kpinnou curves in second hand appear conflicting (Figure 2A). This pattern tends to be confirmed by their LCs (0.87/0.85 and 1.78/1.70) and RRs (54.42/53.59 and 44.94/43.01) which are very closed (Table 2).

The LC₅₀ and LC₉₀ of deltamethrin for Hounde

strain are respectively 0.02% (0.016 to 0.024) and 0.04% (0.029 to 0.054) at 95% CI. They are weaker compared to those of the field samples. The higher LC₅₀ and LC₉₀ among field samples are those of Kpinnou sample followed by Bassila, Gogounou and Okpara samples. Those of Samiondji (0.65 and 1.27%) are the smallest of all field samples.

All of the field samples have their resistance ratios above 10; except that of Okpara which is greater than 4. This leads to admit that Okpara sample shows moderate resistance to deltamethrin whereas the other field samples are highly resistant to all concentrations of deltamethrin (Table 2). Moreover, there is no emerging resistance to deltamethrin but only established resistance.

Table 3. Lethal Concentration (LC50 and LC90) and Alpha-cypermethrin Resistances ratios (RR50 and RR90)

Sites	Alpha-cyperméthrine				Slopes
	LC ₅₀ (CI)	LC ₉₀ (CI)	RR ₅₀	RR ₉₀	
Houde	0.03 (0.03-0.04)	0.09 (0.07-0.12)	-	-	2.191±0.266
Gogounou	1.04 (1.02-1.07)	2.10 (1.98-2.22)	30.42 (27.20-34.75)	22.50 (19.11-28.08)	3.133±0.111
Okpara	1.04 (1.01-1.06)	02.08 (1.97-2.20)	30.33 (27.11-34.65)	22.31 (18.94-27.84)	3.159±0.112
Bassila	2.29 (1.86-2.73)	13.36 (6.89-19.82)	66.96 (63.64-69.43)	143.0 (97.61-170.59)	1.247±0.137
Samiondji	0.07(0.05-0.09)	0.21(0.08-0.34)	01.96 (01.68-02.17)	02.28 (01.18-02.96)	1.902±0.445
Kpinnou	1.24 (1.18-1.30)	3.38 (3.01-3.77)	36.20 (33.23-40.19)	36.24 (32.40-42.55)	2.189±0.110

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios.

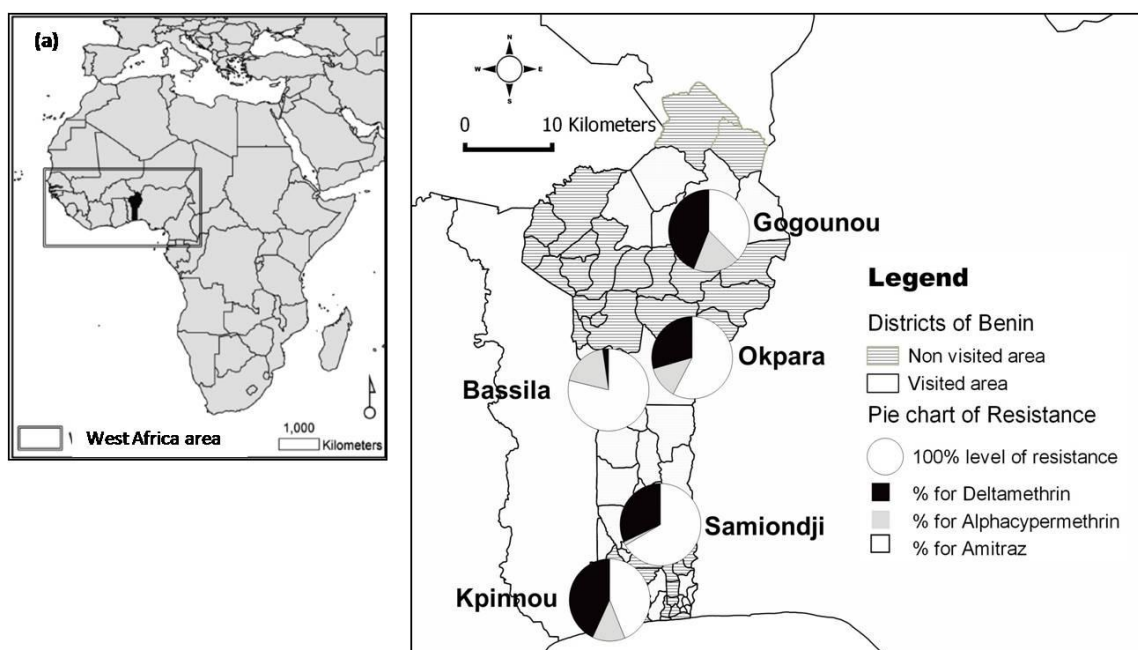


Figure 1. (a) The geographical location of Benin in West Africa and (b) the distribution map of the resistance of *R. microplus* resistance in Benin.

Kpinnou strain is characterized by exceptional very high values of RR₅₀ and RR₉₀, respectively 143.34 and 338.50.

Resistance to alpha-cypermethrin

Houde and Samiondji curves occupy the backward positions (Figure 2B) with Samiondji slightly behind Houde; forwardest position is that of Bassila curve. Gogounou and Okpara curves appear conflicting, slightly behind and very close to that of Kpinnou (Figure 2B).

Lowest LC₅₀ and LC₉₀ are those of Houde (0.03 and 0.09) and Samiondji (0.07 and 0.21) whereas the highest are those of Bassila (2.29 and 13.26). All the RRs values related to the field samples are above 10 except that of Samiondji strain which is less than 4. Consequently,

Samiondji strain is susceptible whereas the other strains are resistant. Moreover, for resistant strains, there is no emerging resistance, but only established resistance. Bassila shows the highest level of resistance to alpha-cypermethrin with RR₅₀ and RR₉₀, respectively equal 66.96 and 143.

Resistance to amitraz

Houde and Samiondji curves are close although Houde curve is slightly behind the second one (Figure 2C). The forwardest curve is that of Kpinnou. Okpara and Bassila curves occupy a medium position with Gogounou curve that is slightly behind but more forwardly open than the two others (Figure 2C). Curves slopes of

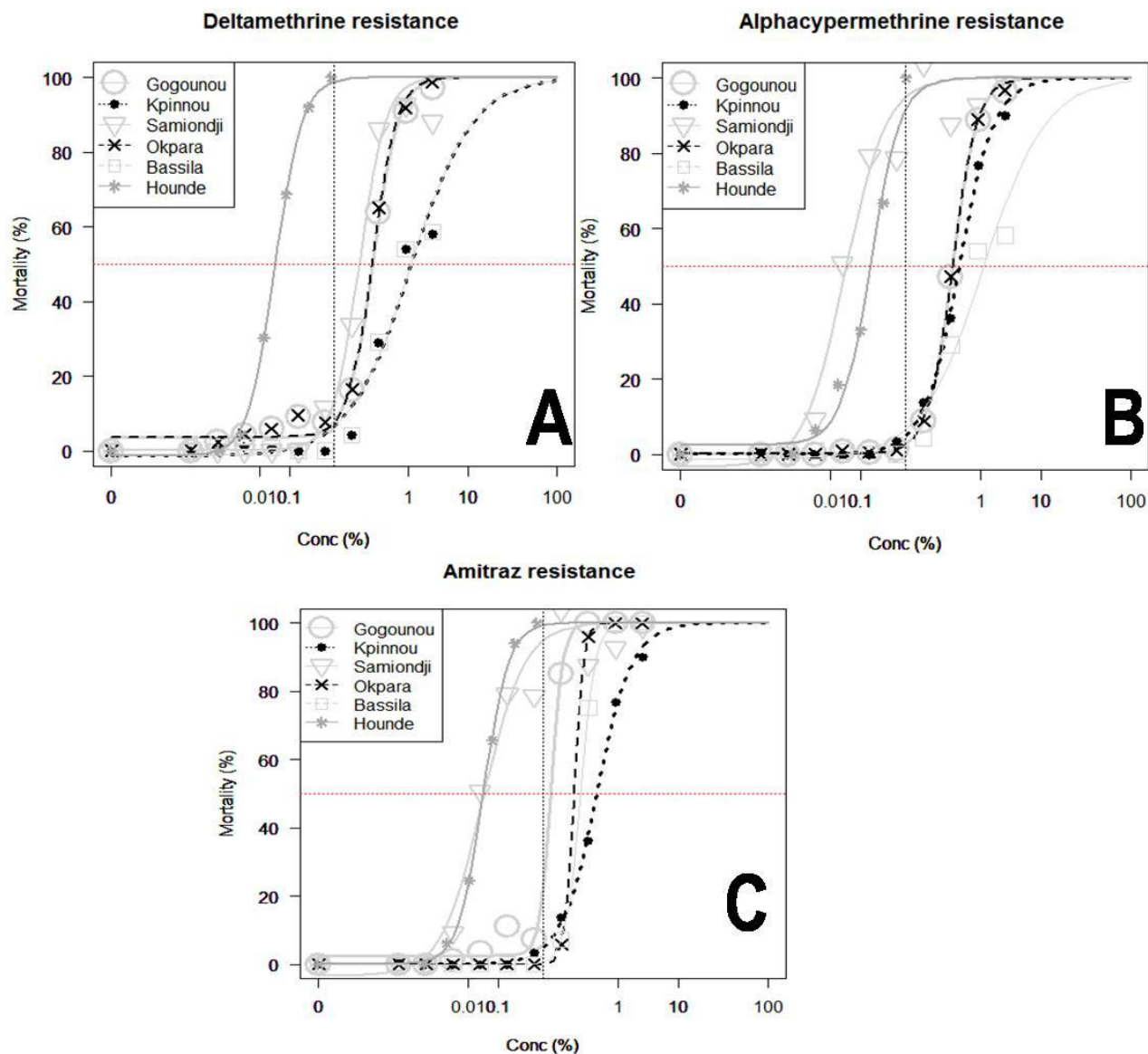


Figure 2. Dose-response curves of five Benin field populations in comparison to the susceptible reference strain of Houndé when tested with three acaricides (The gray dotted horizontal lines indicate 50 and 90 % mortalities)

field samples are among the highest of response curves; up to three or four times greater than that of reference strain.

The lowest values of LC are those of Houndé strain (0.02 and 0.04) and Samiondji sample (0.07 and 0.21) whereas the highest is that of Kpinnou (1.24 and 3.39).

All the RRs values related to the field samples are above 10 except that of Samiondji strain which is greater than 4 and less than 10. Consequently, Samiondji strain is moderately resistant to amitraz whereas the other strains are highly resistant to this acaricide. All the cases of resistance are established resistance; there is neither emerging resistance nor susceptibility. Kpinnou sample expresses the higher level of resistance with resistance ratio about 78.5 and 92.89, respectively for RR₅₀ and

RR₉₀. Bassila sample shows the second resistance ability with RR₅₀ and RR₉₀ values about 51.38 and 33.7.

Relatively to amitraz, RR₅₀ and RR₉₀ values are greater than 10. RR₅₀ values are very high and comprehended between 4.25 (03.23 - 05.21) and 78.53 (77.16 - 79.82), whereas RR₉₀ values vary between 05.84 (02.43 - 08.89) and 92.89 (87.82 - 97.37). Resistance to amitraz for all the samples was concluded. In details, apart from Samiondji strain that shows a moderate resistance, all the other field samples show high resistance to amitraz. The little susceptibility of Samiondji sample to amitraz appear in the graph through the proximity between Samiondji and Houndé curve. All the over curves are forwardly open from the two last (Figure 2C). Moreover, in general response, curves to Amitraz are more

Table 4. Lethal Concentration (LC₅₀ and LC₉₀) and Amitraz Resistances ratios (RR₅₀ and RR₉₀).

Sites	Amitraz				Slopes
	LC ₅₀ (CI)	LC ₉₀ (CI)	RR ₅₀	RR ₉₀	
Hounde	0.02 (0.02-0.02)	0.04 (0.03-0.04)	-	-	2.628±0.088
Gogounou	0.38 (0.36-0.42)	0.54 (0.50-0.57)	24.58 (23.60-25.49)	14.77 (14.70-14.83)	6.724±0.847
Okpara	0.69 (0.69-0.70)	0.90 (0.89-0.90)	43.82 (42.54-45.19)	24.57 (23.31-26.01)	8.524±0.055
Bassila	0.81 (0.77-0.86)	1.23 (1.14-1.32)	51.36 (50.26-52.38)	33.70 (33.27-34.08)	5.297±0.538
Samiondji	0.07 (0.05-0.09)	0.21 (0.08-0.34)	04.25 (03.23-05.21)	05.84 (02.43-08.89)	1.902±0.445
Kpinnou	1.24 (1.18-1.30)	3.39 (3.01-3.77)	78.53 (77.16-79.82)	92.89 (87.82-97.37)	2.189±0.110

LC (%): Lethal concentration at 95%; CI: Confidence intervals at 95%; RR: Resistance ratios.

distinguishable one from the other comparatively to those of the other acaracides.

DISCUSSION

Confirmed tick resistance to acaracide in Benin

Results of study make find out resistance among most of the ticks field samples. Apart from the Samiondji sample, which is still susceptible to alpha-cypermethrin, all the other samples showed moderate resistance ($4 < RR_{50} < 10$) to strong resistance ($RR_{50} > 10$) to all three acaricides (Tables 2, 3 and 4). Similar cases of resistance have been reported by Andreotti (2007); especially, resistance to pyrethroids (SP), namely alpha-cypermethrin and cypermethrin of several samples of *R. microplus* collected from farms in the state of Matto Grosso do soul Brazil. Following recent results obtained by Lovis et al. (2013), the resistance (moderate or high) to deltamethrin and alpha-cypermethrin of Gogounou, Okpara, Bassila and Kpinnou sample and also resistance to deltamethrine of Samiondji strain can be considered as fully established. Since their RR₅₀ and RR₉₀ follow the same trend (in comparison with the cut off values that are 4 and 10). Therefore, no emerging resistance is noticeable; indeed, emerging resistance is admitted when RR₉₀ and RR₅₀ are respectively higher and lower than 4 with the slope of the field population small than that of the reference as published by FAO (2004) cited by Lovis et al. (2013).

Similar results, related to established resistance has been found for the *R. microplus* strains ST27, ST26, ST25, ST22 in Argentina and other strains in South Africa and also Australia with Fluomethrin and Cypermethrin which are synthetic pyrethroids (Lovis et al., 2013). Miller et al. (2003) also mentioned resistance to pyrethroids of a strain from Texas.

Overall, some countries concerned with systematic treatments based on pyrethroids and where resistance studies has been performed encountered resistance to pyrethroids (Leite, 1988; Laranja et al., 1989; Rodriguez-Vivas et al., 2006; Rodriguez-Vivas et al., 2007; Chevillon et al., 2007; Kearney, 2011). Considering the levels of

resistance, examples of deltamethrin resistance are reported in New-Caledonia (Chevillon, 2007) with RRs values smaller than 30; these levels of resistance are lower than those of Samiondji and Gogounou and especially at least five times lower and up to ten times lower than that of Kpinnou (RR₅₀=143.34 and RR₉₀=338.5). It leads to the conclusion that levels of resistance in Benin reach high levels compared with that of the main countries where resistance studies has been performed.

Gogounou and Okpara host the most homogeneous samples

Although Gogounou sample expresses high level resistance to all acaricides; the slopes of dose responsive curves of Gogounou sample are steeper than that of reference; this is an indicator of either a certain high susceptibility of Gogounou sample or a very significant dose-mortality relationship. In fact, small slope make it difficult to discriminate between susceptible and resistant individuals (Miller et al., 2002). An extreme low slope should be the sign of a clearly resistant sample which consist of homogeneous resistant individuals. At the opposite, the steeper the slope is, the higher the response from the sample is; that is a significant and noticeable increment in tick mortality according to increasing doses. An extreme high slope should be the sign of a clearly susceptible sample which consist of homogeneous susceptible individuals. Since LC₅₀ and LC₉₀ related to Gogounou are higher than that of the reference and on the bases of RRs values, Gogounou sample which is concerned with an established resistance to the three acaricides, might consist of resistant individuals that should be less resistant and more homogeneous than other field samples, with regard to the resistance ability. Indeed, an opposite situation is that of Lovis et al. (2013) who find out emerging resistance to flumethrin associated with an heterogeneous population, on the base of smaller slope with RR₉₀ greater than RR₅₀. Okpara appears to be the second point of homogenous sample.

Kpinnou and Bassila respectively as first dissemination point and abundance point of ticks host most resistant tick samples

Moreover, the gap between the resistance levels in Kpinnou and those of the other sites in Benin might be interpreted as an increase of resistance since Kpinnou is the first introduction point in Benin (Madder et al., 2012). Increase of resistance level for Kpinnou sample is particularly spectacular since *R. microplus* control in Kpinnou is at least fourteen years recent than that of New-Caledonia (Chevillon et al., 2007).

Bassila that is recognized as a point of abundance of ticks in Benin (De Clercq et al., 2013; De Clercq et al., 2012), appears to be the second point of relatively high level of resistance behind Kpinnou. This point of abundance of ticks is a grouping point of transhumance. However, it is curious how this grouping point of transhumance show no emerging resistance and instead, an evenly established high level of resistance to deltamethrin and alpha-cypermethrin. Therefore, Bassila sample does not show large phenotypic diversity to deltamethrin and alpha-cypermethrin. The situation should be caused by a rapid regeneration and high number of reproductive cycles of this parasite under favourable environment conditions or continuous deltamethrin and alpha-cypermethrin tick control actions by farmers. This explanation might be the same for the high level resistance of Kpinnou strain relatively to Pyrethroids and it is likely that long time exposure to the related acaricide since the first introduction is of importance. At this step, it raised the necessity of assessing the mechanism of resistance to well understand the comparative evolution of Bassila and Kpinnou samples respectively as transhumance grouping point and first introduction or dissemination point. Many studies used synergists (Chevillon et al., 2007) to master the mechanism, further related experiments should be achieved for Benin sample. Comparatively to New caledonian resistant strain, the samples that shows punctual relatively high level of resistance like Gogounou sample which expressed resistance to deltamethrin and alpha-cypermethrin and Okpara sample that expressed resistance to alpha-cypermethrin, look more resistant. This resistance of Benin samples might not be explained by long time exposure but mainly as consequences of misuses of acaricides (Achukwi et al., 2001). In fact, the repeated use of the same acaricide by farmers in a given area eventually led to serious problems; resistance ticks to some of these acaricides has been reported (Musonge and Tanya, 1987); moreover, it improves fast selection of resistance monotropic and monophasic short-cycle species that are much more likely to develop resistance mechanisms. Indeed, several authors have reported that when generations succeed rapidly for a given species of parasite, the selection of resistant subpopulations is easy; particularly evident in cattle tick *R. microplus* whose

parasitic phase on the host is twenty-one days (Barré and Uilenberg, 2010; Guerrero et al., 2012).

Samiondji host most susceptible samples

Taking into account the susceptibility of Samiondji sample to alpha-cypermethrin and the moderate resistance of the sample to amitraz, Samiondji sample shows the lower resistance ability. The susceptibility of Samiondji sample is also confirmed by the highest slope of deltamethrin dose responsive curve behind the reference strain. However, the change in level of resistance of Samiondji sample according to the acaricide express variation in the mechanisms of tick resistance in one hand and also in the acaricide mode of action in the second hand. This issue is particularly interesting if they take into account the change in level of resistance of Samiondji according to the two different pyrethroids (alpha-cypermethrin and deltamethrin). Assessing this particular change in the mechanism of resistance or susceptibility to the two pyrethroids will help understand the base of mechanisms.

Particular trend of amitraz dose-response relationship

Since the slope of amitraz dose-responsive curves are among the highest of all the dose-responsive curves, it can be concluded that the amitraz dose responsive relationship is particularly significant with LPT. Samiondji, Bassila, and Okpara samples express the same trend than that of the results obtained by Lovis et al. (2013) with LPT. Opposite trend to those results have been found with amitraz by Kemp et al. (1998) and reported by Mendes et al. (2013). In fact, these related authors point out the lower efficiency of LPT test with Amitraz in comparison with organophosphat, pyrethroid and chlorinated hydrocarbon. Therefore, Samiondji, Bassila and Okpara samples show some specificity in comparison with the strains mentioned by Kemp et al. (1998), Miller et al. (2002) and Mendes et al. (2013). Another particularity is the lower size of RR_{90} confidence interval in comparison with RR_{50} confidence interval for the three samples of Benin. Again, this trend is the same than the results reported by Lovis et al. (2013). In fact Lovis et al. (2013) argues inadequacy of RR_{90} to identify amitraz resistance with LTT test. All in one, the differences observed with amitraz should be object of more investigation; especially to access effect of strain and acaricide interactions on dose responsive curves and also on the efficiency of amitraz resistance assessment with the different tests.

Conclusion

The results showed that most of the ticks populations

collected were resistant to alpha-cypermethrin, deltamethrin and amitraz, apart from the sample of Samiondji which is susceptible to alpha-cypermethrin. However, the level of resistance vary according to the sample and the acaricide in such a way that the real challenge remains to get more knowledge about the history and process of the resistance, particularly through deeper comparisons between Bassila and Kpinnou strains. Also, the particular sensitivity of dose response relationship demand more exploration.

All in one, in order to solve resistance phenomenon, there is a great need to consider other alternatives for an efficient and safe control of ticks especially for *R. microplus* currently invading the West African sub-region and Benin. Some molecules of plant extracts have been shown to be effective organs on *R. microplus* and should be further tested. Also, molecules of the latest generation with growth regulators like fluazuron or Spinosyns (spinosad) are little known in the West African sub-region and against which *R. microplus* have not yet developed resistance should receive the same attention.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Socio-economic attributes of guinea fowl production in two districts in Northern Ghana

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The search for a sustainable solution to the growing household food insecurity in many Ghanaian homes calls for an effective harnessing of locally available resources for increased agricultural production. The study was conducted against the background that, although several studies have been conducted on the guinea fowl in Northern Ghana, very little attention has been paid to the socio-economic attributes of production. Such information is critical to understand the behaviour of farmers, especially, about technology adoption by understanding the opportunities and constraints confronting farmers. This allows a more holistic and informed approach to solve the problems of farmers. The study employed descriptive statistic by means of percentages, statistical means and frequency tables. The results indicate that guinea fowl keepers in both districts keep the birds for economic and socio-cultural reasons. Like any other economic venture, guinea fowl production is not without constraints. The main constraints to guinea fowl production include high keet mortality rates, inadequate access to veterinary services, low productivity of local breeds, unstable prices and poor management practices. It is recommended that there should be increased research to increase the productivity of local breeds of guinea fowls.

Key words: Guinea fowl, Ghana, socio-economic.

INTRODUCTION

Guinea fowls in the Ghanaian economy

The domestic guinea fowl (*Numida meliagriss*) is a poultry bird that derives its name from the guinea coast of West Africa where it originated (Moreki and Seabo, 2012; Annor et al., 2012). The commonest variety of guinea fowl raised in Ghana is the Peal helmeted guinea fowl (FAO, 2014). Its origin notwithstanding, the commercial viability of the guinea fowl on the African continent is yet to be realised in full (Moreki and Radikara, 2013). On the

contrary, guinea fowl production has proven to be commercially viable and is raised in large numbers in Europe and the United States of America where it has been successfully commercialized (Cassius and Radikara, 2013; Nahashon et al., 2006). In Africa, Guinea fowls are still raised as free range scavenging birds and have seen little improvement (Dougnon et al., 2012). Guinea fowls are easier to manage by resource poor farmers with hardly any access to formal veterinary services because they are resistant to most poultry

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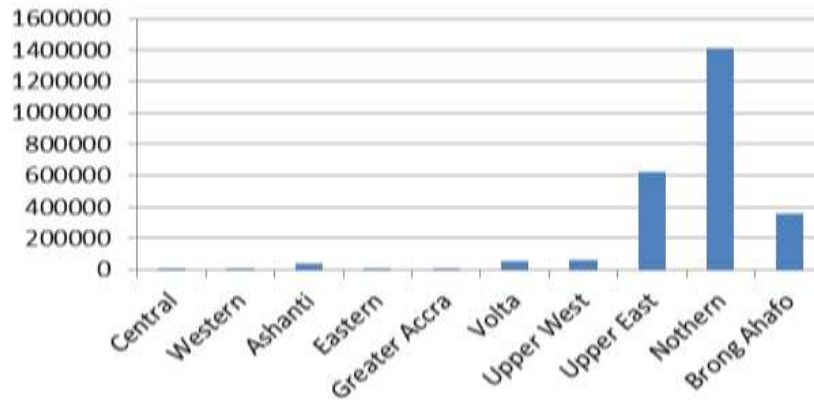


Figure 1. Regional distribution of guinea fowls in Ghana. Source: Adapted from FAO (2014).

diseases at the adult stage (Sayila, 2009). Housing is rudimentary and health management practices depend, largely, on ethno-veterinary medicine (Moreki and Radikara, 2013). Konlan and Avornyo (2013) have shown that 98% of guinea fowl farmers in Benin house their birds only at night and offer them a few handfuls of grains in the morning with the birds scavenging for feed the rest of the day. In Ghana, Guinea fowl production is restricted, generally, to the Northern Savannah zones of the country and is an integral part of the farming system in these areas (FAO, 2014). Guinea fowls are said to be the commonest poultry species in Northern Ghana (Agbolosu et al., 2012). The birds, apart from contributing to household income, play an important role in the socio-cultural lives of the people of Northern Ghana (Teye and Adam, 2000). The bulk of guinea fowls produced in Ghana are raised by subsistence farmers mainly from the rural areas in the Upper West, Northern and Upper East regions all in northern Ghana. The guinea fowl population has been estimated to constitute 7.1% of the total poultry population and 81% of all guinea fowls produced in Ghana (FAO, 2014) (Figure 1).

Guinea fowl meat has assumed a delicacy and meat of choice for most people in Ghana. This has led to increased demand for guinea fowl meat in other parts of Ghana. From the rural areas, in northern Ghana, traders transport large numbers of guinea fowls to the cities and big towns where they are sold to individual consumers and restaurants. It is a common sight to observe grilled guinea fowl at bars and along the roadside for sale in most cities in Ghana. Guinea fowl eggs are a common sight in most rural markets during the laying season. Thus, guinea fowl production plays a significant role in the economies of these districts. In recognition of this, government, under the Savannah Accelerated Development Authority, targeted the promotion of guinea fowl as a strategy to improve household income and to reduce poverty in Northern Ghana. Some other rural intervention programs have targeted guinea fowl

production as a means to poverty alleviation in Northern Ghana. These include the Northern Rural Growth Program that is currently supporting actors in the Guinea fowl value chain. Between 2009 and 2012, GIZ contributed to efforts to improve guinea fowl production in Northern Ghana by addressing key constraints in the guinea fowl value chain. Development of the Ghanaian poultry sector has centred on large-scale industrial chicken production. This has resulted in the neglect of smallholder poultry which produce a considerable proportion of domestic protein needs, especially, among rural folks. Large-scale industrial production of guinea fowls under intensive management is yet to be developed in Ghana. Lately, however, a few commercial Guinea fowl ventures have emerged in southern Ghana (Annor et al., 2012). Although some attempts at large scale, industrial production of guinea fowls have been made in northern Ghana these have failed largely and there is not much to show for it. According to the FAO (2014), exotic guinea fowl production is negligible in Northern Ghana.

Role of socio-economic factors in stimulating technology adoption

The role of socio-economic factors in determining technology adoption has been proven by several studies. A vast literature also exists in the specific case of the influence of socioeconomic factors on the adoption of technology among livestock farmers. Mlote et al. (2013) found that marital status, awareness and attitude towards technology influenced the willingness of pastoralist to adopt beef fattening technology in Tanzania. Mafimisebi et al. (2012) also found a positive relationship between a farmer's age, household size, distance to the nearest veterinary hospital and extent of travel on the utilization of plant medicine among livestock farmers in South-West Nigeria. In a particularly revealing article, Zander et al. (2013) have demonstrated that lack of knowledge,

inadequate support and failure to target local needs and conditions are potentially limiting to technology adoption. Thus, analysing the context within which a technology is diffused via socioeconomic analysis is critical in ensuring improve technology uptake. Several studies have been carried out on guinea fowls in Ghana. Most of these studies, however, failed to address the socio-economic aspects of guinea fowl production. Most studies carried out on the local guinea fowl in Ghana have been in the area of biology and reproductive performance. These include the works of Adjetei et al. (2014), Alidu (2014), Agbolosu et al. (2012), Donkoh and Zanu (2010), Teye et al. (2003), Dei et al. (2009), Dei and Nsawah (2009), Dei et al. (2006), Awotwi (1972) and Laate (1974). This trend was confirmed when several more recent studies that have been carried out by the animal science department of the University for Development Studies were reviewed. Consequently, the purpose of this study is to fill this important gap by examining key social and economic characteristics of guinea fowl production in the Tolon and Builsa North districts in the Northern and Upper East regions of Ghana which are noted for guinea fowl production. Understanding the economic and socio-cultural dynamics of guinea fowl production is important since these economic and social factors have been proven to affect technology adoption among farmers.

METHODOLOGY

Study areas

The Builsa north and Tolon districts are major production and marketing centres for guinea fowl in Northern Ghana. The two districts share many common characteristics. Both are rural in nature with majority of their respective populations depending on crop and livestock farming as their main source of livelihood. They share similar vegetation and climatic conditions which are common to the Northern Savannah Zones of Ghana. The Northern Savannah Zone is largely made up of the Northern, Upper West and Upper East regions. Apart from the regional capitals the area is largely rural with majority of its population depending on subsistence agricultural as the main source of livelihood. The Builsa north and Tolon districts are located in the Upper East and Northern regions respectively.

Builsa North District

The Builsa North District is predominantly rural with agriculture as the main economic activity undertaken by self-employed farmers. About ninety percent (89.2%) of the population is rural. The population of the Builsa North District according to the 2010 Population and Housing Census is 56,477. Females constitute 50.8% and males represent 49.2%. The district lies between longitudes 1° 05' West and 1°35' West and latitudes 10° 20' North. The district covers an estimated land area of 816.44030 km² and has mean monthly temperatures ranging between 21.9 and 34.1°C.

The highest temperatures are recorded in March and this can rise to 45°C, whereas the lowest temperatures are recorded in January. The dry season is characterized by dry Harmattan winds. There is only one rainy season, which builds up gradually from little rains in April to a maximum in August-September, and then

declines sharply coming to a complete halt in mid-October when the dry season sets in. The district is characterized by Savannah woodland and consists mostly of deciduous, widely spaced fire and drought resistant trees of varying sizes and density with dispersed perennial grasses and associated herbs. Common tree species include *Adonsonia* spp., *acacia* spp, *Vitellaria* spp. and *Parkia* spp. which have been retained with time due to their economic importance (Ghana Statistical Service, 2014).

Tolon District

The Tolon District lies between latitudes 9° 15" and 10° 0 02' North and Longitudes 0° 53'and 1° 25' West. The district is characterised by a single rainy season, which starts in late April with little rainfall, rising to its peak in July-August and declining sharply and coming to a complete halt in October-November. The dry season starts from November to March with day temperatures ranging from 33 to 39°C, while mean night temperature range from 20 to 26°C. The mean annual rainfall ranges between 950 to 1,200 mm. The main vegetation is grassland, interspersed with guinea savannah woodland, characterised by drought-resistant trees such as *Vitellaria* spp., *Parkia* spp. and *Adonsonia* spp. The majority of the district's inhabitants are peasant and subsistent farmers (Ghana Statistical Service, 2014).

Sampling and source of data

Five communities each in the Tolon and Builsa North Districts were purposively sampled for the study. The main criterion employed in the selection of communities for inclusion was the relative abundance guinea fowl keepers in the each district. Six guinea fowl keepers were selected randomly from each community making sixty guinea fowl keepers in both districts.

Methods of data collection

A combination of quantitative and qualitative methods of data collection was employed in collecting data for the study to allow for statistically reliable information by way of triangulation. Primary data were collected by means of semi-structured questionnaire. Following the questionnaire administration one focus group discussion was conducted in each community to collect additional qualitative information and to ascertain aspects of data collected via questionnaires with a view to obtaining better understanding of issues. Each focus group discussion consisted of a minimum of 15 and a maximum of 20 persons per community. In order to capture intergenerational and gender differences participants in the focus group discussions were selected carefully to ensure that gender and generational parity was achieved as much as possible. Questionnaires were administered to all the 60 guinea fowl keepers in both districts. The main focus of the questionnaire was to generate quantitative data on guinea fowl performance and socio-economic characteristics of guinea fowl keepers. On the other hand, the focus group discussions served to collect background information and common practices of guinea fowl rearing in addition to helping to clarify the qualitative basis of the quantitative data collected through the questionnaires. Secondary data were obtained from the revenue departments of the respective District Assemblies on the numbers of guinea fowls traded in both districts. From the Ministry of Food and Agriculture, information was obtained on the nature of guinea fowl diseases and extension delivery to guinea fowl farmers. Key informant interviews were conducted among community elders to ascertain historical trends and to seek their perspectives on the findings from both the questionnaires and focus group discussion. A major problem encountered during data

collection was the fact that farmers do not keep records. Consequently, the quality of quantitative data obtained depended very much on farmers' ability to recall. This was especially so with production costs and expenditure on guinea fowl production. This notwithstanding, a great deal of effort was made by the researchers to improve data quality by triangulation. Hence, it became necessary to conduct in-depth interviews with experienced and prominent guinea fowl keepers in both districts to ascertain the reliability of data collected. This yielded positive result as the results, generally, follows trends obtained elsewhere in Ghana.

Method of data analysis

Quantitative data were analysed statistically using frequency tables, statistical means and percentages while qualitative data were analysed simultaneously during data collection. Profitability of guinea fowl production was analysed using Benefit-Cost ratio.

RESULTS AND DISCUSSION

Gender and guinea fowl production

Males dominate guinea production in both districts. Ninety-eight percent of guinea fowl farmers in both districts are male. This is not much different from findings by Konlan and Avornyo (2013) who also reported that males constitute the majority (89.4%) among guinea fowl keepers in the Tolon District. Most females rearing guinea fowls were household heads. Male household heads indicated that their wives do not rear guinea fowls because of disputes over ownership while married women indicated that they do not rear guinea fowls because they do not have full control over them. However, married women usually help their husbands in taking care of guinea fowls, especially keets, while male children provide termites to feed them. Moreki et al. (2010) indicate that majority of beneficiaries of various guinea fowl projects supported by the government in Botswana were female. Although this situation appears to have been influenced by the possibility that females were deliberately targeted, it is an important lesson to the extent that it has demonstrated that females are capable of rearing guinea fowls if given the adequate support. Some married women, in the study area, did not see the need to rear guinea fowls as they prefer to concentrate on off-farm activities such as trading. Thus, it must be recognised that females must not be considered a homogenous group. Females have different needs, priorities and capabilities and the extent to which they participate in livestock rearing have been shaped by culture, tradition and history (Mupawaenda et al., 2008).

Contrary to the situation of women in small-scale crop farming, not much research has been conducted on the role of women in livestock production and this has often hindered effort to address inherent constraints affecting effective participation of women in livestock production (Quisumbing and Pandolfelli, 2010). The right questions must therefore, be asked taking into consideration

contextual and related factors such as culture in shaping responsive policies and interventions for a more inclusive female participation in guinea fowl production.

Age distribution

The age distribution of guinea fowl keepers in both districts is similar. Majority of guinea fowl farmers in both districts are between the ages of 30 and 50 years. This is not surprising since household heads, who are usually advanced in age, are the ones that undertake guinea fowl production. The situation, however, portrays a seeming lack of interest by the youth in undertaking commercial production of guinea fowls. This is reflected in the fact that only 10% of guinea fowl keepers are between the ages of 21 to 30. Further interrogation revealed lack of appreciable commercialization as a reason why the younger farmers are not undertaking guinea fowl production as an income generation activity. High keet mortality also means that it is not possible to predict income from guinea fowl production making guinea fowl as a commercial venture precarious, unreliable and unattractive to the youth who would rather migrate elsewhere to engage in other income generating activities. Younger farmers are more likely to adopt agricultural technology (Sezgin et al., 2011). Sustaining interest in commercial guinea fowl production therefore, requires more effort to make guinea fowl production more attractive to the youth (Table 1).

Literacy level of guinea fowl keepers

Majority (67%) of guinea fowl keepers have no formal education. This result is not peculiar to guinea fowl keepers as illiteracy rate among smallholder farmers in Ghana is generally (Table 2). Access to formal education is an important determinant of livestock production technology adoption (Zipora et al., 2011; Sezgin et al., 2011) to the extent that it has a positive influence on technology adoption. Therefore, the high rate of illiteracy among guinea fowl keepers is a potential drawback to large-scale commercialization of guinea fowl production (Table 3).

Rearing experience

Overall, about 55% of guinea fowl keepers have more than 10 years of experience rearing guinea fowls. It is obvious that guinea fowl farmers have a great deal of experience implying that farmers are repository of knowledge in guinea fowl rearing which can be exploited. In the Tolon district 66.7% of guinea fowl keepers have, at least, 10 years of experience rearing guinea fowls compared to 43.3% in the Builsa district. This is possibly because higher numbers of new entrants are attracted to

Table 1. Age distribution of guinea fowl keepers.

Age (Years)	Frequency	%
21-30	6	10.0
31-40	16	26.7
41-50	27	45.0
>51	11	18.3
Total	60	100

Source: Field survey (2013).

Table 2. Literacy level of guinea fowl keepers.

Educational level	Frequency	%
No formal education	20	67
Basic education	7	23
Secondary Education	2	7
Tertiary Education	1	3
Total	30	100

Source: Field survey (2013).

Table 3. Experience in guinea fowl rearing.

Experience (Years)	Frequency	%
Up to 5	14	23.3
6-10	13	21.6
11-15	9	15
16-20	11	18.3
>21	13	21.6

Source: Field survey (2013).

guinea fowl rearing in the Builsa North district.

Socio-cultural factors

In northern Ghana Guinea, fowls play an important role in the socio-cultural lives of the people (Teye and Adam, 2000). The study revealed that guinea fowls play a significant role in the social and cultural lives of the people in both districts. All the respondents interviewed cited social and cultural obligations among the reasons why they rear guinea fowls. Guinea fowls provide meat for traditional festivals, as well as, other celebrations like Christmas, Easter, and Eid. The birds play important roles in marriage ceremonies and are presented as gifts to important guests. In the both districts, parents-in-law are usually welcome to the homes of their children with guinea fowls. Thus, Guinea fowl production constitutes an important aspect of the culture and tradition of the people in both districts and every farmer keeps a few

birds to meet social and cultural obligations. The major problem with the commercialization of guinea fowls in both districts therefore, is a matter of how to increase production beyond subsistence level.

Flock size

Fifty percent of respondents kept flock sizes of up to 50 birds, 30% kept between 50 and 100 birds and 20% kept more than 100 birds. The mean flock size is 33 in the Tolon District and 40 in the Builsa North District. About forty-three percent of guinea fowl farmers in the Tolon district had flock sizes of up to 50 birds. Another 43.3% had flock sizes of between 50 and 100 with 13.3% of farmers having flock sizes of more than 100. Comparatively, 56.7 of guinea fowl keepers in the Builsa North district had flock sizes of up to 50 while 16.6 and 26.7% had flock sizes of 50 to 100 and above 100 respectively. By all indications therefore, guinea fowl production is on a relatively larger scale in the Builsa North District than in the Tolon District. High keet mortality rates largely accounts for the relatively small flock size. However, the wide disparity in flock size among respondents recorded during the study is a reflection of the level of skill and experience among guinea fowl keepers. The study revealed that some farmers are noted in, every community, as gifted with livestock rearing in general. The study confirmed that such farmers are, indeed, highly skilful in guinea fowl production and usually inherit rearing knowledge from their forbearers.

Management practices

Farmers in both districts raise guinea fowls under the free-range system with varying levels of supplementary feeding. The birds are left to scavenge for food in a boundless area and return home in the evenings to sleep in coops, usually small huts with thatch roofs, roost in trees or rooftops. Keets are provided intensive care for the first six weeks of age. The common practice in both districts is that keets are carried along to the farm daily and left to scavenge for feed while the keepers work on their farms until evening when they are brought home. This saves farmers much time in caring for the keets. Keets' diet is supplemented with sorghum grit or ground maize and termites. On the other hand, less attention is given to adult birds. Adult birds are fed with a few handfuls of sorghum or maize thrown on the ground for the birds to pick. Adult birds are fed with the main aim of taming them or attracting them to return home. Overall, 71.7% of guinea fowl keepers feed their birds three times a day. Ninety-three per cent of respondents fed their birds three times daily in the Builsa North District compared to 50% in the Tolon district. 43.3% of guinea fowl keepers in the Tolon District fed their birds two times

daily. Comparatively, guinea fowl keepers are more commercially oriented in the Builsa North District. This, possibly, accounts for the reason why farmers in the Builsa North District attach more importance to feeding their birds more regularly.

Access to extension services

Access to extension services is a major driver in the adoption of agricultural innovation. However, access to extension services by guinea fowl farmers is very poor as revealed by this study. Agricultural extension, in general, has witnessed dwindling prospects over the years as a key driver of agricultural innovation in Ghana due to a major drift in agricultural policies over the years. The result is that public delivery of veterinary services, like other sectors, has received progressively dwindling financial and human resources while there has been little investment in physical infrastructure (Amankwah et al., 2014). Employment in the agricultural sector, like other sectors, has stagnated in recent years. The expectation was that private service providers would fill the gap. Unfortunately, this has not been the case as rural poultry farmers in Ghana still rely, to a large extent, on public veterinary services (FAO, 2014). All the respondents interviewed in both districts decried the lack of veterinary extension services as a major constraint to guinea fowl production. Unlike crop production the participation of private extension services, notably NGOs, is lacking in the case of guinea fowl production. The recent state sponsored interventions aimed at improving guinea fowl production in Ghana have rather concentrated on increasing production without adequate attention to the provision of complementary services such as veterinary services. Thus, poor access to veterinary services by guinea fowl farmers in Ghana is rather institutional.

Reproduction

The best time to raise keets in both districts is from March to June and September to October when there is abundance of feed for the birds to scavenge on and the warm weather makes brooding conducive. Using brooding chicken hens to incubate and raise keeps is the common practice in both districts. Guinea fowl keepers in both districts indicated that they do not allow their birds to hatch and raise keets because they would lose all keets in the bush while scavenging through carelessness and long treks. In addition, keets hatched and raised by guinea fowls are difficult to tame. Farmers also indicated that when guinea fowls are allowed to go broody it curtails their ability to lay eggs early. Breeding stocks are usually birds left over from the previous season's flock. Respondents revealed that the hatchability rate when ducks are used to incubate guinea fowl eggs is much

higher than that of chicken hens. A duck is capable of incubating an average of 30 guinea fowl eggs compared to an average of 20 for chicken hens. The disadvantage of using ducks to incubate guinea fowl eggs, however, is that there is the likelihood of losing all the keeps through drowning if they are not taken away immediately upon hatching as ducks would usually take the keeps out to swim. Moreover, ducks often peck and kill keeps. A male: female ratio of 1:4 to 1:6 is the case in the Talon and the Builsa North Districts respectively. Adam (1997) reported the commonest male: female ratio in the Damango District, also in northern Ghana, is 1:4. Guinea fowl keepers in both districts indicated that higher ratios are possible. This, however, is not desirable as the flock is dispersed making it difficult to trace eggs and also results in fighting among males over the females.

Breeding season

The laying season for guinea fowls in both districts is during the months of March to September when temperatures are relatively mild. The egg laying ability of eggs by guinea fowls is influenced by environmental factors and the feeding regime. Some farmers indicated that guinea fowls are capable of laying outside the main laying season if fed well. In addition, guinea fowls raised near large water bodies are said to be capable of laying continuously throughout the year due to favourable temperature conditions. This is supported by Konlan and Avornyo (2013) who reported that the egg laying ability of guinea fowls found in communities near water bodies in the Tolon District was significantly higher than those found in drier environments were.

Hatchability

Hatchability appears to be directly related to egg fertility rate since the hatchability rate of 72% reported in this study compares favourably with a fertility rate 77.4 and 80.4% for the Upper East and Northern Regions respectively as reported by Agbolosu et al. (2012). However, the study could not confirm this since it is based on respondents' accounts. More than 97% of respondents in both districts indicated that they could not achieve their desired flock size mainly due to poor hatchability of eggs and high keet mortality rate. The commonest source of eggs for incubation is the market. Farmers, usually do not have any idea of the source of eggs or how long eggs have been kept prior to sale. Guinea fowl keepers also indicated that eggs laid early in the laying season are more viable than those laid later in the season are. Some farmers attributed the poor hatchability of eggs to the inefficiency of some brooding hens. These reasons notwithstanding, the most important reason cited by farmers for the poor hatchability is the

Table 4. Main causes of keet mortality.

Cause of mortality	Frequency	%
Diseases	57	95.5
Rain/Cold	12	20.0
Predators/theft	15	25.0

Source: Field survey, 2013.

low fertility of eggs. Thus, it is important that any serious attempt to commercialize guinea fowl production in Northern Ghana should be accompanied by a breeding program that will ensure the availability of fertile and certified eggs for incubation.

Keet survival

The most delicate period for keets is from day-old up to six weeks of age when high keet mortality rates are recorded. On the average, 34 and 32 keets are lost per keeper annually in the Tolon and Builsa North Districts respectively. These figures are significantly high compared to the mean flock size of 33 and 40 in the Tolon and Builsa North Districts respectively. It is common for a farmer to lose all of his/her stock of keets. Teye and Gyawu (2001) have reported keet mortality of up to 100% in Northern Ghana. This suggests that losses to guinea fowl keepers, due to keet mortality are enormous. Three factors were found to be responsible for the high keet mortality. These are indicated in Table 4.

Diseases constitute the most important cause of keet mortality. Apart from 10% of farmers in the Tolon district who employ veterinary services in treating their birds, all other respondents mentioned diseases as the major cause of keet mortality. Although bad weather and predators/theft were mentioned these did not constitute major causes. Access to veterinary services is therefore, critical to reducing the high keet mortality rates experienced by farmers.

Laying performance

The average age of first lay in both districts is 29 weeks. Guinea fowls raised under intensive management in Ghana start laying between 32 and 40 weeks of age (Awotwi, 1987). Teye and Gyawu (2001) reported in their study of the performance of guinea fowls in northern Ghana that guinea fowls are capable of laying at 22 weeks under improved management conditions. In the Tolon District, farmers reported age of first lay of between 24 and 32 weeks with 70% of farmers reporting 28 weeks. In the Builsa North District, farmers reported the age of first lay of between 24 to 40 weeks with the majority (47%) reporting 32 weeks. Majority of farmers in

both districts (58 and 75% of farmers in the Tolon and Builsa North Districts respectively) reported collecting up to 100 eggs per guinea fowl per annum. Number of eggs collected ranged between 50 and 250 eggs and 51 to 210 eggs per guinea fowl per annum in the Tolon District and Builsa North Districts respectively. An estimated 20% of eggs are lost to theft and predators as most of the eggs are laid in nearby bushes. This brings the total number of eggs laid to between 252 and 300 eggs annually. The result is similar (301) to that reported by Agbolosu et al. (2012) for the Upper East region but lower (362) than that of the Northern region. Teye and Gyawu (2001) in an earlier study of the guinea fowl in northern Ghana recorded 200 to 300 eggs per guinea fowl per annum. Generally, free ranging birds have been reported to lay fewer eggs (Sonaiya, 1998). As discussed earlier guinea fowl, keepers in the Builsa North districts feed their birds more frequently than those in the Tolon districts. There is therefore, the possibility that the feeding regime has contributed to better laying ability of guinea fowls in the Builsa North district.

Marketing

Beside the need to keep a few guinea fowls to meet social and cultural obligations, guinea fowls are kept mainly for cash in both districts. This reflects in the fact that only 3.3 and 6.7% of guinea fowl keepers in the Tolon and Builsa North District respectively, mentioned meat and eggs for home consumption among reasons why they rear guinea fowls. The rest of the respondents in both districts mentioned income as their main reason for keeping guinea fowls. It is common practice for rural people in northern Ghana to rear guinea fowls, like other livestock, as a traditional option for investment as they provide a readily convertible source of immediate cash for most households. The study confirmed that the sale of guinea fowls and eggs provide substantial income for farmers in both districts. Majority of farmers in both districts indicated that their birds are usually ready for the market at about 28 weeks of age. The need for immediate cash is the major determinant of the age at which guinea fowls are sold. While some farmers sold their birds after 20 weeks of age, others kept their birds up to 52 weeks. An average of 17 and 26 birds were sold per keeper annually in the Tolon and Builsa North District respectively. This accounts for 52 and 65% of total flock size in the Tolon and Builsa North Districts respectively. Thus, this confirms the fact that commercialization is not the sole reason why farmers keep guinea fowls as discussed above. Moreover, farmers rely on their current stock for breeding and therefore retain some birds for that purpose. Guinea fowls are sold mainly live in local markets, usually, on market days. The urban centres and major cities are main destinations for guinea fowls in Ghana. The birds are usually sold live. Information collected from the revenue collection unit of both district

Assemblies show that during the peak production season an average of 1,498 and 1,150 live guinea fowls are transported by traders from the Builsa North and Tolon Districts, respectively, every week for sale in other parts of the country. This brings to a total of, at least, 77, 896 and 58,800 guinea fowls traded annually in the Builsa North and Tolon District respectively. Like other livestock guinea fowl meat is hardly processed for sale. This marks a major challenge confronting local agricultural production in general. Thus, they are unable to compete favourably with processed imported meat products that have seen an increase in recent years.

Profitability of guinea fowl production

Average net returns from the sale of guinea fowls ranged from USD 532 to UDS 1,750 per annum. The sale of live guinea fowls constituted 70.7% while sale of eggs constituted the remaining 39.3% of total revenue from guinea fowl production. Returns from the sale of eggs constituted a smaller proportion because a substantial number of eggs are lost to predators in the bush and theft. Some eggs are also reserved for incubation. Of the total cost of production, cost of eggs used for incubation constituted 76.4% while the cost of housing constituted 14.6%. The cost of supplementary feeding was 10% of total cost of production. The cost of medication and treatment were assumed negligible as keepers in both districts hardly purchased drugs for treating their birds or employed the veterinary services. Overall, the benefit cost ratio of 8.2 indicates that guinea fowl production is profitable and provides a good return on investment.

Conclusion

The study has demonstrated that farmers rear guinea fowls mainly for cash beside the need to meet social and cultural obligations. Therefore, the fundamental conditions exist for effective commercialization guinea fowl production in both districts since the will exists among farmers to commercialize. Income from guinea fowl production can result in considerable reduction in household food insecurity in the rural areas of northern Ghana if efforts are made to improve the management conditions under which they are currently raised. The potentials of the guinea fowl production as a commercial venture are enormous due to high and increasing demand of guinea fowl meat as meat of choice by most Ghanaians. Besides, it is profitable as shown by this study. This development is significant against the background that the production and demand for exotic guinea fowl production in Ghana is low (FAO, 2014). These potentials can be realised if guinea fowl production is developed under smallholder farmer conditions and not necessarily large scale intensive management conditions.

Therefore, research should rather be directed at improving guinea fowl guinea fowl production under farmer conditions, as these systems are well adapted to the farming system and is profitable. Any attempt to promote guinea fowl production without providing the necessary conditions such as access to adequate veterinary service, drugs, feed supplements and proper housing conditions will alienate large numbers of smallholders who do not have the resources to invest adequately in an intensive production system. Again, as demonstrated by this study, socio-economic and cultural parameters, largely defined by contextual factors, are important in innovation and technology development. These and institutional constraints such as inadequate access to extension services must be taken into consideration to enhance technology adoption by guinea fowl farmers.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Bentonite application in the remediation of copper contaminated soil

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The concern on heavy metals from commercial fertilizers for fertilization of crops when they are intended for human consumption has increased specifically, horticultures and grains. Several procedures have been proposed to reduce the concentration of heavy metals in the soil; among these are, the application of materials that are able to adsorb these elements, making them less available to plants. This study aimed to evaluate the bentonite for remediation of artificially copper contaminated soils, grown with beets, radish and corn. The experiment was conducted in a greenhouse in a completely randomized design, with four replicates. A loamy sand soil planted with radish and corn was contaminated with 100 mg kg⁻¹ of copper, while for beets, the soil was contaminated with 250 mg kg⁻¹ of copper as copper sulphate (Cu₂SO₄). Bentonite treatments consisted of four doses of bentonite: 0, 30, 60 and 90 t ha⁻¹. The copper content in the soil and in plants, as well as the translocation index in the plants was evaluated. The results were analyzed by the F test and polynomial regression was used for adjustment of significant data. Bentonite decreased the copper content in the dry phytomass of the plants, affected significantly the copper accumulated in the roots of beets and radish, and in the aerial part of radish. The copper translocation index in beets reduced with bentonite doses, and consequently the quantity of copper on beets was higher than those levels permitted for human consumption. Application of bentonite in contaminated soils grown with radish and corn improved their amelioration; on the other hand, the soil grown with beets did not present any amelioration.

Key words: Heavy metal, accumulation, vegetables.

INTRODUCTION

The concern on heavy metals from commercial fertilizers is still more preoccupant when they are intended for

human consumption, specifically horticultures and grains. Plants are the main entrance of heavy metals in the

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feeding chain due the relative absorption ability of their roots (Guimarães et al., 2009).

Soil contamination by heavy metals can occur immediately by the great release of heavy metals to the environment. In addition, the contamination can be observed with the accumulation of metal in nature throughout decades, producing irreversible damages in most cases. Thus, several researches have been conducted to study, prevent or minimize health problems due to heavy metals contamination and to remediate areas already contaminated.

Remediation of soils contaminated with heavy metals needs the adoption of technics that provide a decrease in the availability of these metals for the plants. These technologies vary according to the characteristics of the soil, the nature of the contaminant, the degree of contamination and the financial conditions (Tavares et al., 2013). Several adsorbent materials, such as clay minerals have been evaluated to reduce the contaminant effect of heavy metals (Ghorbel-Abid et al., 2010; Jiang et al., 2010; Bhattacharyya and Gupta, 2007), indicating some advantages of these materials such as low cost, availability and efficiency when compared with other adsorbent materials. Bentonite can also be used as a chemical and physical conditioner due to the high cationic exchange capacity, as it has been already reported by Tito et al. (1997) and Tito et al. (2001).

Mainly, minerals from the smectite clay group and quartz impurities constitute bentonite clays, and some bentonites present caulinite and illite (Sdiri et al., 2011). In the State of Paraíba, Brazil, mainly in Boa Vista Municipal region, there are great amounts of a bentonite mineral known as “bofe”, whose characteristics are not adequate to the industry requirements, and therefore less commercialized. In this sense, this “bofe” bentonite could be used to meliorate contaminated soils with heavy metals.

Copper is an essential element for plant growth; however, elevated concentrations can produce drastic alterations in plant cells, affecting plant development (Giroto, 2010). According to Kabata-Pendias and Pendias (1992), copper content greater than 100 mg kg^{-1} is considered excessive in the soil and may cause phytotoxicity. The maximum content of Cu from which there is risk to human health and to the environment varies greatly and depends on the laws of each country. In Brazil, the permissible maximum Cu value for agricultural soils is 200 mg kg^{-1} (CONAMA, 2009). Copper moves slowly in the soil, generally as an organic complex and remains at the soil surface (Paganini et al., 2004). Tito et al. (2012) evaluating the effect of the bentonite on the zinc and copper mobility in an Argissol verify that the copper was strongly adsorbed as a soil/bentonite complex.

The objective of the present work was to evaluate the contamination of soils and plants with copper and the effect of “bofe” bentonite application on the melioration of

copper contaminated soils, by evaluating the production of beets, radish and corn.

MATERIALS AND METHODS

This study was carried out under semi controlled greenhouse conditions, from March 2014 to June 2014 at the Agricultural Engineering Department, Federal University of Campina Grande, Paraíba, Brazil. The experiments were conducted with beets (*Beta vulgaris*), radish (*Raphanus sativus*) and corn (*Zea mays* L.) on a loamy sand soil classified as a Red Eutrophic Latossol (Embrapa, 2006), collected in Campina Grande region at a 0-20 cm soil depth. After collecting the soil, samples were air-dried, crushed, sieved through a 2 mm sieve and analyzed using the procedures recommended by Embrapa (1997). The following attributes were found: pH (H_2O) = 6.0; electrical conductivity = $0.16 \text{ (mmhos cm}^{-1}\text{)}$; Ca = $2.10 \text{ cmol}_c \text{ kg}^{-1}$; Mg = $2.57 \text{ cmol}_c \text{ kg}^{-1}$; Na = $0.06 \text{ cmol}_c \text{ kg}^{-1}$; K = $0.14 \text{ cmol}_c \text{ kg}^{-1}$; H+ Al = $1.78 \text{ cmol}_c \text{ kg}^{-1}$; organic carbon = 5.5 g kg^{-1} ; P = 45.0 mg kg^{-1} and Cu = 0.355 mg kg^{-1} .

Soil samples were placed in 5 kg plastic pots for beets and radish; and for corn, 14 kg pots were used. The doses of copper used in this study were; for beets, 250 mg kg^{-1} ; for radish and corn, 100 mg kg^{-1} . Nitrogen, phosphorus and potassium fertilization for beets and radish was 1.11 g of urea, 1.25 g of potassium chloride (KCl) and 8.3 g of super phosphate (P_2O_5). Nitrogen (N), phosphorus (P) and potassium (K) fertilization for corn was 3.11 g of urea, 3.5 g of potassium chloride (KCl) and 23.33 g of super phosphate (P_2O_5). The 100 mg kg^{-1} copper dose applied to the soil was based on not published results of the effect of copper doses on the emergency of radish and corn.

After the NPK fertilization, copper and bentonite were applied to the soil, which was conditioned in the plastic containers. Water was applied to the soil until the field capacity of soil and it remained incubated during 20 days under this soil moisture. After the incubation period, the seeds of each crop were sown and, 8 days after the emergency of seedlings, a thinning was conducted leaving two plants per pot. Four doses of bentonite were used: 0.0; 10.7; 21.4 and 32.1 g kg^{-1} , corresponding to 0, 30, 60 and 90 t ha^{-1} , respectively. The bentonite used in this study was “bofe” bentonite clay from a Paraíba State region. The X-rays diffractogram of this bentonite is presented in Figure 1.

The diffractogram picks observed are typical of the smectite (S) clays, and picks of tridymite (T), a silicate mineral and polymorph of high temperature of quartz. Picks of quartz are observed although in a low quantity.

The irrigation was carried out using tap water to maintain the soil moisture to field capacity. At 30 and 60 days of experimental period, the plants were harvested and separated into aerial part and roots, washed with distilled water, and placed in paper bags in order to be dried in forced air stove at 65°C during 48 h. After drying, the plants were triturated and samples were weighed for foliar analyses. Plant samples were submitted to copper determination conducted after nitric-perchloric digestion, according to Embrapa procedures (Embrapa, 1997), using a Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES), as described by Oliva et al. (2003). The translocation index (TI) was determined by using the follow expression (Abicheque and Bohnen, 1998):

$$\text{TI} = \frac{\text{Amount of copper accumulated in the aerial part of the plant}}{\text{Amount of copper accumulated in the complete plant}} \times 100$$

Soil samples were collected from each experimental unit and the copper content was determined using the Mehlich-1 extractor (Embrapa, 1997).

The experimental design was a completely randomized design

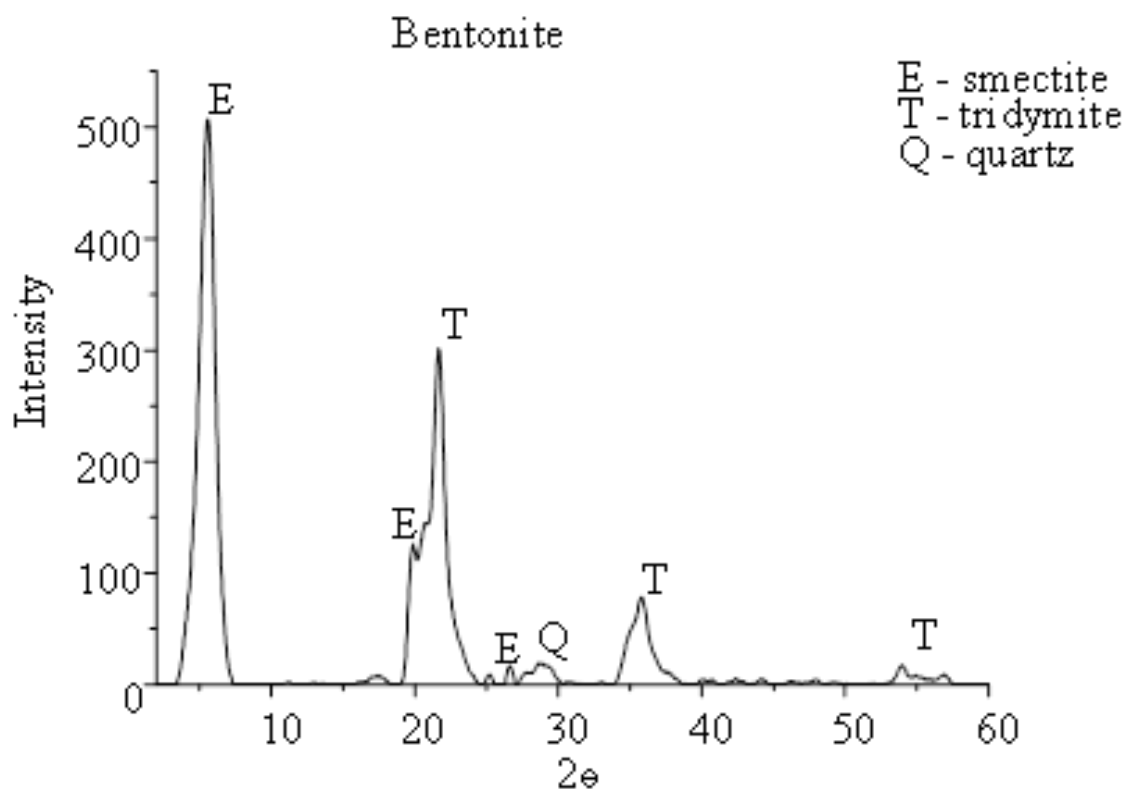


Figure 1. X-rays diaphactogram of the “bofe” bentonite clay.

Table 1. Summary of the analyses of variance for the dry phytomass of the aerial part and roots of the beets, radish and corn according to the different bentonite treatments.

Source of variation	DF	Mean Square					
		Beets		Radish		Corn	
		ADPB	RDPB	ADPR	RDPR	ADPC	RDPC
Bentonite	3	0.514 ^{ns}	43.02 ^{**}	0.80 ^{**}	1.01 ^{**}	84.50 ^{**}	4.85 ^{ns}
Linear	1	1.169 ^{ns}	120.95 ^{**}	1.53 ^{**}	2.48 ^{**}	236.25 ^{**}	14.12 [*]
Quadratic	1	0.154 ^{ns}	4.67 ^{ns}	0.68	0.005 ^{ns}	6.91 ^{ns}	0.02 ^{ns}
Error	12	0.579	1.69	0.60	0.14	5.09	2.86
VC (%)		10.17	19.58	9.35	19.71	2.53	10.49
Mean (g)		7.48	6.64	2.70	1.90	89.29	16.14

DF = Degree of freedom, ^{ns}, * and ** not significant, significant at 5 and 1% levels, respectively. VC = variation coefficient. ADPB, RDPB, ADPR, RDPR, ADPC and RDPC: Aerial dry phytomass of beets, root phytomass of beets, aerial dry phytomass of radish, root phytomass of radish, aerial dry phytomass of corn and root phytomass of corn, respectively.

with four replicates, totaling 16 experimental units (plastic pots). SISVAR statistical program (Ferreira, 2011) was employed to analyze the obtained results, by using the F test and regression polynomials, which were used to adjust the data when significant.

RESULTS AND DISCUSSION

With the exception of beets, the dry phytomass of the

aerial part of the radish (ADPR) and corn (ADPC) was significantly affected by the bentonite application at 1% significance level. With the exception of the corn, the bentonite treatments affected significantly the dry phytomass of aerial part of the beets (ADPB) and radish (ADPR) at 1 and 5% significance levels, respectively (Table 1).

With the exception of the dry phytomass of the aerial

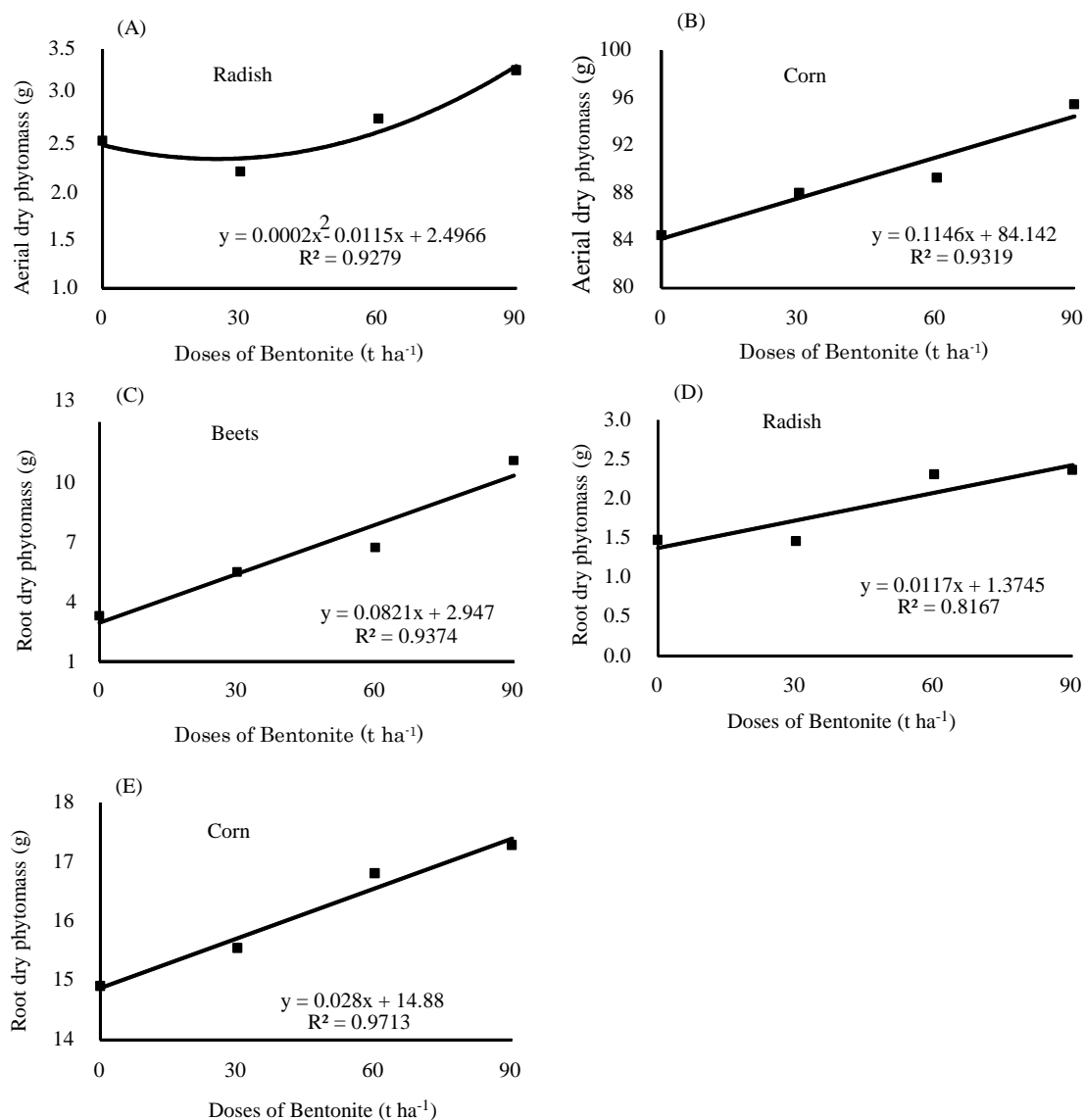


Figure 2. Dry phytomass of the aerial part for the radish (2A) and corn (2B) and dry phytomass of the roots for beets (2C), radish (2D) and corn (2E) according to bentonite doses.

part of the corn, which was adjusted to a quadratic model, the significant bentonite effects were adjusted to linear regression models (Figure 2). The dry phytomass of the aerial part of the radish (ADPR) presented the maximum value with the highest bentonite treatment, which was 23.43% greater than the control (dose 0) (Figure 2A). The dry phytomass of the aerial part of corn (ADPC) presented the maximum value (17.40 g) with the highest bentonite treatment; and the lowest value (14.88 g) with the control, an increase of 14.48% (Figure 2B). The dry phytomass of beet beets, radish and corn roots increased with the bentonite application, reaching increases of 250.00, 76.61 and 16.93% when comparing the zero bentonite dose (the lowest phytomass) to the higher bentonite dose (Figures 2C, D and E,

respectively). The increase of the dry phytomass of the aerial part and roots for beets, radish and corn with the bentonite application can be explained because the clay increased the adsorption of the copper in soil reducing its availability to the plant roots. The results corroborate with Kabata-Pendias and Sadurski (2004), who found that the mobility of Cu from soil to plant decreased due the presence of bentonite in the soil.

The increase of the soil adsorption capacity for copper due to bentonite application reduced the availability of copper in the soil solution, and therefore favored the growth of the crops and increased the dry phytomass of the aerial part and roots, corroborating Llorens et al. (2000) and Qian et al. (2005). According to these authors, the presence of high copper concentrations in

Table 2. Summary of the analyses of variance for the copper concentration in the aerial and root part of the beets, radish and corn for the different bentonite treatments.

Source of variation	DF	Mean Square					
		Beets		Radish		Corn	
		BACC ¹	BRCC	RACC	RRCC	CACC	CRCC
Bentonite	3	0.31 ^{ns}	178.37*	2.20**	1615.77**	2.86 ^{ns}	2046.37*
Linear	1	0.33 ^{ns}	365.51**	5.55**	3158.59**	2.58 ^{ns}	5474.74**
Quadratic	1	0.0003 ^{ns}	169.26 ^{ns}	0.24 ^{ns}	364.49**	4.12 ^{ns}	664.09**
Error	12	0.43	37.49	0.10	24.47	1.46	518.52
VC (%)		8.43	9.97	18.49	14.05	19.13	19.93
Mean (mg kg ⁻¹)		7.80	61.44	1.70	35.21	6.32	114.23

^{ns}, * and ** not significant, significant to the 5 and 1% level, respectively, VC = Variation coefficient. ¹ Data transformed in \sqrt{x} . BACC, RACC, CACC = Copper concentration in the aerial part of beets, radish and corn, respectively. BRCC, RRCC, CRCC = Copper concentration in the roots of beets, radish and corn, respectively.

soil can influence the plant metabolism and the proper absorption of others nutrients, affecting negatively, the growth of plants.

The bentonite doses had significant effect on beets, radish and corn roots at 5, 1 and 5%, respectively, but there was no significance for beet and corn aerial part (Table 2). According to Marques et al. (2002), copper concentration in roots that is toxic for plants ranges from 60 to 125 mg kg⁻¹, thus the high concentrations found on the beet roots (mean of 61.44 mg kg⁻¹) and corn roots (mean of 114.23 mg kg⁻¹) can be considered as non-toxic for the plants. However, the root concentrations of copper found in the beets and corn are toxic for human consumption, according to the Brazilian Association of Feeding Industries (ABIA) (ABIA, 1985). According to the ABIA (1985), the tolerant limit of copper for roots, horticultures, tubercles and other fresh foods is 30 mg kg⁻¹.

The copper concentration of the beet roots decreased linearly from 69.95 to 54.43 mg kg⁻¹ when bentonite application varied from 0 to 90 t ha⁻¹ (Figure 3A). For the aerial part, copper concentration also decreased linearly with the bentonite application decreasing from 2.5 to 0.91 mg kg⁻¹ when the bentonite doses varied from 0 to 90 t ha⁻¹ (Figure 3B). The copper concentration in the roots of the radish varied exponentially with the bentonite doses, by increasing from 44.96 to 50.60 mg kg⁻¹ for doses of 0 to 30 ton ha⁻¹; and decreased to 7.34 mg kg⁻¹ when the bentonite dose was 90 ton ha⁻¹ (Figure 3C). In the corn roots, copper concentration varied linearly with the bentonite doses, by decreasing from 139.05 to 89.41 mg kg⁻¹ when the bentonite dose was 90 ton ha⁻¹ (Figure 3D). It is shown in Figures 3A, C and D that copper concentration in the roots of beets, radish and corn were higher than the permitted levels for human consumption recommended by ABIA (1985). According to ABIA (1985), the tolerant limit of copper for roots, horticultures, tubercles and other fresh foods is 30 mg kg⁻¹. It is also observed that the copper concentration of the roots of

radish decreased to below 30 mg kg⁻¹ for bentonite doses greater than 60 t ha⁻¹, pointing out that this application increase the soil adsorption capacity of copper, decreasing the availability of copper in the soil solution and, consequently, decreasing the absorption of copper by plants.

The results found for corn corroborates Marques et al. (2002) and Mantovani (2009). Mantovani (2009) evaluated corn grown in a soil contaminated with 202 mg kg⁻¹ Cu and a great copper concentration was observed in the roots (502 mg kg⁻¹); however, the aerial part presented low copper concentrations, below 30 mg kg⁻¹, which is the toxic limit for human consumption according to the ABIA (1985).

The copper concentration in the roots was much higher than in that the aerial part of the plants as evaluated in this study (Table 2). This fact can be attributed to physiological mechanisms presented by plants in order to prevent the translocation of the copper from the roots to the aerial part (Cornu et al., 2007). According to Marsola et al. (2005), this phenomenon would be a tool that plants present as a protection for copper intoxication. Loneragan (1981) and Tiffin (1972) observed that root tissues present a higher capability to hold copper and prevent the copper translocation to shoots, both for copper deficiency and excess. These authors concluded that the copper excretion from root cells to xylem and phloem is a key process for plant nutrition. Bentonite affected significantly at 1% probability level the accumulation of copper in roots of beets and on the aerial and roots of the radish (Table 3). The regression curve presented in Figure 4A shows a linear increase for copper accumulation in the beet roots with bentonite doses, ranging from 0.219 mg with the 0 t ha⁻¹ to 0.562 mg for 90 t ha⁻¹, corresponding to an increase of 156.16%. It is important to highlight that although the copper concentration in the root decreased with the bentonite application, the accumulated copper found in the roots increased, this is because the accumulative

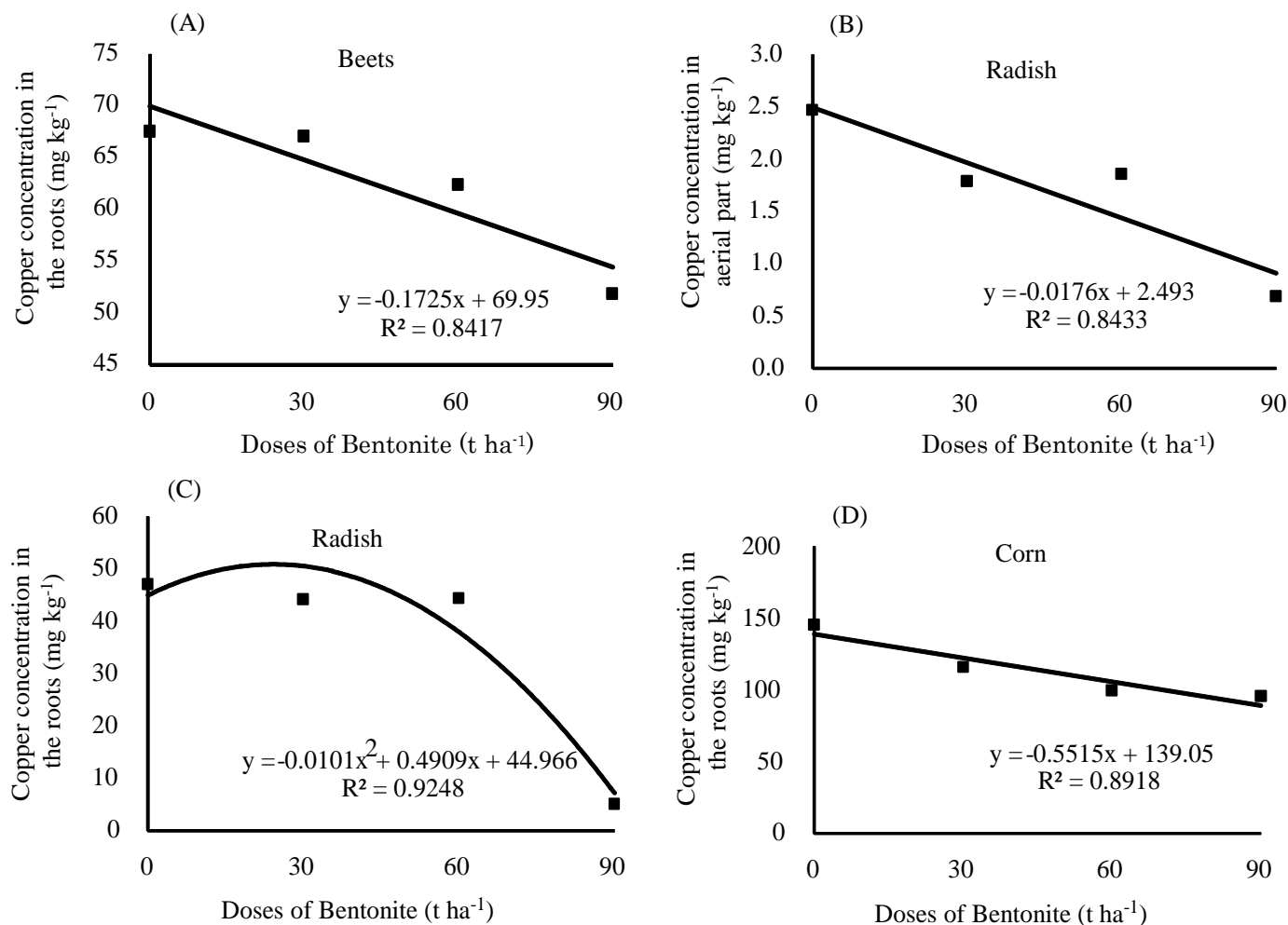


Figure 3. Copper concentration in the roots of beets (3A), in the aerial part of the radish (3B), in the roots of radish (3C) and in the roots of corn (3D) at the end of the experiment, according to bentonite doses.

copper was calculated based on the plant dry phytomass, which increased with bentonite application. The regression curve in Figure 4B shows an increase of the copper accumulated in beet roots until 40 ton ha⁻¹ approximately, and a decrease of 68.35% for the 90 ton ha⁻¹ when compared with the results. The regression curve in Figure 4C shows a linear decrease of copper accumulated in the aerial part of the radish with the bentonite doses, varying from 0.0873 mg with 0 t ha⁻¹ to 0.051 for the 90 t ha⁻¹ of bentonite, corresponding to a decrease of 41.58%. As a result of the accumulated copper calculated based on the dry phytomass of the plant, similar amount of copper accumulation was observed in the roots and shoots because the dry phytomass in the aerial part of the plant (2.70 g) was much higher than that in the roots (1.90 g).

Bentonite application affected significantly at 1% level of probability, the translocation index of the copper in beets and radish (Table 4). The translocation index is the percentage of the metal absorbed by the plant and

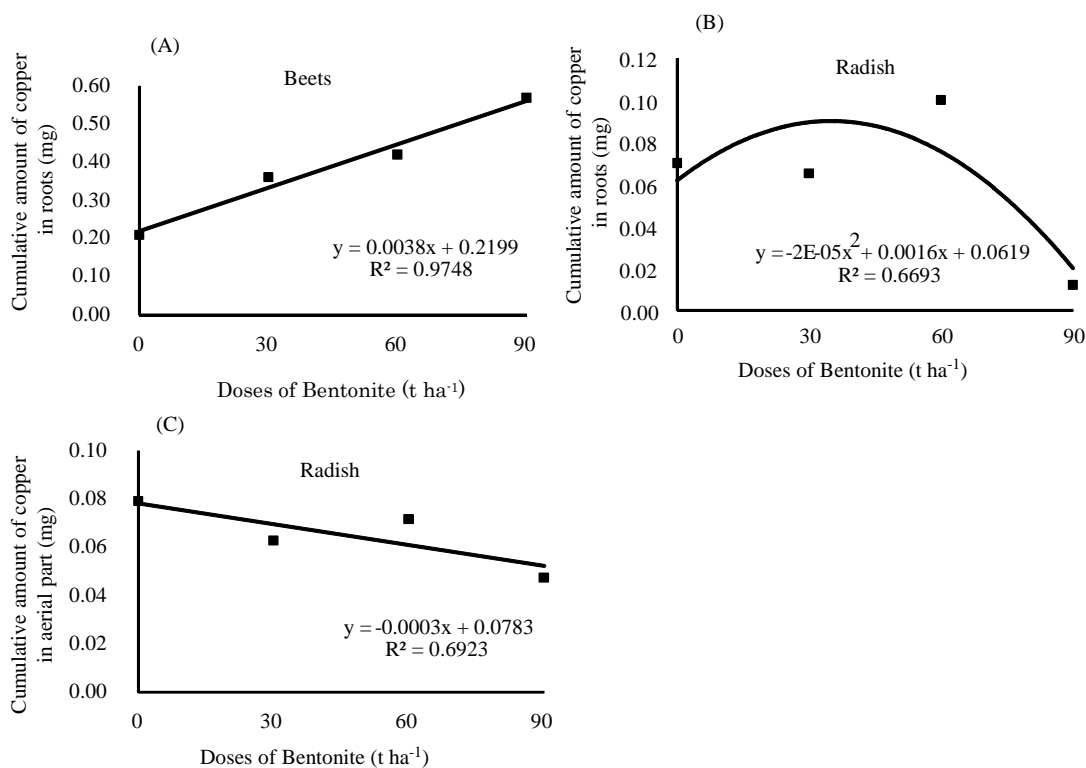
transferred to the aerial part (Abichequer and Bohnen, 1998). The bigger the index, the greater the translocation. The translocation index of the copper in the beets decreased linearly with bentonite application, varying from 67.17% for the 0 ton ha⁻¹ to 43.62% for the 90 ton ha⁻¹ of bentonite application, a decrease of 35.06% (Figure 5A). Based on the definition of the translocation index and the results of the significant regression (Figure 5A), it is observed that the translocation of copper on beets decreased with bentonite application, being accumulated in the roots and not transferred to the aerial part of the plant. The results corroborate Kabata-Pendias and Pendias (1992) who said that the copper is an unmoved element because it is strongly fixed by the root cellular walls. This is probably the reason why a great quantity of copper was found in the beet roots making it inappropriate for human consumption, higher than the permitted levels for human consumption as recommended by the ABIA (1985).

The application of bentonite in the soil grown with

Table 3. Summary of the analyses of variance for the accumulated copper in the aerial and root part of the beets, radish and corn for the different bentonite treatments.

Source of variation	DF	Mean square					
		Beets		Radish		Corn	
		BACAP	BACR	RACAP ¹	RACR	CACAP	CACR
Bentonite	3	0.005 ^{ns}	0.089**	0.0007**	0.006**	0.020 ^{ns}	0.220 ^{ns}
Linear	1	0.00002 ^{ns}	0.259**	0.002**	0.004**	0.001 ^{ns}	0.523 ^{ns}
Quadratic	1	0.0002 ^{ns}	0.00001 ^{ns}	0.00006 ^{ns}	0.007**	0.038 ^{ns}	0.133 ^{ns}
Error	12	0.009	0.006	0.0001	0.0001	0.010	0.139
VC (%)		20.86	20.01	14.06	19.23	18.43	20.45
Mean (mg)		0.460	0.390	0.065	0.060	0.560	1.821

^{ns} not significant, ** significant at 1% probability. VC = Variation coefficient. ¹Data transformed in \sqrt{x} . BACAP, RACAP, CACAP = Copper concentration in the aerial part of beets, radish and corn, respectively. BACR, RACR, CACR = Copper concentration in the roots of beets, radish and corn, respectively.

**Figure 4.** Accumulated copper in the root of beets and radish and in the aerial part of radish according to bentonite doses.

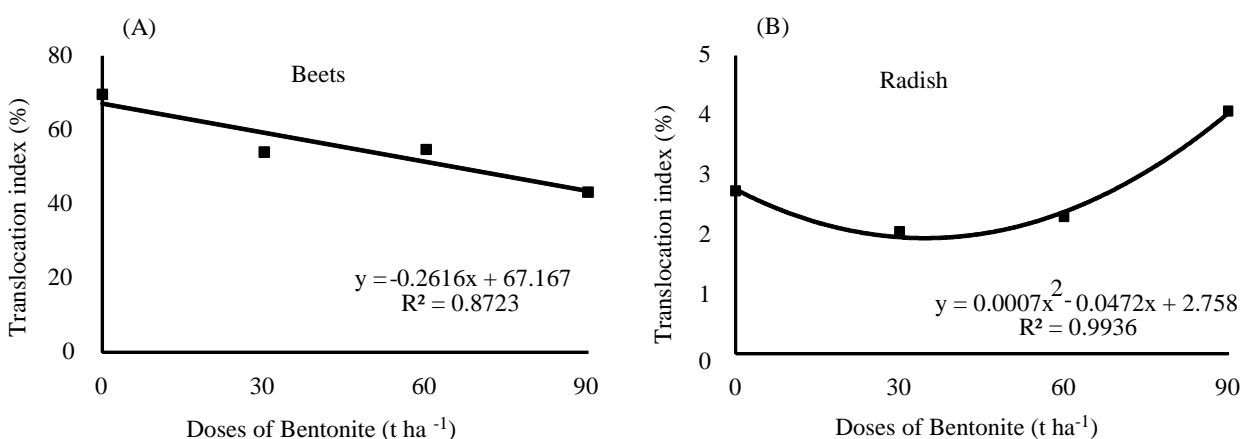
radish affected significantly the translocation index of the copper; however, the indices were very small: 1.97% for the 30 ton ha⁻¹ and 5.67% for the 90 ton ha⁻¹ (Figure 5B). There was no defined pattern of variation for the bentonite application. The available copper content in soil at the end of experimental period, whose means for radish and corn were 28.33 and 23.93 mg kg⁻¹, respectively, when submitted to bentonite applications, they were lower than 35 mg kg⁻¹, which is the reference

value corresponding to the quality level for a clean soil, with absolute no copper contamination (CETESB, 2005). It also was lower than the copper intervention level (60 mg kg⁻¹) also reported by the CETESB (2005), corresponding to the copper content in which there are risks for human health when this soil is used for human food production. Thus, the application of bentonite to these contaminated soils favored their amelioration. The mean available copper found in the soil after harvest of

Table 4. Summary of the analyses of variance for the copper translocation index of the beets, radish and corn for the different bentonite treatments.

Source of Variation	DF	Square root		
		Beets	Radish ¹	Corn
Bentonite	3	470.60**	3.25**	0.23 ^{ns}
Linear	1	1231.54**	3.66**	0.41 ^{ns}
Quadratic	1	17.07 ^{ns}	6.64**	0.08 ^{ns}
Error	12	33.83	0.24	0.28
Variation Coefficient (%)		10.50	17.55	10.91
Mean (%)		55.40	2.78	4.88

^{ns} not significant; ** significant at 1% probability. ¹ Data transformed in logx.

**Figure 5.** Translocation index for the beets and radish according to bentonite doses.

the beets was 116.49 mg kg⁻¹, corresponding to a contaminated soil, inappropriate for agricultural use because the risks for human health when used for human food production. Thus, the application of bentonite to this contaminated soil did not ameliorate it, probably due to the great quantity of copper added to the soil at the beginning of the experiment.

Conclusions

The increase of dry phytomass of the aerial part and roots of beets, radish and corn with bentonite application showed that the bentonite reduced the copper content of the plants, probably because the adsorption capacity of the soil increased with the application of bentonite. Thus, for the conditions under which the study was conducted, bentonite application favored crop development. With the exception of aerial part of the beets and corn, the copper concentration of the plant decreased significantly with the bentonite application. Bentonite affected significantly at 1% probability, the copper accumulated in the roots of beets and radish and in the aerial part of the radish.

The translocation index of copper in the beets was

reduced with the bentonite application, to find a great quantity of copper in the beet roots, higher than the permitted levels for human consumption and making it inappropriate for human consumption.

The application of bentonite to the contaminated soils planted with radish and corn favored their amelioration; the application of bentonite to the contaminated soil planted with beets did not ameliorate it.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Multivariate analysis on dry mass variables in cupuassu progenies (*Theobroma grandiflorum*) in function of the plant age

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The aims of this study was to evaluate the dry matter production in different parts of cupuassu plant (*Theobroma grandiflorum*), such as leaves, stems, primary and secondary branches in progenies resistant to pests and diseases (Codajás and Manacapuru) in function of the plant age, through the application of multivariate statistical analysis. The experiment was conducted at Embrapa Amazônia Oriental for a period of four years, in a randomized design. Samples were taken of five trees of each progeny, resulting in a total of 40 plants, which were separated into leaves, stems, primary and secondary branches. In each group of variables, the assumptions of canonical correlation analysis were tested, such as multivariate normal, the homoscedasticity deviations, multicollinearity, linearity in the dry mass of plant parts and correlation matrices that test the significance of the experimental data. Plants of PMI186 and PMI215 showed significant canonical correlations between the most variable groups of leaves, stems, primary branches and secondary branches, indicating that the groups are not considered independent, showing a linear relationship between them. In the dry matter of leaves, stems, primary and secondary branches index showed a high degree of significance for the Pearson test when related to parts of cupuassu plants. The largest production of total dry matter was obtained in PMI186 with 4332.92 g plant⁻¹ in secondary branches (4th year) and the lowest production showed in PMI215 with 4086.26 g plant⁻¹ in the secondary branch (4th year).

Key words: Linear relationships, vegetative growth, cluster analysis.

INTRODUCTION

The cupuassu plant (*Theobroma grandiflorum*) shows social and economic importance in the state of Pará, especially in family farming in areas that has been

improving their farms, with the introduction of consortium systems with other fruit trees, or through the introduction of agro-forestry systems involving planting a tree species,

such as mahogany intercropped with cupuassu and other fruit trees (Vieira et al., 2007). The introduction of tree species in future shows as selling wood and seeds, while cupuassu plant are included as part of the system, enabling farmers to have the economic profitability in the short term, while the timber species is in vegetative development, producing shading for cupuassu (Vieira et al., 2007). In addition of high economic and social importance in the Amazon region, there is little research on the agronomic performance of cupuassu, limiting the expansion of the cultivated area in the region (Alfaia and Ayres, 2004). The plant has high potential, with average fruit yield around 16.8 and 13.1 fruits plant⁻¹ for the PMI Codajás and PMI Manacapuru cultivars, respectively (Alves and Cruz, 2003). As a result of most soils in which the plantations are established in the Amazon region having low fertility (Vale Júnior et al., 2011), a research that takes into account this situation is needed to be carried out.

A proper mineral nutrition of cupuassu plants is critical to maintaining a vigorous growth and increase fruit yield (Ayres and Alfaia, 2007), since successive harvests, without the replacement of nutrients, promote soil depletion, resulting in low soil fertility, thus, decreasing the supply of nutrients to the culture. Although there are limitations due to the influence of other factors, unresolved, these influence the production, such as genetic (Alves et al., 2010), incidence of pests (Thomazini, 2002) and diseases (Alves et al., 2009), farmers whose economic activity with the cupuassu recognize the importance of fertilization to increase productivity, but find the problem of lack of research to enable the recommendation of agronomically correct and economically viable rates. Most of the information used for cupuassu cultivation comes from studies of cocoa by the proximity taxonomy of these plants (Alfaia and Ayres, 2004). However, this situation is reversed with fertilization and liming recommendations for the management of cupuassu plant (Viégas et al., 2010).

For the mass production of dry of cupuassu there is little information about which part of the plant could be used in order to study new mechanisms that relies not only on fruits to obtain any information about the plant nutrition, since although the study of the fruit is important, as well as accumulation and exportation of nutrients by fruit, it is important to report that it is not always possible to obtain this type of plant material on the occasion of not having control in the study of possible loss of fruit during the experiment period, such as stealing fruit.

Multivariate statistics in this evaluation process of dry matter production in the cupuassu progenies is an important tool in data analysis. The multivariate statistical consists of set of statistical methods allowing the

confrontation of several variables of each sample element simultaneously. The technique aims to simplify or facilitate the interpretation of the phenomenon studied and its development has enabled the accurate study of complex phenomena, used in order to construct alternative indexes or variables and groups of sampling elements, analyze the dependence relationship of the variables and compare populations (Bakke et al., 2008).

The aims of this study was to evaluate the dry matter production in different parts of cupuassu plant, such as leaves, stems, primary and secondary branches in progenies resistant to pests and diseases (Codajás and Manacapuru) in function of the plant age, through the application of multivariate statistical analysis.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in Belém city, State of Pará, Brazil, in the Embrapa Amazônia Oriental, with coordinates 48° 26' 55" and 48° 26' 40" Latitude (North-South) and 01° 26' 30" and 01° 26' 10" Longitude (East-West) of Greenwich. The climate, according to Köppen classification, is the type Afi, characterized by being hot and humid, with annual average temperature of 26.7°C, relative humidity of 80% and average annual rainfall of 3,001 mm (Bastos et al., 2002).

Area preparation for planting

At the beginning of 2002 until the year 2003, soils were prepared with pit fertilizers, organic manure and covered with triple superphosphate, castor bean, potassium chloride, urea, limestone and fertilizer with micronutrients (g plant⁻¹). In the experimental area, mowing and harrowing was practiced.

Study area

The area has 4300 m² with PMI186 (Codajás) and PMI215 (Manacapuru) progenies of cupuassu planted in alternating rows, with banana as temporary shading and açaí as permanent shade. The study was carried out during the four years, with annual evaluations of five plants of each progeny, totaling 40 plants.

Planting system

The planting system used was the cupuassu consortium with banana, açaí and mahogany. The cupuassu presented spacing of 5.0 × 5.0 m, the banana with 2.5 × 2.5 m and açaí with 5.0 × 5.0 m. Mahogany was located only on the sides of the experiment.

Plant material evaluations

The plant material used in the evaluations were leaves, stems, primary and secondary branches of half-brothers of cupuassu

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Table 1. Chemical characterization of soil before the implementation of the experiment, in the year of 2002.

Site	Depth (cm)	pH (H ₂ O)	P	K	Ca	Mg	Al
			-----mg dm ⁻³ -----		----- cmol _c dm ⁻³ -----		
Yellow Latosol	0-20	4.3	4.0	19.0	0.4	0.2	0.8

progenies (PMI 186: Codajás; PMI 215: Manacapurú), from the Embrapa Amazônia Oriental.

Collection of plant material and determination of dry matter

Plant samples were collected from each cupuassu progeny (PMI186; PMI215) in the experimental area of Embrapa Amazônia Oriental, during the four years (2004 to 2007). The collected material was dried in a forced-air oven at 70°C and weighed on a digital scale to determine the dry mass of leaves, stems, primary and secondary branches.

Collection and determination of soil analysis

The soil of the experimental area was classified as Yellow Latosol (Embrapa, 2013). In the areas of cupuassu plants, two samplings were carried out, the first at the beginning of the experimental implementation in 2002, and the second during the conduct of the study in 2005. The soil sampling was performed at a depth of 0 to 20 cm (Tables 1 and 2) and then submitted to the laboratory for subsequent chemical analysis of the soil according to the methodology described by Embrapa (2009).

Experimental design

The experimental design was completely randomized in a factorial triple, 2 × 4 × 5, so specified: two half-brothers of cupuassu progenies (PMI186: Codajás; PMI215: Manacapurú); four years of evaluation (2004, 2005, 2006, 2007); five vegetative parts of cupuassu evaluated [leaves (F), stems (C), the primary branches (RP), secondary branches (RS) and total dry matter (MST)], with 5 replications, totaling 40 experimental units, in which each experimental unit consisted of one plant of each progenies.

Data analysis

Initially, the assumptions of canonical correlation analysis were tested in each of the groups of variables, in the following order, multivariate normality, homoscedasticity of standard deviations, multicollinearity and linearity. Multivariate normality was measured by the Box Plot software (Mingoti, 2005); while the homogeneity of variances was made by check scatter diagrams and outliers (deviations > +5 or < -5) (Corrar et al., 2009), measured in Microsoft Office Excel 2007 spreadsheets; multicollinearity was calculated through the condition number (Montgomery and Peck, 1981), eliminating variables until getting the low degree of multicollinearity; linearity significance was calculated by the Pearson correlation coefficient, eliminating variables that showed no significant correlation coefficient or showed low correlation ($r < |0,3|$) (Carvalho et al., 2004), both by using the SPSS Statistics 17.0 software (Corrar et al., 2009). In the canonical correlation analysis were related in neighboring groups, that is, Group 1 with Group 2 and the Group 3, thus, consecutively. When a group showed significant canonical correlation to be related to the neighboring group, this has been correlated to the nearest second group etc. The canonical

correlation analysis was performed by using SPSS Statistics 17.0 software (Corrar et al., 2009). The data were submitted to analysis of multivariate variance, Box plot figures and dendrogram, selecting the variables leaves, stems, primary branches, secondary branches, and total dry mass in two half-brothers cupuassu progenies (PMI186: Codajás; PMI215: Manacapurú) (Silva et al., 2013).

RESULTS AND DISCUSSION

Multivariate analysis of variance

Table 3 shows a significant ordinal interaction, which carried out an assessment on both independent variables, such as X₁₁; X₂₁; X₃₁; X₄₁; X₅₁; X₆₂; X₇₂; X₈₂; X₉₂ and X₁₀₂ that showed significant main effects when considered simultaneously with the X₁ (A: PMI186 - Codajás) and X₂ groups (B: PMI215 - Manacapurú). Table 1 shows the results of the multivariate analysis of variance for the main purpose of X₁ and X₂ as well as the tests for the interaction effects, characterized significance for dependent variables, PMI186 (X₁) and PMI215 (X₂). The dependent variables X₁ (PMI186) and X₂ (PMI215) have significant impact (main effect) of the ten variables of the plant parts, both together as separately, according to the multivariate test. The impact of the ten independent variables resulted in the formation of combined groups, in which the multivariate model follows its normal size, both quadratic form as linear, but always explaining that two functions were formed that justified the formation of the canonical functions, dendograms and distance Euclidean two-dimension and correlations.

During the comparison of the dependent variable with any independent variable, the size of the effect attributable to the study the interaction effect (progeny*year*plant parts) is large, therefore, application of multivariate testing is possible. Thus, the effect of the difference between variables was the dry mass of each progeny produced, in which the PMI186 justifies the hypothesis of the study due to more productivity and which required larger amount of total dry mass in the leaves (FAT) with 3768.62 g plant⁻¹; 1666.44 g plant⁻¹ of total stem (CAT); 3017.02 g plant⁻¹ of total primary branch (RPAT); 4442.92 g plant⁻¹ of total secondary branch (RSAT); 12785.00 g plant⁻¹ of total dry matter (MSTA), compared to PMI215 which showed values of total dry mass in the leaves (FBT) of 3646.64 g plant⁻¹; 2183.82 g plant⁻¹ of total stem (CBT); 2634.28 g plant⁻¹ of total primary branch (RPBT); 4086.26 g plant⁻¹ of total secondary branch (RSBT); 12551.00 g plant⁻¹ of total dry

Table 2. Chemical and particle size characterization during the experimental period, in the year of 2005.

Site	Depth cm	Granulometry (g kg ⁻¹)			
		Coarse sand	Fine sand	Silt	Clay
PMI186	0-20	605	265	31	100
PMI215	0-20	649	231	41	80

Site	Depth cm	pH	OM	P	K	Na	Ca	Mg	Al	H+Al
		H ₂ O	g kg ⁻¹	---- mg dm ⁻³ ----			---- cmol _c dm ⁻³ ----			
PMI186	0-20	4.2	8.36	193	49	16	0.8	0.5	0.9	3.96
PMI215	0-20	4.0	8.91	206	61	21	1.0	0.7	1.1	5.45

Table 3. Multivariate Analysis of variance of the results of measurements of plant parts: leaves (F), stem (CA), primary branches (RP), secondary branches (RS) and total dry matter (MST) (X₁₁; X₂₁; X₃₁; X₄₁; X₅₁; X₆₂; X₇₂; X₈₂; X₉₂; X₁₀₂) to X₁ group (A = PMI186: Codajás) and X₂ group (B = PMI215: Manacapuru).

Independent variable	Combined groups (PMI186*PMI215*age)	Sum of squares	df	Mean square	Sig.
X ₁₁ (FA)	Linear	119475.64	2	59737.82	**
	Quadratic	46.976.76	1	46976.76	**
X ₂₁ (CA)	Linear	47451.38	2	23725.69	**
	Quadratic	1575.27	1	1575.27	**
X ₃₁ (RPA)	Linear	10607.11	2	5303.56	**
	Quadratic	27.40	1	27.40	**
X ₄₁ (RSA)	Linear	482268.35	2	241134.17	**
	Quadratic	1672.35	1	1672.35	**
X ₅₁ (MSTA)	Linear	95054.12	2	47527.06	**
	Quadratic	87225.04	1	87225.04	**
X ₆₂ (FB)	Linear	15354.36	2	7677.18	**
	Quadratic	2834.32	1	2834.32	**
X ₇₂ (CB)	Linear	22630.60	2	11315.30	**
	Quadratic	17977.15	1	17977.15	**
X ₈₂ (RPB)	Linear	17300.48	2	8650.24	**
	Quadratic	2657.38	1	2657.38	**
X ₉₂ (RSB)	Linear	237009.68	2	118504.84	**
	Quadratic	906.80	1	906.80	**
X ₁₀₂ (MSTB)	Linear	78546.94	2	39273.47	**
	Quadratic	38206.52	1	38206.52	**

matter (MSTB). The results obtained in this study are in agreement with Alves and Cruz (2003) who in studies

with cupuassu progenies observed that the Codajás (PMI186) showed higher production of fruits than the

Manacapuru (PMI215).

In studies with *Eucalyptus grandis* presenting age of 4.3 years were founded values of 3047.53 g plant⁻¹ dry mass of stems; 2475.00 g plant⁻¹ dry mass of leaves; 2182.10 g plant⁻¹ dry mass branches (Bellote et al., 1980). Compared to progeny cupuassu, PMI186 and PMI215, dry mass value was lower, featuring their quality probably attributed to greater leaf area of cupuassu plant, which promoted increments in the production of assimilates and plant biomass accumulation (Nunes et al., 2013).

Correlation matrices of PMI186 (Codajás) and PMI215 (Manacapuru)

To the values of the correlation matrices of PMI186 (Codajás) and PMI215 (Manacapuru) observed a high correlation index (≥ 0.90) between the indicators (Table 4), enabling visualization of the explanatory power of the factors (MSF; MSC; MSRP; MSRS and MST) in each of the variables analyzed. These factors are represented by values (1.00) founded on the main diagonal which are indicated by colors, in which the pink color characterizes the correlations of parts of cupuassu with the PMI186, and green colors characterize the parts of cupuassu with the PMI215, while colors isolated demonstrate the interrelationships between the progeny and parts of plant (RPA*FB), (RPA*RPB) and (RPB*FB). The results obtained of the interrelationships between progeny and parts of plant observed a well-adjusted correlation, in which the adjustment explains that primary branch of PMI186 with the leaf PMI215 (RPA*FB); primary branch of PMI186 with primary branch of PMI215 (RPA*RPB); primary branch of PMI215 with a leaf of the same progeny (RPB*FB) showed increments of dry matter, a fact that, probably, is related to constant releases of leaves and branches by cupuassu plant. In the present study were not observed values lower than 0.50 in the correlation matrix, which is considered that a small data measurement (Corrar et al., 2009) should be removed from the analysis.

The values obtained in this study are above 0.90 and considered ideal for the analysis, thus, without removing any variable statistical context, enabling high power of explanation of indicators, taking into account all factors obtained (commonalities).

Scores of the dry mass of cupuassu progenies in relation to two canonical variables

To obtain a satisfactory interpretation of the variability manifested among Progenies *Parts of plant* years it would be necessary that the first two canonical variables allow a minimum estimate of 80% of the total variance contained in the data set, and in this study the two canonical variables were explained for about 99.33% of

the variance (Table 5). By using the scores of the first and second canonical variable Figure 1 was obtained with two-dimensional display (model of Euclidean distance) of PMI's analyzed. The dispersion allowed the separation of the progenies in groups with parts of interrelated plants in the years 1, 2, 3 and 4, providing a strategy to select the progeny and plant part of this divergent progeny used to quantify the contribution of dry matter accumulation.

Model of Euclidean distance of dry matter production

Figure 1 shows the selection of significant variables constituting part of a factor, selected based on the magnitude of factor loadings. Initially, a cutoff point was adopted, considering only the loads above 0.50. Subsequently, the significant variables were selected and chosen to the factor loadings of greater value. Later, this process was made to Figure 1, in which the observed Factor 1 showed two significant loads, and the Factor 2 showed eight loads. In the Factor 1, secondary branch of PMI186 (RSA) and secondary branch of PMI215 (RSB), both with positive signs, demonstrating the occurrence of variation, showed consistency with the dry matter production process [4332.92 g plant⁻¹ of RSA in the 4th year (PMI 186); 4086.26 g plant⁻¹ of RSB in the 4th year (PMI 215)]. The increase in the secondary branch provided an increase in dry matter yield of the progenies 186 (Codajás) and 215 (Manacapuru), possibly, due to increased formation of conducting vessels, which directly influenced the plant production capacity, with greater expressiveness in PMI186. In Factor 2, the progeny 186 and 215 of cupuassu, the variables (CB, RPA, CA, RPB, FA, FB) showed the better adjustment in the same quadrant, and away from Factor 1, which means that the isolation of Factor 2 has a higher power of explanation in comparison to Factor 1, by having a greater involvement on the dry mass production. Although its effects are visible when comparing high-yielding progenies, thus, the PMI186 showed higher production of total dry matter compared to progeny PMI215, during the trial period of four years. The difference in yield of progenies is related to genetics of plant and its nutrient absorption capacity of the soil.

Dendrogram of Ward's clustering method

The average distance of Mahalanobis between progenies and their plant parts (F, C, RP, RS, MST) according to the age ($D^2=5.3$) was used as a criterion for the formation of groups, as well as applied to bean cultivars (Cargnelutti Filho et al., 2009). By dendrogram information shown in Figure 1, the formation of three groups was observed, the first group consisted RPA, FB and RPB, the second group consisted MSTA and MSTB, while the third group consisted the variables CB, FA, CA, RSA and RSB.

Table 4. Matrix of correlation between the half-brothers progenies of PMI186 (Codajás) and PMI215 (Manacapuru) of cupuassu plant (*Theobroma grandiflorum*), according to the age.

	FA	CA	RPA	RSA	MSTA	FB	CB	RPB	RSB	MSTB
FA	-	0.99	0.99	0.96	0.99	0.99	0.98	0.99	0.97	0.99
CA	0.99	-	0.99	0.93	0.98	0.99	0.97	0.99	0.95	0.98
RPA	0.99	0.99	-	0.97	0.99	1.00	0.99	1.00	0.98	0.99
RSA	0.96	0.93	0.97	-	0.98	0.97	0.98	0.97	0.99	0.98
MSTA	0.99	0.98	0.99	0.98	-	0.99	0.99	0.99	0.99	0.99
FB	0.99	0.99	1.00	0.97	0.99	-	0.99	1.00	0.98	0.99
CB	0.98	0.97	0.99	0.98	0.99	0.99	-	0.99	0.99	0.99
RPB	0.99	0.99	1.00	0.97	0.99	1.00	0.99	-	0.98	0.99
RSB	0.97	0.95	0.98	0.99	0.99	0.98	0.99	0.98	-	0.99
MSTB	0.99	0.98	0.99	0.98	0.99	0.99	0.99	0.99	0.99	-

FA (Leaves of PMI186); CA (stem of PMI186); RPA (primary branches of PMI186); RSA (secondary branches of PMI186); MSTA (total dry matter of PMI186); FB (Leaves of PMI215); CB (stem of PMI215); RPB (primary branches of PMI215); RSB (secondary branches of PMI215); MSTB (total dry matter of PMI215).

Table 5. Scores of the dry mass of progenies cupuassu (*Theobroma grandiflorum*) for the two canonical variables obtained in the evaluation of dissimilarity of PMI186 and PMI215 with plant parts, according to the age.

Progenies*Plant parts*Years	Canonical variables	
	1°	2°
FA	0.4142	-0.1062
CA	1.2014	-0.0980
RPA	0.7225	-0.0495
RSA	0.3833	0.3142
MSTA	-2.8664	0.0286
FB	0.4704	-0.1088
CB	1.0429	-0.0386
RPB	0.8578	-0.0696
RSB	0.4330	0.2102
MSTB	-2.6599	-0.0824
Variance (%)	98.36	99.33
Accumulated variance (%)	98.36	99.33

The MSTA and MSTB access (GII) highlights compared to the others showing increased production of dry matter, due to the total dry weight of the progeny A (PMI 186) and progeny B (PMI 215) is the sum of leaves (F), stems (C), primary branches (RP) and secondary (SR) within each progeny. The total dry mass of the PMI186 corresponds to a production of 486.78 g plant⁻¹ (1th year), 1214.68 g plant⁻¹ (2th year), 5080.71 g plant⁻¹ (3th year), 12785.00 g plant⁻¹ (4th year), while PMI215 obtained a production in total dry mass of 610.18 g plant⁻¹ (1th year), 1847.16 g plant⁻¹ (2th year), 4799.36 g plant⁻¹ (3th year), 12164.54 g plant⁻¹ (4th year).

The first group (GI), which corresponds to RPA (primary branch of PMI186), FB (leaf of PMI215) and

RPB (primary branch of PMI215) showed the order of placement due the similarities between them (Figure 2), such as the total dry matter production, the GI showed increase from the second year, checking that the RPA showed an increase of 429.54 g plant⁻¹ (1th year) to 3017.02 g plant⁻¹ (4th year); the variable FB showed the same tendency of dry matter increase from the second year with values of 677.12 g plant⁻¹ reaching values in the fourth year of 1688.04 g plant⁻¹; RPB showed higher dry matter yield in the second year (461.08 g plant⁻¹) until the fourth year (2634.28 g plant⁻¹).

The third group (GIII) of dendrogram consisting CB, FA, CA, RSA, RSB showed a satisfactory level of explanation that the positions on the same set (Figure 2), due to the

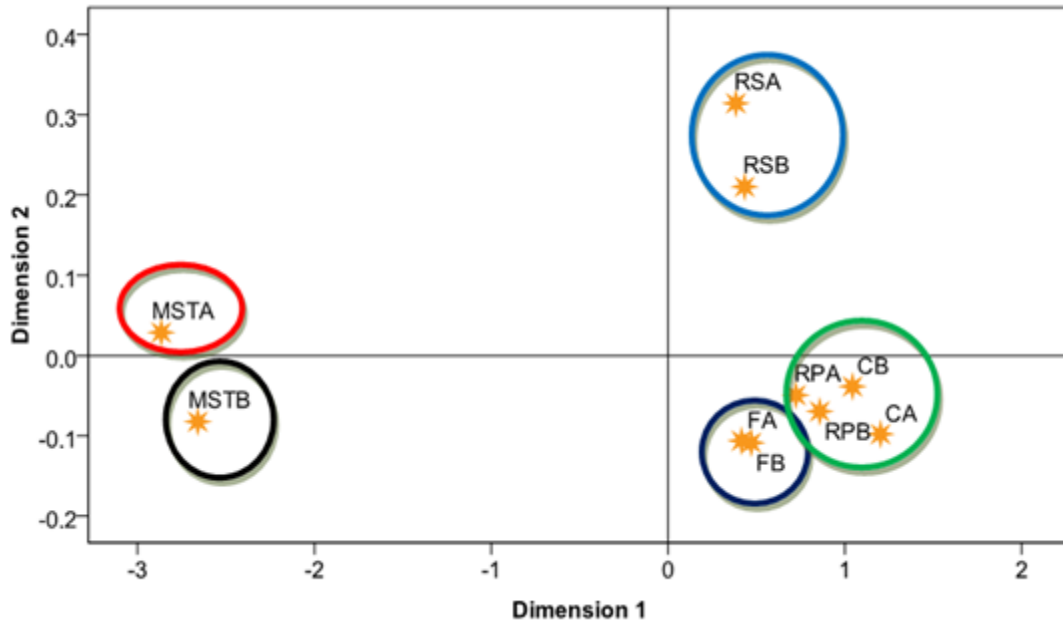


Figure 1. Euclidean distance model of the dry mass production (g kg^{-1}) of PMI186 (Codajás) and PMI215 (Manacapurú) in cupuassu plant (*Theobroma grandiflorum*), according to the age.

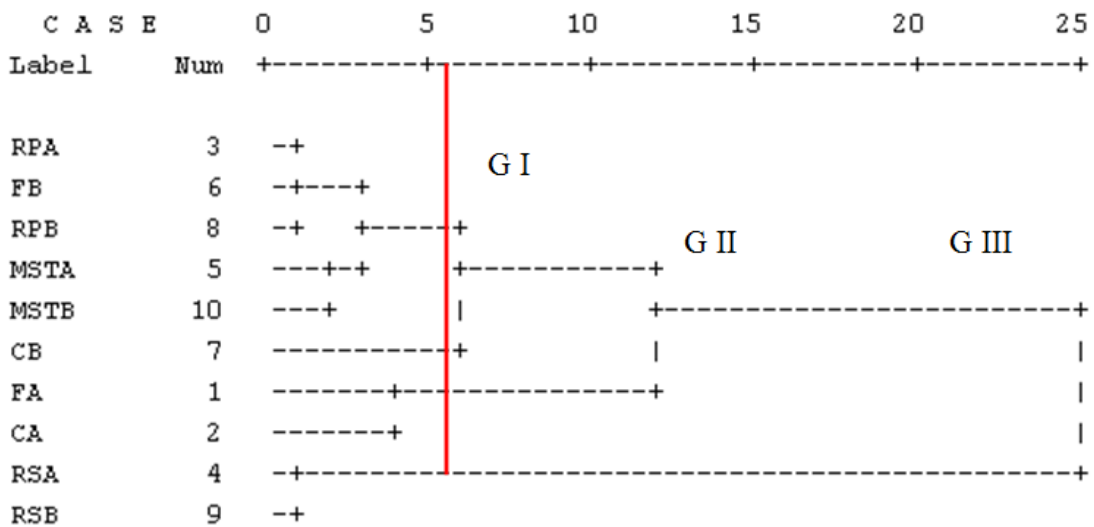


Figure 2. Dendrogram of Ward's clustering method, obtained in two half-brothers progeny (A, B) of cupuassu plant (*Theobroma grandiflorum*), PMI186 and PMI215, according to age, from the Mahalanobis distance with parts of the plant (F, C, RP, RS, MST).

variables from the second year present a dry matter yield more than five times compared to any components of cupuassu plants, such as FA in the second year which were of $396.14 \text{ g planta}^{-1}$ and the fourth year of $3768.62 \text{ g planta}^{-1}$; CA in the second year were of $304.02 \text{ g planta}^{-1}$ and the fourth year of $1666.44 \text{ g planta}^{-1}$; RSA in the second year were of $84.98 \text{ g planta}^{-1}$ and the fourth year

$4332.92 \text{ g planta}^{-1}$; CB in the second year were $501.52 \text{ g planta}^{-1}$ and the fourth year $2183.82 \text{ g planta}^{-1}$; and RSB in the second year were of $207.32 \text{ g planta}^{-1}$ and the fourth year $4086.26 \text{ g planta}^{-1}$.

The formation of three groups was due to the characteristics already mentioned which indicate the similarity between the variables. The formation of two

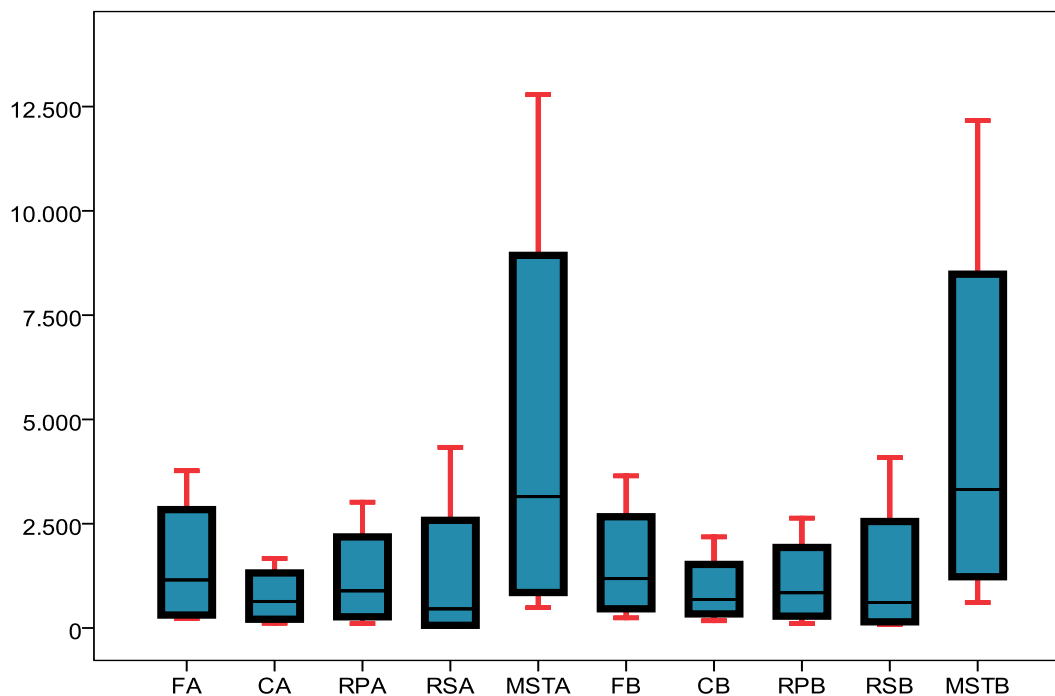


Figure 3. Box plot of dry mass production (g kg^{-1}) in cupuassu plant (*Theobroma grandiflorum*), PMI186 and PMI215, according to the age.

groups obtained in this study are consistent with those obtained by Bento et al. (2007), in a study of phenotypic variability in *Capsium annum*, that observed differences in the number of groups formed. In this study, the formation of groups constituted to multicategoric data related to Years*Progeny*Plant parts, while for Sudré et al. (2006) characterized the collection of data as multicategoric practice to demand less time in the studies with pepper and chili.

Box plot of dry matter production

Figure 3 shows the box plot analysis of PMI186 (Codajás) and PMI215 (Manacapurú) cupuassu of plants, which represents the degree of variation between the groups (FA, CA, RPA, RSA, MSTA, FB, CB, RPB, RSB and MSTB) for a qualitative or categorical variable (PMI186 or PMI215), therefore, the length of the box and its extensions describe the variation of the data within each progeny. The information obtained, found that PMI186 showed the highest amount of MST compared to PMI215, the same happened to other variables, except CA and CB which were lower in the two PMIs, during the four years of study. All variables dry mass obtained different values, in which variations were observed between them in relation to the total dry mass in the PMI186 of $486.78 \text{ g plant}^{-1}$ of MSTA (1th year), 1214.68 g

plant^{-1} of MSTA (2th year), $5080.71 \text{ g plant}^{-1}$ of MSTA (3th year), $12785.00 \text{ g plant}^{-1}$ of MSTA (4th year), which was higher than the total dry matter in the PMI215 of $610.18 \text{ g plant}^{-1}$ of MSTB (1th year), $1847.16 \text{ g plant}^{-1}$ of MSTB (2th year), $4799.36 \text{ g plant}^{-1}$ of MSTB (3th year), $12164.54 \text{ g plant}^{-1}$ of MSTB (4th year). The difference in values reinforces the justification that has differences in the absorption of nutrients provided by the genetic trait of progenies.

The PMI186 showed higher dry biomass compared to PMI215. The largest biomass plant was promoted by greater photosynthesis capacity and production photoassimilates, providing greater accumulation of biomass therefore, higher level of carbon was absorbed due to the ease in capturing light and assimilate production, which allows a higher flow of carbohydrates in the root system in which a part is stored in nutrition and accumulated in reserve structures (vesicles type) in formation with mycorrhiza and the other is accumulated in the plant storage tissues, in the form of reserve substance (Ozdemir et al., 2010).

The PMI186 progeny showed a higher stem mass compared to the PMI215. Probably because the system in which the PMIs were conducted during the four years of study was characterized by being an intercropping system with other fruit trees, together with occurring successive incorporations of plant materials at the time of falling leaves and branches originating from the

Table 6. Estimate of Pearson correlation coefficient of the Progenies*Plant parts*Years between dry matter production variables in PMI186 (A) leaves (FA), stems (CA), primary branches (RPA), secondary branches (RSA), total dry matter (MSTA) and dry matter in PMI215 (B) leaves (FB), stems (CB), the primary branches (RPB), secondary branches (SBR), total dry mass (MSTB) of cupuassu (*Theobroma grandiflorum*), according to the age.

	FA	CA	RPA	RSA	MSTA	FB	CB	RPB
FA	-	0.99**	0.99**	0.96*	0.99**	0.99**	0.98*	0.99**
CA	0.99**	-	0.99**	0.93ns	0.96*	0.99*	0.97*	0.99*
RPA	0.99**	0.99**	-	0.97*	0.99**	1.00**	0.99**	1.00**
RSA	0.96*	0.93*	0.97*	-	0.98*	0.97*	0.98*	0.97*
MSTA	0.99**	0.98*	0.99**	0.98*	-	0.99**	0.99**	0.99**
FB	0.99**	0.99*	1.00**	0.97*	0.99**	-	0.99**	1.00**
CB	0.98*	0.97*	0.99**	0.98*	0.99**	0.99**	-	0.99**
RPB	0.99**	0.99**	1.00**	0.97*	0.99**	1.00**	0.99**	-
RSB	0.97*	0.95*	0.98*	0.99**	0.99*	0.98*	0.99*	0.98*
MSTB	0.99*	0.98*	0.99**	0.98*	0.99**	0.99**	0.99**	0.99**

** : significant ($p \leq 0.01$); * : significant ($p \leq 0.05$); ns : not significant ($p > 0.05$) by the t test.

cultivation, which allowed the cycling of plant material, it is a beneficial process to the cultivation system for providing the plants a nutrient reserve and soil friability, and facilitating the exchange of elements, allowing the root system to present good development, thereby, improving the uptake of water and nutrients.

Pearson correlation

The Pearson correlation presented in Table 6 shows the effects of PMI186 (Codajás) and PMI215 (Manacapuru) presenting a statistical difference between the PMIs, probably, by plants showing distinctive vegetative structure on the dry matter production in its components (leaves, stems, primary and secondary branches), as well as the size of the sum of this material for intercropping conditions with other fruits, such as açai and banana plants. In order to understand the interactions between the components with their respective PMIs analyzed, the Pearson correlation test was analyzed, a positive correlation (+) indicated very strong PMI186 with their respective components, as leaves (FA), stems (CA) primary branches (RP) and secondary branches (RS). The positive correlation occurred in all PMIs, except for the correlation between CA and RSA that was not significant, possibly, this occurred because the dry matter intake did not have much difference during the four years of the study. Unfortunately, there are no parameter studies which serve to explain these results, but in accordance with the field observations and laboratory production of dry matter there was no difference in stem and secondary branch.

The Pearson correlation is considered in the analysis as a very strong correlation (+) and almost perfect, when their values are above 0.92, which occurred in this study.

The degree of perfection is very characteristic when plant parts relate to each other, thus, when the value reaches 1.00, which means that their explanatory power is at 100%. In Table 6, the explanatory power is not 100% for all parameters, but their fit is almost perfect, meaning that the analysis is correct and that these interactions exist because the plants originate from the same mother, but there is no knowledge of who the father is. The father of knowledge would enable a good understanding of certain parameters, such as FB*CA which correlate very well, probably, due to the ability of a plant, compared to another, to produce more dry matter in plant tissues, without affecting production. Similar results were founded by Locatelli et al. (2001) in the consortium of Brazil nuts and cupuassu.

Conclusion

The progeny PMI186 (Codajás) obtained the highest production of dry matter compared to PMI215 (Manacapuru). The largest production of total dry matter occurred in 2007, in which the PMI186 progeny (Codajás) had the highest production of dry matter, with 4332.92 g plant⁻¹ in the primary branch (in the year of 2007), while PMI215 progeny provided a production of 4086.26 g plant⁻¹ of dry matter in the primary branch.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The influence of protected kapok seed oil supplementation on *in vitro* ruminal fermentability and linoleic acid status with Etawah crossbred goat rumen fluid and elephant grass as feed

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This investigation was conducted to study the effect of protected kapok (*Ceiba pentandra*) seed oil supplementation upon the *in vitro* relative proportion of linoleic acid and ruminal fermentation parameters using rumen fluid of Etawah crossbred goat and elephant grass (*Pennisetum purpureum*) as feed. Research materials used were elephant grass (EG), kapok seed oil (KSO), lactating Etawah crossbred goat (ECG) rumen fluid, potassiumhydroxide (KOH) and calciumchloride(CaCl₂). There were two treatment factors, namely KSO supplementation as factor I (S) and protection as factor II (P). The first factor consists of four levels, namely 5% (S1), 10% (S2), 15% (S3) and without KSO supplementation (S0), while the second factor consists of 5 levels, namely: 0% (P0), 25%(P1), 50% (P2), 75% (P3) and 100% (P4), thus there were 15 combinations of treatment and one control (S0P0). Measured variables consisting of: *in vitro* neutral detergent fiber digestibility (IVNDFD), the molar proportion of acetic acid, propionic acid, butyric acid, acetic acid: propionic acid (A/P) ratio, the relative proportion of linoleic acid, stearic acid and ruminal lipid iodine number (IN). The results showed that 5% of unprotected KSO supplementation increased the molar proportion of propionic acid from 21.30% in the EG without supplementation becomes to 23.67% (P <0.05) without any negative effect on IVNDFD. Supplementation of protected KSO at 10 and 75% protection level increased (P <0.05) the molar proportion of propionic acid significantly (35.49%) with A / P ratio: 1.68 without significant IVNDFD variation. Increasing levels of KSO supplementation (up to 15%) increased the relative proportion of ruminal linoleic acid, followed by increasing in iodine number. The combination between KSO supplementation and protection increased the ruminal linoleic acid relative proportion and lipid iodine number, were 47.14% and 51.09, respectively. The KSO supplementation at the level of 10% with 75% protection level can resulted in the same proportion of linoleic acid to 15% KSO supplementation with 75% protection level (45.39 and 46.50 and 97%, respectively) with higher IVNDFD (P<0.05), namely 50.97 vs. 45.44%).

Key words: Kapok seed oil, protection, linoleic acid, ruminal fermentability, elephant grass, Etawah crossbred goat, *in vitro*, linoleic acid.

INTRODUCTION

Consumer demand for improved quality of livestock products recently is higher not only in terms of nutritional

value, but also for healthy products (Nuernberg et al., 2006). The increasing of goat products, both meat and

goat milk consumption still often face constraint such as cholesterol phobia issue, because of the high saturation degree of fatty acid and cholesterol levels in the livestock products. Feed manipulation was required to overcome this, among other with the supplementation of linoleic acid -rich oil in this case was kapok seed oil (KSO), because of lower lipid levels in goats conventional feed (forage), which was only about 3% despite a high proportion in linoleic acid (Jalc et al., 2007). Oil supplementation may also increase the energy density of the ration (Cieślak et al., 2010).

Linoleic acid makes up about 23% of fatty acids in phosphatidylcholine, a major phospholipid constituent of high density lipoprotein (HDL) (Bauchart, 1993). That lipoprotein plays an important role in cholesterol controlling by cholesterol transporting from extrahepatic tissues and other lipoproteins to the liver (reverse cholesterol transport), for various processes. The role of linoleic acid in the control of cholesterol also occurred through the increasing of low density lipoprotein (LDL) receptors number and the increasing of LDL catabolic rate (Murray et al., 1997).

Linoleic acid as part of phosphatidylcholine also plays an important role in the maintenance of membrane integrity, both the cell membrane and mitochondria membrane as well as nuclei membranes, to maintain the membrane fluidity. These conditions allow the maintenance the normal activity of membrane bounded enzymes, receptors affinity and membrane permeability. It makes possible for the increasing of the nutrients transport into the cells and activation of intracellular enzymes which is reflected in the stimulation of growth, maintenance of reproduction performance and other aspects of the livestock production that in turn increases the livestock productivity (Sardesai, 1992). Supplementation of the linoleic acid-rich oils also allow deposition of essential polyunsaturated fatty acids occur in animal products so that can make the consumers healthy (Jayanegara, 2013).

Level of linoleic acid in ruminant products is low, due to low level of that essential fatty acid in conventional feed, it is also due to microbial biohydrogenation in the rumen (Varadyova et al., 2013). Thus, protection needs to be done to protect linoleic acid from ruminal biohydrogenation, thus ensuring the sufficient supply of these nutrients for the post-rumen absorption (Jayanegara, 2013). Protection is also required to minimize the negative effect of unsaturated fatty acids upon the fiber degradation by cellulolytic microbes. Partial protection is applied as an attempt to capitalize on the positive influence of polyunsaturated fatty acids (PUFA) to raise the ruminal fermentation efficiency up to a certain level that does not cause the decreasing of

fibrous feed utility, significantly (Renno et al., 2014). Linoleic acid can affect the ruminal fermentation, which lowers the availability of substrates for methanogenesis so that the methane formation is reduced, which means reduce the waste of energy that to go to waste through the formation of methane gas. The decreasing of methanogenesis has impact on the increasing of molar proportion of propionic acid and / or the decreasing of acetate / propionate ratio which also means the increasing of energy efficiency (Cieślak et al., 2009). Metabolism of propionic acid will produce higher adenosine triphosphate (ATP) while the heat increment (HI) per mole lower than acetic acid metabolism (Banerjee, 1978).

In the view of above concepts, this study was conducted to assess the effect of supplementation of linoleic acid source (that is, KSO) upon ruminal fermentation. The study also yields the technology of protected KSO supplementation and information about its potency to increase the supply of linoleic acid for absorption in the post-ruminal digestive tract, which is reflected in the status of ruminal lipids, especially the relative proportions of ruminal linoleic acid, without reducing the utility of fibrous feed as the main feed ingredient for ruminants.

MATERIALS AND METHODS

The materials used in this study was the elephant grass in air dry state as a single feed, KSO as supplements and rumen fluid from lactating Etawah crossbred goat which fed a standard diet with elephant grass as a basal feed. Reagents used among other reagents to protect KSO, such as KOH and CaCl₂.

The results of the analysis of fatty acid composition of KSO listed in Table 1, the saponification number was 119.06 whereas the iodine number was 53.93. Nutrient composition of elephant grass showed in Table 2. Goat rumen fluid taken from four fistulated lactating goats on 3 h after the morning feeding.

Research method

There were 2 factors in this study, namely KSO supplementation as factor I and KSO protection as a factor II. The factor I consists of three levels, namely 5 (S1); 10 (S2) and 15 (S3)%, while the factor II consists of 5 levels of protection, namely 0 (P0); 25 (P1); 50 (P2); 75 (P3) and 100 (P4)%. Both of these factors resulted 15 combinations of treatment and 1 control, namely without supplementation and without protection (S0P0).

Protection procedure of KSO

Protection of KSO was conducted through saponification with KOH, which then transformed into calcium salt by using CaCl₂. The amount of KOH used was suitable to the protection level and

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Table 1. Fatty acid composition in kapok seed oil (KSO).

Fatty acid composition	Proportion (%)*
Palmitic acid (C16:0)	23.62
Stearic acid(C18 :0)	2.38
Oleic acid (C18 :1)	24.59
Linoleic acid (C18 :2)	43.68
Linolenic acid (C18 :3)	2.92

*Proportion from total fatty acids in KSO.

Table 2. Nutrient composition of experimental feed (%) (dry matter basis).

Feed	CP ^a	CF ^a	NFE ^b	EE ^a	Ash ^a	TDN ^c
Elephant grass	9.52	31.13	41.82	2.27	15.26	55.43

Explanation : CP : crude protein ; CF : crude fiber ; NFE : nitrogen free extract ; EE : ether extract ; TDN : total digestible nutrient : ^a Determined, ^b DM – Ash- CP – CF – EE, ^ccalculated based on TDN formulation for goat.

calculated based on the saponification number of KSO that were determined according to the method of Cabatit (1979). A certain amount of KSO (based on the supplementation levels) put into the beaker glass, then heated in a water bath until the temperature reached 90°C. A number calculated KOH were weighed, diluted with distilled water and then added to KSO which was being heated while stirring for 10 min to form a suspension of potassium soap. To transform potassium soap into calcium salt, a number of CaCl₂ were calculated using stoichiometry, weighed and diluted with distilled water. The CaCl₂ solution was added to the suspension of potassium soap while heated in a water bath at the temperature of 90°C and stirred until the precipitation of calcium salts then formed. After centrifugation at 2500 rpm for 10 min, the supernatant was removed, the precipitate was mixed with a portion of KSO which was not protected (suitable to the supplementation level), ready to be used as a supplement.

Variables were measured and chemical analysis of samples

The measured variables included fiber digestibility, in this case the *in vitro* neutral detergent fiber digestibility (IVNDFD), molar proportions of volatile fatty acids (VFAs), the relative proportion of linoleic acid and stearic acid, and ruminal lipid iodine number (IN). Elephant grass IVNDFD was determined according to the method of Tilley and Terry one stage (1963). Amount of 0.5 g sample were incubated in 50 ml of a mixture of rumen fluid and McDougall's buffer solution (1: 4), for 48 h. Fermentation was stopped after 48 h using 2 ml of 50 g / kg HgCl₂. To the initial sample, residue and blank also boiled (refluxed) in neutral detergent solution to determine NDF level (Goering and Van Soest, 1970), for calculating IVNDFD.

Amount of 9 ml of supernatant from each fermentation tube was taken, preserved by the addition of 1 ml of 50% (vol. / vol.) H₂SO₄, then stored at -20°C, prior to analysis. Towards the analysis, then sample was thawed and centrifugated (15000 g for 15 min). Concentration of volatile fatty acids (acetic acid, propionic acid and butyric acid) were determined by gas chromatography (Galyean, 1980).

After incubation the content of fermentation tubes were dried by freeze-drier at a temperature of -50°C. Lipids were extracted from 0.5 g of culture that has been dried by a freeze-drier using mixture

of chloroform: methanol 2:1, the samples were purified using a 20% HCl (Bligh and Dyer, 1959). Concentrations of long chain fatty acids were determined by gas chromatography (Galyean, 1980). Iodine number (IN) of the extracted lipid was determined by the method of Plumer (1971).

Statistical analysis

The collected data included IVNDFD, partial VFAs molar proportions, A / P ratio, the relative proportion of linoleic and stearic acids and iodine number variables were processed statistically by analysis of variance (ANOVA) with factorial treatment patterns, in a completely randomized design (Steel et al., 1997). Data processing was done by using co-stat program.

RESULTS AND DISCUSSION

In vitro neutral detergent fiber digestibility (IVNDFD)

The IVNDFD and molar proportions of partial volatile fatty acids in this study reflected the fibrous feed utility, in this case the elephant grass (EG), and the effect of protected KSO supplementation upon these variables (Narimani-Rad et al., 2012). Data of those variables were summarized in Table 3. Elephant grass IVNDFD without supplementation (S0P0) was 51.63%, whereas the treatment group of KSO supplementation with 5% level and 25; 50; 75 and 100% protection level (S1P0, S1P1, S1P2, S1P3, and S1P4) were 50.87; 50.93; 51.03; 51.09 and 51.12%, respectively.

Between the elephant grass with no supplementation and supplemented by 5% KSO with and without protection, there were no significant difference in IVNDFD. According to Byers and Schelling (1988) and Patra (2013), levels of fat in the diet up to 5%, did not inhibit the microbial fermentation in the rumen. Elephant

Table 3. The *in vitro* NDF digestibility (IVNDFD), VFAs molar proportion (%) and acetic acid/propionic acid ratio.

Treatment	IVNDFD	Acetic acid	Propionic acid	Butyric acid	Acetic acid/Propionic acid ratio
S ₀ P ₀	51.6±1.3 ^a	72.3±1.0 ^a	21.3±0.5 ⁱ	6.4±1.0 ^{cd}	3.4±0.1 ^a
S ₁ P ₀	50.9±0.3 ^a	64.2±1.3 ^d	23.7±0.7 ^h	12.1±1.4 ^a	2.7±0.1 ^d
S ₁ P ₁	50.9±0.4 ^a	67.5±1.5 ^c	22.5±0.5 ^{hi}	8.0±1.9 ^{ab}	3.0±0.1 ^c
S ₁ P ₂	51.0±0.6 ^a	68.4±1.3 ^{bc}	23.0±0.8 ^h	6.9±2.2 ^b	3.0±0.1 ^c
S ₁ P ₃	51.1±0.6 ^a	71.3±1.9 ^a	22.4±0.6 ^{hi}	6.3±1.4 ^{cd}	3.2±0.2 ^b
S ₁ P ₄	51.1±0.5 ^a	72.0±0.9 ^a	21.3±1.8 ⁱ	6.7±2.6 ^c	3.4±0.3 ^a
S ₂ P ₀	40.9±0.3 ^{cd}	55.8±1.1 ^g	42.1±0.6 ^b	2.0±0.9 ^{fh}	1.3±0.1 ^{hi}
S ₂ P ₁	45.6±0.6 ^b	56.0±1.2 ^g	40.7±1.1 ^c	3.2±2.1 ^{efgh}	1.4±0.1 ^h
S ₂ P ₂	45.9±0.3 ^b	58.7±0.5 ^{ef}	37.3±1.0 ^d	3.2±1.4 ^{effh}	1.6±0.1 ^{fg}
S ₂ P ₃	51.0±0.4 ^a	59.9±2.0 ^e	35.5±0.9 ^e	4.6±2.8 ^{cde}	1.7±0.1 ^f
S ₂ P ₄	51.0±0.3 ^a	69.5±1.3 ^b	26.6±0.6 ^g	4.0±1.3 ^{defgh}	2.6±0.1 ^d
S ₃ P ₀	40.3±0.7 ^d	52.7±0.9 ^h	45.2±1.2 ^a	2.1±1.3 ^{fgh}	1.2±0.1 ^j
S ₃ P ₁	41.4±0.9 ^c	52.9±1.4 ^h	42.6±0.7 ^b	4.5±1.6 ^{cdef}	1.2±0.1 ^{ij}
S ₃ P ₂	41.6±0.9 ^c	56.3±0.5 ^g	42.2±0.8 ^b	1.6±0.7 ^h	1.3±0.1 ^{hi}
S ₃ P ₃	45.4±0.9 ^b	58.1±0.8 ^f	37.5±0.7 ^d	4.4±0.6 ^{cdefg}	1.5±0.1 ^g
S ₃ P ₄	46.0±1.3 ^b	63.9±1.0 ^d	29.9±1.7 ^f	6.2±2.4 ^{cd}	2.1±0.1 ^e

The values were arithmetic means (n=5); IVNDFD : *in vitro* neutral detergent fiber digestibility; VFAs: volatile fatty acids. S₀,S₁,S₂, S₃ : KSO supplementation levels : 0; 5; 10; 15%, respectively. P₀, P₁, P₂, P₃, P₄ : protection levels : 0; 25; 50; 75 and 100% respectively. Different superscript within column denote significantly different means (P <0.05).

grass fat content was 2.27%, with KSO supplementation, levels of fat in the ration became to more than 5%. The high proportion of fibrous feed in the ration could reduce the negative effect of fat on the rumen microbial metabolism (Delgado et al., 2013). Feedstuff that was used as a research material in this study was fibrous feed as a single feed, so that 5% KSO supplementation did not influence the IVNDFD significantly.

The IVNDFD began to decrease at the level of 10% of KSO supplementation without protection (S₂P₀), namely 40.93% and the decreasing tend to be larger at the level of 15% of KSO supplementation (S₃P₀), namely 40.28%. The decreasing of IVNDFD primarily could occurred due to the influence of unsaturated fatty acids (UFA) contained in KSO. Unsaturated fatty acids could inhibit fibrolytic bacteria causing reduced fiber digestibility (McGinn et al., 2004; Messana et al., 2013). Anaysis of KSO in this study showed the proportion of unsaturated fatty acids in KSO (from total fatty acid in KSO) was 71.19%, which consist of 43.68% linoleic acid, 24.59% oleic acid and 2.92% linolenic acid (Table 1).

The IVNDFD of elephant grass supplemented by 10% KSO with protection level of 25, 50, 75 and 100%, were 45.60; 45.92; 50.97 and 51.05%, respectively while elephant grass supplemented by 15% KSO were 41.37; 41.64; 45.44 and 45.96%, respectively. Protection of KSO could reduce the negative effect of these supplements on ruminal fermentation, which were reflected in the increasing of IVNDFD, both at 10% as well as 15% supplementation levels. Combination

between 75 as well as 100% protection level and 10% KSO supplementation level resulted in not significantly different IVNDFD from elephant grass without supplementation (50.97 and 51.05 vs 51.63%). Binding of the carboxyl group with the calcium in the protection process reduced the unsaturated fatty acids toxicity on rumen microbe resulting reduced ruminal metabolism inhibition (Bhatt et al., 2013)

Molar proportions of volatile fatty acids

Analysis of variance showed the effect of KSO supplementation and protection as well as its interaction upon the molar proportions of partial volatile fatty acids . The major volatile fatty acids as ruminal fermentation product were acetic acid, propionic acid and butyric acid.

Acetic acid

Most of the acetic acid produced from ruminal fermentation of fibrous feed, by fibrolytic bacteria, among other Ruminococcus, Butyrivibrio and Bacteroides (Hungate, 1966). The rumen microbes which were depressed by unsaturated fatty acid supplementation were primarily fibrolytic bacteria (Patra and Yu, 2013), thus will resulted in a decreasing of ruminal acetic acid production (Al-Dobaib and Kamel, 2012; Sun et al., 2013). It was seen from the molar proportion of acetic acid in the fermentation of elephant grass supplemented

with 5, 10 and 15% KSO without protection (S1P0, S2P0 and S3P), namely 64.22; 55.84 and 52.68% which lower ($P < 0.05$) than the molar proportion of acetic acid in the treatment group without KSO supplementation (S0P0), namely 72.26%.

Protection of KSO reduced the cytotoxic effect of unsaturated fatty acids upon fibrolytic microbes, so that the molar proportion of ruminal acetic acid from the fermentation of elephant grass supplemented with protected KSO were higher than the treated group with unprotected KSO supplementation. The molar proportion of acetic acid were more and more high along with the increasing of protection level. Protection level of 75 and 100% at 5% KSO supplementation level (S1P3 and S1P4) resulted in molar proportion of acetic acid which were equivalent to the treatment group without KSO supplementation (S0P0), namely 71.34 and 72.03% vs. 72.26%. This phenomenon showed that protection of KSO began the 75% level could eliminate the inhibition upon fibrolytic microbes.

Propionic acid

Unsaturated fatty acids also have depressing effect on the methanogenic microbes, which have an impact on the decreasing of methane production (Patra, 2013; Thanh and Suksombat, 2013). Decreasing of methane production resulted in the increasing of external hydrogen pressure that could potentially inhibit the thermodynamics of reoxidation reaction of the reduced coenzyme ($\text{NADH}_2 \rightarrow \text{NAD}^+ + \text{H}_2$), so that the microbes are encouraged to reduce pyruvic acid to propionic acid, in order to maintain hydrogen balance (Baldwin and Allison, 1983). That mechanism was reflected in the increasing ($P < 0.05$) of molar proportion of propionic acid due to KSO supplementation. The molar proportion of propionic acid in the treatment group without supplementation (S0P0) was lower ($P < 0.05$) than the treatment group of KSO supplementation at the level of 5% (S1P0), 10% (S2P0) and 15% (S3P0), which were 21.30 vs. 23.67; 42.13 and 45.24%.

Protection of KSO could reduce the pressure on methanogenic microbes, so that the molar proportion of propionic acid in protected KSO treatment group was lower than KSO supplementation without protection treatment group ($P < 0.05$). In line with the increasing of protection level, the decreasing in the molar proportion of propionic acid became greater. Ruminal propionic acid molar proportion in 5% KSO supplementation level with 100% protection level treatment group (S1P4) did not significantly different from without supplementation treatment group (S0P0), namely 21.30 vs 21.27%.

Butyric acid

Ruminal butyric acid production essentially one of reaction mechanism to allow reoxidation of reduced

coenzyme (in this case NADH_2 become to NAD^+) in for ensure the continuity of fermentation in anaerobic systems (Baldwin and Allison, 1983). Pyruvic acid as the central intermediate compound in ruminal fermentation will be converted into various end products, among other butyric acid (Hungate, 1966). Reaction process of pyruvic acid to butyric acid involves the formation NAD from NADH_2 , namely in reduction of acetoacetyl-CoA to betahydroxybutyryl-CoA and reduction of crotonyl-CoA to butyryl-CoA (Baldwin and Allison, 1983). The reaction increases for reduced coenzyme reoxidation, along with the decreasing of methane production as a result of pressure on methanogenic microbes by unsaturated fatty acids in the KSO, because hydrogen production in the butyric acid formation is lower than in the acetic acid formation.

The change of butyric acid production was appeared significantly at 5% KSO supplementation level. The molar proportion of butyric acid increased sharply ($P < 0.05$) from 6.43% in the without supplementation treatment group to 12.10% in 5% KSO supplementation without protection treatment group (S1P0). The molar proportion of butyric acid decreased gradually along with the increasing of protection levels, because the decreasing of methanogenesis pressure. The molar proportion pattern of butyric acid at the higher levels of KSO supplementation (10 and 15%), was not specific. This phenomenon might be occurred because in the high level KSO supplementation, the main hydrogen controlling mechanism was through the increasing of propionic acid production.

Acetic acid / propionic acid ratio

The molar proportion ratio of acetic acid to the butyric acid (A / P) can be used as one of the indication in the energy efficiency of ruminal metabolism. Decreasing of A / P value means the increasing of energy efficiency of ruminal metabolism, which in line with the increasing of the molar proportion of propionic acid and decreasing of methane production (Rahbar et al., 2014). In general A / P data in Table 3 illustrated that KSO supplementation improved the energy efficiency of ruminal metabolism, as reflected by the decreasing of A / P value. The decreasing of A / P were occurred in the KSO supplemented treatment group, either unprotected or protected. Protection level of 100% resulted in the higher A / P value than the lower protection level for each level of KSO supplementation. The value of A/P in 5% KSO supplementation with 100% protection level treatment group (S1P4) was not significantly different from without supplementation treatment group (S0P0), namely 3.39 vs. 3.41.

Status of ruminal linoleic acid

The relative proportion of ruminal linoleic acid, stearic

Table 4. Relative proportion of linoleic and stearic acids (%) and IN.

Treatment combination	Linoleic acid	Stearic acid	IN
S0P0	nd	nd	nd
S1P0	18.5±1.6 ⁱ	8.2±1.1 ^c	11.2±0.5 ^{ij}
S1P1	22.5±1.1 ^g	7.9±1.1 ^{cd}	13.5±1.0 ^j
S1P2	31.5±1.2 ^f	7.4±0.6 ^{cd}	20.4±1.2 ^h
S1P3	34.9±1.1 ^e	6.0±1.2 ^{ef}	26.2±0.5 ^g
S1P4	38.0±1.1 ^d	3.4±0.6 ^h	28.5±1.0 ^f
S2P0	20.0±1.3 ^h	15.7±1.2 ^a	13.4±1.6 ⁱ
S2P1	38.9±0.8 ^d	6.3±0.6 ^{ef}	29.0±0.7 ^f
S2P2	43.6±0.7 ^c	5.4±0.8 ^{fg}	37.8±1.5 ^d
S2P3	45.4±1.1 ^b	4.6±0.5 ^g	42.8±1.1 ^c
S2P4	46.1±0.8 ^{ab}	3.0±0.6 ^h	48.6±1.0 ^b
S3P0	33.5±1.6 ^e	10.0±0.9 ^b	35.5±1.6 ^e
S3P1	43.5±0.5 ^c	6.9±0.9 ^{de}	47.2±1.6 ^b
S3P2	43.4±0.9 ^c	5.7±1.3 ^{fg}	48.2±2.2 ^b
S3P3	46.6±1.1 ^{ab}	5.5±0.5 ^{fg}	49.0±1.8 ^b
S3P4	47.1±1.1 ^a	3.2±0.4 ^h	51.1±2.0 ^a

The values were arithmetic means (n=5); nd : non detected; IN : iodine number. S0,S1,S2, S3 : KSO supplementation levels : 0; 5; 10; 15%, respectively. P0, P1, P2, P3, P4 : protection levels : 0; 25; 50; 75 and 100% respectively. Different superscript within column denote significantly different means (P <0.05).

acid and iodine number (IN) were showed in Table 4. In the without supplementation treatment group (S0P0), linoleic acid and stearic acid were undetected. In general, forage lipid content is low, and main fatty acid in forages was linolenic acid (Byers and Schelling, 1988; Mir et al., 2006). Lipids were metabolized and used rapidly by rumen microbes, so that its metabolic products were not detected (Varadyova et al., 2007). The lowest relative proportion of ruminal linoleic acid in 5% KSO supplementation treatment groups was found in S1P0, namely 18.53% whereas in S1P4 was highest, namely 38.02%. The portion of the hydrogenated linoleic acid in S1 treatment groups was in S1P0, because it was not protected. It can be seen from the relative proportions of stearic acid as biohydrogenation product which higher than other treatment combinations in S1 groups, namely 8.21% (S1P0). Protection decreased the relative proportions of stearic acid that the value of it was getting the lower, it was in line with the decreasing the level of protection at S1P4 namely 3.41%. The magnitude of biohydrogenation in S1P0 treatment combination was also visibled from the lower IN value (11.21), than that in protected S1 combination. Protection at the level of 100% gave a higher degree of unsaturation compared to unprotection and protection at a lower level (the value of IN was 28.46).

Phenomenon as described above was also found in the 10% KSO supplementation treatment groups (S2). Treatment combination of 10% KSO supplementation

without protection (S2P0) showed the lowest linoleic acid relative proportion and IN value (20.04 and 13.42%), with the highest relative proportion of stearic acid (15.71%), while the opposite value was seen in the treatment combination between 10% KSO supplementation and 100% protection (relative proportions of linoleic acid was 46.05% and IN value was 48.63) whereas the relative proportion of stearic acid was 2.99%. It showed that the treatment combination of KSO supplementation with protection could increase the proportion of available linoleic acid for the post-ruminal absorption, for productive purpose.

Treatment combination of 15% KSO supplementation without protection (S3P0) supplied the linoleic acid more than S2P0, but its biohydrogenation products, namely stearic acid, had lower relative proportion (P <0.05) than S2P0 (10.04 vs. 15.71%). That were presumably because the increasing of uncomplete biohydrogenation process and its product were accumulated as intermediate compounds, namely cis 9, trans 11 linoleic acid and trans-11 vaccenic acid (Khamal and Dhiman, 2004; Panatuk et al., 2013; Castano et al., 2014). That assumption was supported by the higher IN of S3P0 (P <0.05) than S2P0 (35.46 vs. 13.42). That intermediate compounds were unsaturated fatty acids derivatives, thus contributed to the magnitude of ruminal lipid unsaturation degree which was reflected on the magnitude of the iodine number (IN). Uncomplete biohydrogenation was occurred because inhibition in second step of

biohydrogenation, the change of trans vaccenic acid into stearic acid by the increasing of free linoleic acid as a result of trilinolein lipolysis by ruminal *Anaerovibrio lipolytica* that increased due to the increasing of KSO supplementation level (15%) without protection (Jenkins et al., 2008).

Conclusion

Kapok seed oil supplementation on elephant grass as fibrous feed lowers the ratio of acetic acid / propionic acid. Protected kapok seed oil supplementation up to 10% supplementation level and 75% protection level increased the molar proportion of ruminal propionic acid without affected the *in vitro* fiber digestibility with lactating Etawah crossbred goat rumen fluid .

The Increasing of KSO supplementation level (to 15%) increased the relative proportion of ruminal linoleic acid, followed by the increasing of ruminal fatty acid unsaturation degree (IN). Combination of KSO supplementation with protection increased the ruminal linoleic acid proportion and lipid iodine number. The inhibition of biohydrogenation by free linoleic acid significantly occurred at the level of 15% unprotected KSO supplementation, with the decreasing of ruminal stearic acid proportion. The KSO supplementation at the level of 10% with 75% protection level could resulted in the same linoleic acid proportion with 15% of KSO and 75% of protection level with a higher *in vitro* NDF digestibility.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional assessment of potassium in tomato (*Lycopersicon esculentum* Mill.) by direct reading of fruit sap

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Analysis of content made available to the plants and their relationship with what is present in plant tissue, are great alternative for proper handling of fertilizer, but the methods currently used, are expensive and require time. This study aimed to implement a new methodology for its determination and thus develop a correlation with the conventional method. It also evaluated the influence of the increase of K doses in production, plant development and relationship with the other nutrients. For the study we used two experiments with fertirrigated tomato having five different doses of potassium, grown in a greenhouse and analyzed according to the methodology developed. In the experiments were evaluated productivity, illuviated electrical conductivity and nutritional content in the fruit and leaf immediately above each bunch. Potassium was analyzed by conventional methodology and for comparison purposes. The values were submitted to polynomial regression analysis to the second degree and correlation. The results showed that increased potassium levels significantly influenced the productivity, illuviated electrical conductivity and K contents. After comparing the two methods was reached a relationship where reading by the conventional method is 20.085 times higher than the values presented by reading without digestion if the sap of the fruit and 9.6857 in the sap of the leaf petiole. The new methodology has proved capable of replacing the conventional of quick, cheap and effective form. Increased potassium levels had significantly influence in the illuviated electrical conductivity, productivity and nutritional content of the plant tissue.

Key words: Plant tissue, methodology, analysis, nitric perchloric acid digestion.

INTRODUCTION

Potassium (K) is one of the most required nutrients by plants, being responsible for the stomatal opening, which is related to photosynthesis and, consequently, to the

synthesis of photoassimilates, and act as an enzyme activator (Taiz and Zeiger, 2013).

In the case of tomato, K is the nutrient most required by

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the plant about 180 mg dm^{-3} (Almeida et al., 2015), mainly after the onset of the breeding season (Faquin, 2001), where this becomes important nutrient translocation of photoassimilates, especially for fruits, interfering in their sugar content and the synthesis of lycopene pigment (Marschner, 2011).

Given the difficulties encountered by the tomato production, such as low prices, high competition, pests and diseases, alternatives are necessary to increase profitability. The growing, in greenhouse, in substrates and fertigation become feasible to increase productivity and fruit quality. However, they are expensive techniques that rely on extensive attention and time. In the case of alert to the electrical conductivity of fertigation nutrient solution, and the content of each nutrient in accordance with the development of the plant.

Seeking to better monitor the development of plants and their nutrition, the evaluation of the nutrient content in leaves and fruits, compared with the levels available for absorption, can, especially in the case of fertirrigated crops, determine which nutrient should have his concentration managed to achieve better productivity (Braga, 2010).

There are some devices on the market that estimate the present potassium content in the plant by liquid extracted mainly from the petiole, but they are inaccurate and in some cases lead to greater errors than hits. The most feasible method for such determination is by further extraction of the leaf, which is dried, crushed and then digested with a nitric and perchloric acid solution based on the methodology developed by Bataglia et al. (1983). This method, although more accurate, demand more time, work and cost. Aiming to seek an alternative that enables the potassium reading in the plant in order to clarify its content quickly and cost effectively has been prepared for this work a direct reading of this nutrient, without going through any process of digestion, for only in the case of this element, there is no formation of molecular bonds within the plant, the remaining ionic form (Taiz and Zeiger, 2013), which is easy to read in a flame photometer. The aim was to determine the K content of fruit and leaves of tomato through direct reading without digestion, and so develop a correlation with the conventional method and also evaluate the influence of K levels in the production and development of the plant.

MATERIALS AND METHODS

First there was the study of the best method for extracting the sap of the fruit and tomato leaf. Fruits of different maturity stages were used beyond the leaves just above the bunches where these fruits were collected in order to reach the best way of extracting and her best dilution for direct reading in a flame photometer.

It was coming to the conclusion that the best method to be used would be to slightly squeeze the fruits cut in half to collect only their sap, which was collected in a test tube and then filtered, allowed to decant the tube itself, or collected for dilution and immediate analysis. Of the three extraction methods, the choice was the decanting tube as not different values of others and proved to be

the most practical method. The dilution was determined after several attempts not to exceed the last photometer reading point (100 ppm). For extracting the petiole was required great pressure on it because of its consistency, in this case with the aid of pliers. For dilution, both the fruit and the petiole to the best approach was to collect the extract and 0.1 ml diluted in 19.9 ml of deionized water and subsequently performing the direct reading in a flame photometer.

Plant materials

For the analysis of K and the new methodology testing levels, we used two experiments conducted in a greenhouse, arch type, with 6 m wide, 30 m long, 3 m high and transparent polyethylene cover, the Department of agronomy, and the samples analyzed in the Soil Laboratory, both from the State University of Londrina, Londrina city, State of Paraná, Brazil (latitude $23^{\circ} 23'S$, $51^{\circ} 10'W$ longitude, at an altitude of 580 m).

These experiments consisted of K rate of increase in tests with two varieties of fertirrigated tomato grown in pots with sand, being in a randomized block design with five treatments and 10 repetitions, totaling 50 vases side by side and 60 cm between rows, with borders around and fertirrigated drip.

Plants were grown in plastic pots with 9 L of capacity ($23.5 \times 26 \times 19.5 \text{ cm}$), using sand as substrate. The sand test results were obtained: $H + +Al + 3 = 1.89 \text{ cmolc dm}^{-3}$; organic matter = 0 g dm^{-3} ; $K + = 0 \text{ cmolc dm}^{-3}$; Mehlich P = $0.02 \text{ cmolc dm}^{-3}$; $Mg + 2 = 1.44 \text{ cmolc dm}^{-3}$; $Ca + 2 = 0.29 \text{ cmolc dm}^{-3}$ and $Al + 3 = 0.08 \text{ cmolc dm}^{-3}$.

The seedlings were tomato type Pizzadoro for the experiment 1 (E1) and Carina to experiment 2 (E2), from certified commercial vivarium, which were transplanted to pots with 25-30 cm high, on 23 March 2013. The experiment was conducted until July 29 of that year.

The pest control was performed preventively and the following insecticides were applied from the start of cultivation: Cypermethrin (pyrethroid), 1 ml L^{-1} , every 15 days, large fruit borer (*Helicoverpa zea*) and Dipel® (biological) 1 ml g^{-1} once a week for tomato leafminer (*Tuta absoluta*). The fungicides applied from the reproductive stage were: Chlorothalonil, 5 ml L^{-1} once a week for early blight, septoria and powdery mildew and Amistar Top®, 1 ml L^{-1} once every 15 days also to early blight.

Fertirrigation system

The experiments were conducted with five treatments consisted of five concentrations of K in the nutrient solution (60, 120, 180, 240 and 300 mg dm^{-3} of K) applied after the opening of the first flowers, with 29 days after transplanting, and until that stage of development, the nutrient solution was standard (180 mg dm^{-3} of K) for all treatments. These doses were established from prior knowledge of the mean dose of K which is recommended for the tomato crop, it would be 180 mg dm^{-3} (Almeida et al., 2015). From this information, it was decided to test doses, starting at 60 to 300 mg dm^{-3} .

The doses were of essential nutrients (mg dm^{-3}): N: 198, P: 43.6, C: 152.4, Ca 233, Mg: 27 S: 39 were used as fertilizers: the MAP ($200 \text{ g } 1000 \text{ L}^{-1}$); Ca (NO_3) 2 ($800 \text{ g } 1000 \text{ L}^{-1}$); CaCl_2 ($300 \text{ g } 1000 \text{ L}^{-1}$); MgSO_4 ($300 \text{ g } 1000 \text{ L}^{-1}$) and KNO_3 ($400 \text{ g } 1000 \text{ L}^{-1}$). Micronutrients were supplied through REXOLIN BRA® (11.6% K_2O , 1.28% S, 0.86% Mg, 2.1% B, 0.36% Cu, 2.66% Fe, 2.48% Mn, 0.036% Mo and 3.38% Zn) and REXOLIN M48® (65% to chelated Fe EDDHMA), both at a concentration of $25 \text{ g } 1000 \text{ L}^{-1}$ (Table 1).

Monitoring the concentration of each nutrient present it was given by periodically measuring the electrical conductivity of the solution

Table 1. Nutrient Concentration (mg dm^{-3}) and electrical conductivity (EC) (dS m^{-1}) of the nutrient solutions used in the treatments.

Nutrient	Treatments containing K (mg dm^{-3}) and of the nutritive solution conductivities ***						
	Compleat nutrient solution (100%)*	Solution* (70%)**	60 (2.07)***	120 (2.14)***	180 (2.15)***	240 (2.26)***	300 (2.42)***
N	198	138.6	198.0	198.0	198.0	198.0	198.0
P	43.6	30.52	43.6	43.6	43.6	43.6	43.6
K	152.4	106.68	60.0	120.0	180.0	240.0	300.0
Ca	233	163.1	233.0	233.0	233.0	233.0	233.0
Mg	27	18.9	27	27	27	27	27
S	39	27.3	39	39	39	39	39
B	0.5	0.35	0.5	0.5	0.5	0.5	0.5
Fe	5	3.5	5	5	5	5	5
Cu	0.07	0.049	0.07	0.07	0.07	0.07	0.07
Mn	0.1	0.07	1	1	1	1	1
Mo	0.075	0.0525	0.075	0.075	0.075	0.075	0.075
Zn	0.4	0.28	0.4	0.4	0.4	0.4	0.4

* Sarruge (1975) modified and used in the UEL Soils Laboratory. ** Nutrient solution used for 15 days for adaptation of seedlings.*** Means of electrical conductivity (EC) measured in the nutrient solution water tanks (dS m^{-1}).

in water tanks and the resulting illuviated the nutrient solution that passed through the vase and was retained on the plate below it, leaving the system conductivity exceed three dS m^{-1} which could adversely affect plant development. When the conductivity exceeded the three dS m^{-1} the fertirrigation was interrupted and the system was irrigated for one day only with water to prevent salinization of the system.

The fertigation system consisted of submersible pumps, with an operating pressure of up to 1.9 of metres of water column (mwc) and power of 38 watts, model AT 203 Atman® in water tank with 80 L capacity for each treatment. The pumps were connected to a timer, driven by a contactor to avoid damage due to oscillation of the amperage. The fertilizer applications were made through irrigation water with variable frequency so that the losses did not exceed 10% by irrigation interval, each dripper was set for maximum flow of 300 ml min^{-1} . The irrigation interval was defined based on climatic conditions how temperature, relative humidity, which were measured inside the greenhouse during the experiment, with datalogger Instrutherm® ht-500 model and the characteristics of culture, ranging from 1 to 5 times a day shift.

Average monthly temperatures were 33.6°C on 27 to 31 March 26°C in April, 27.5°C in May, 23.8°C in June and 27.9°C until the day July 29. The relative humidity was 42% of 27 to 31 March, 64.4% in April, 57% in May, 72.6% in June and 54.1% until July 29. Plants were conducted with three tomato bunches, and after the third bunch counted five leaves and pruned plants to cut the apical dominance.

Sample preparation treatments

After the onset of fruiting was collected a green fruit each repetition, the basal part of the bunch, with a diameter between four and five centimeters. It was also collected the leaf just above the same bunch for analysis, according to the experiment conducted by Almeida et al. (2015). It was determined the mass, diameter, length of the fruit and leaf mass collected over three clusters, each fruit was subsequently cut in half with one half its sap extracted as developed methodology. The leaves collected just above the three clusters had their sap extracted for direct analysis and then were analyzed by conventional methodology.

The other half of the fruit and leaf were placed in appropriately labeled Kraft paper bags and brought to air forced circulation stove at 65°C for three days. After drying was determined the dry weight of leaves and fruits and then crushed in Willey type mill. At the end of the experiments were obtained productivities and all plant material collected was prepared for determination of K by flame photometry for the final results.

Statistical analysis

The final results could be compared by means of polynomial regression to the second degree with 5% significance level, to establish a relationship between the two methodologies. The results of the different levels of K read from the sap of the petiole and fruits with and without digestion provided, as well as productivity and other nutrient levels have been correlated by Pearson correlation to determine which nutritional parameters the new methodology can measure, directly or indirectly.

RESULTS AND DISCUSSION

Productivity

In regard to yield the highest value found in the two experiments was at a dose of 300 mg dm^{-3} K, with 80.00 t ha^{-1} at Experiment 1(E1) and 98.20 t ha^{-1} at Experiment 2(E2) (Table 2), agreeing with Sara et al. (2007) also observed an increase in the fresh weight production according to the concentration of nutrients until it reaches a saturation point where the output decreases considerably.

Cook and Sander (1991) also observed a significant increase in tomato yield with the increase in the K doses, according to the results of E2 that had linear adjustment as increasing doses. However, E1 had a quadratic adjustment with the trend towards greater productivity for

Table 2. Average of production in t ha⁻¹ in function of K doses in both experiments.

Productivity of tomato in t ha ⁻¹	Doses of K (mg dm ⁻³)					Cv (%)	p>F*
	60	120	180	240	300		
Experiment 1	46.13	59.85	76.23	71.54	80.00	28.34	*
Experiment 2	56.47	78.69	88.48	92.21	98.20	27.39	*

*: Significant data to 5% by regression analysis. ns: not significant data for 5% by regression analysis.

Table 3. Means of electrical conductivities of the two experiments in five treatments in dS m⁻¹.

illuviated EC in dS m ⁻¹	Doses of K (mg dm ⁻³)**					Cv (%)	p>F*
	60	120	180	240	300		
Experiment 1	2.48	2.28	2.7	3.09	3.46	15.14	*
Experiment 2	2.49	2.59	2.67	2.98	3.14	9.87	*

*: Significant data to 5% by regression analysis. **: Treatments with five doses of K (60, 120, 180, 240 and 300 mg dm⁻³) in nutrient solution. ns: not significant data for 5% by regression analysis.

the dose of 180 mg dm⁻³ (76,23 t ha⁻¹) agreement with Coltman and Riede (1992) who obtained similar results working with fertigation under greenhouse conditions with K five levels, with higher productivity at a dose of 200 mg dm⁻³. The total yield obtained in this experiment was similar to the 97.9 t ha⁻¹ recorded by Macedo (2005). According to Fontes et al. (2000), the commercial and total yield of tomato have increased with the increase in the K doses, reaching a maximum of 73.4 and 86.4 t ha⁻¹, with the application of 194 and 198 kg ha⁻¹ K respectively.

Roquejani et al. (2008), in their studies with productivity and quality of tomato hybrids Italian segments and holy cross in the greenhouse when thinned, had an average yield of 106.7 t ha⁻¹ to variety Giuliana.

The increased productivity observed in both experiments shows that when the tomatoes are conducted in the nutrient solution, where all nutrients are supplied in optimum quantity for their full development, increasing the amount of potassium, which is the nutrient required in greater quantity reflects directly in the best plant development and higher fruit production.

Illuviated electric conductivity

The means of illuviated electric conductivity (EC) in each dose of K differ significantly with increasing linear fit to the experiments (Table 3), the largest of which EC was obtained at a dose 300 mg dm⁻³ (3.46 dS m⁻¹ to E1 and 3.14 dS m⁻¹ for the E2) and the lowest was observed with 120 mg dm⁻³ K (2.28 dS m⁻¹) in E1 and 60 mg dm⁻³ with K (2.49 dS m⁻¹) to E2.

The results for the two experiments agree with Genúncio et al. (2006), working with growth and tomato yield under hydroponics in ionic concentration of the

nutrient solution, where they observed values greater than 2.0 dS m⁻¹ in the EC.

According to Maas and Hoffman (1977), the maximum tolerable soil salinity, expressed in terms of EC, the tomato is 2.5 dS m⁻¹, with a reduction of 9.9% in the production unit for each incremented EC. Eloi et al. (2007), testing effect of salinity in tomato grown in sandy loam soil, found the salinity threshold value of 3.03 dS m⁻¹, very close to that seen in E1 and E2, with decreased productivity in 10.95% for each increase of one unit of soil salinity, caused by fertilizer salts. During the conduct of experiments care was taken not to allow the illuviated electric conductivity exceed 3.0 dS m⁻¹, so there was not salinity problems of the system, thus contributing to the full development of the plants.

Comparison of the two methods

After analysis of the K content in both methods of reading, in the sap of the fruit without digestion (SFWD) and K content in the dry matter of fruit with digestion (FRD), the three bunches of two experiments, it is concluded that there was significant difference with increased K content. There was an increase in K content (Tables 4 and 5) to the fruits of the second and third bunch without digestion in the E1 and E2 of the three bunches. The K levels after digestion had difference for the second and third lock of E1 and E2 in all bunches.

As the treatments had concurrent with beginning to flourish, it is appropriate to no significance to the first bunch in E1 for the plants are subject to treatments for a very short period. The results agree with the Blanco and Folegatti (2008), who worked with K levels in tomato hybrid "Facundo" under salt stress, and found that the higher the dose of K, the greater the absorption by fruit,

Table 4. K content in g kg^{-1} of sap fruit without digestion (SFWD) and fruit with digestion (FRD) in the first, second and third bunch in accordance with increasing doses of potassium experiment 1.

K in g kg^{-1}		Doses of K (mg dm^{-3})**					CV(%)	p>F*
		60	120	180	240	300		
1 st bunch	SFWD	3.48	3.45	3.76	3.83	3.64	16.19	ns
	FRD	54.84	84.04	78.21	71.54	75.66	18.87	ns
2 nd bunch	SFWD	2.44	2.68	2.79	2.88	3.46	18.94	*
	FRD	55.88	68.13	70.64	72.64	74.39	12.06	*
3 th bunch	SFWD	3.17	3.81	3.83	3.78	4.18	14.61	*
	FRD	54.88	66.79	67.62	70.95	73.32	7.51	*

*: Significant data to 5% by regression analysis. **: Treatments with five doses of K (60.120.180.240 and 300 mg dm^{-3}) in nutrient solution.

ns: not significant data for 5% by regression analysis.

Table 5. K content in g kg^{-1} of sap fruit without digestion (SFWD) and fruit with digestion (FRD) in the first, second and third bunch in accordance with increasing doses of potassium experiment 2.

K em g kg^{-1}		Doses de K (mg dm^{-3})**					CV(%)	p>F*
		60	120	180	240	300		
1 st bunch	SFWD	3.15	3.17	3.41	3.48	3.68	16	*
	FRD	67.16	78.66	78.64	82.17	88.72	16.37	*
2 nd bunch	SFWD	2.61	3.11	3.55	3.90	4.27	27.82	*
	FRD	50.46	69.80	74.29	81.39	84.40	14.27	*
3 th bunch	SFWD	3.29	3.63	3.70	3.74	3.82	16.29	*
	FRD	59.51	63.60	69.32	77.31	80.93	7.32	*

*: Significant data to 5% by regression analysis. **: Treatments with five doses of K (60.120.180.240 and 300 mg dm^{-3}) in nutrient solution.

ns: not significant data for 5% by regression analysis.

and found that the highest concentration was 35 g kg^{-1} , the highest dose applied ($24 \text{ g plant}^{-1} \text{ K}$). A suitable potassium fertilizer provides tomatoes with more pronounced red color and well formed inside, without the presence of voids. The fruits are firmly stuck in plants, reducing losses by fall. Disability, the fruits have bad coloration and shorter conservation. The excess may result in cracking in fruit (Moraes, 2006).

After comparing the two methods, direct reading from the sap of the fruit and reading after digestion of dry matter through polynomial regression to the second degree, it reached a relationship where reading with digestion, which is the conventional method used in laboratories, is 20.085 times higher than the values presented for reading without prior digestion, namely $y=20,085x$, where y is the value obtained by reading the sample after passing for digestion is the value obtained without prior digestion, as shown in Figure 1. As the levels found in the sap of the leaf petiole (SLP) and

digestion of leaf (LD) collected directly above each bunch also had a significant response with increasing dose for the two methods (Tables 6 and 7).

The results shown in Tables 6 and 7 agree with Calvert (1969) found that increased K content in leaf tissue according to increasing doses of adult citrus plants and Miller et al. (1993) found that the same effect of increasing doses of rootstock citrus in the early stages of growth. Fertilizers with K promote increased concentration in plant tissue, as observed results in Tables 4, 5, 6, and 7, result in superior whole plant due to increased photosynthetic efficiency, resistance to certain diseases. In tomato, as K increases the concentration of the nutrient solution, the concentration of K in the leaf and fruit, as well as the amounts extracted by the same also increase.

The K is absorbed by the roots in the form of ion, the roots being translocated through mass flow to the outer membranes of the cells of the leaves and then stored in

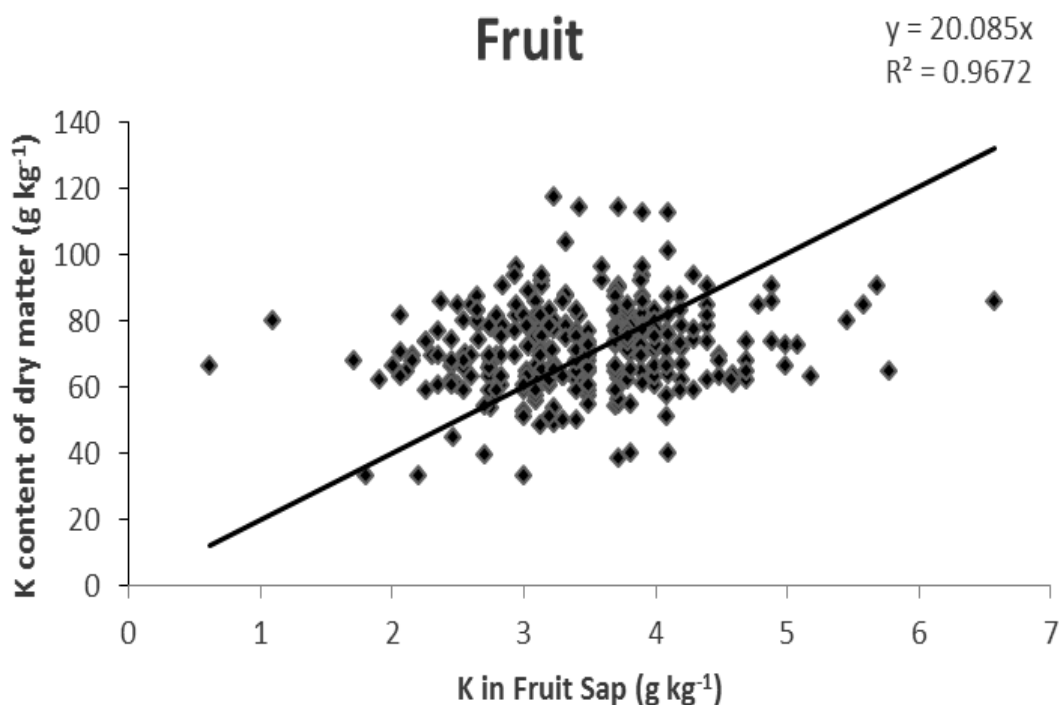


Figure 1. Regression between the readings of the K content in the sap of fruit without digestion and fruit after digestion for both experiments in the fruits of the three bunches. According to five levels of K.

Table 6. K content in g kg^{-1} in the sap of the leaf petiole without digestion (SLP) and in the leaf with digestion (LD) just above the first, second and third bunches in accordance with the increase of K doses of experiment 1.

K in g kg^{-1}		Doses of K (mg dm^{-3})**					CV(%)	p>F*
		60	120	180	240	300		
1 st bunch	SLP	2.79	4.61	5.53	5.74	6.24	25.81	*
	LD	27.64	49.40	52.05	63.12	65.08	17.17	*
2 nd bunch	SLP	1.99	3.14	2.97	4.26	4.97	33.84	*
	LD	30.97	33.62	37.69	48.84	48.41	24.62	*
3 th bunch	SLP	3.41	4.59	6.95	7.46	8.63	13.81	*
	LD	27.73	50.67	56.04	61.09	83.50	11.76	*

*: Significant data to 5% by regression analysis.**: Treatments with five doses of K (60.120.180.240 and 300 mg dm^{-3}) in nutrient solution. ns: not significant data for 5% by regression analysis.

the vacuole. When required for the tomato plant growth, it is transferred to the phloem and then mobilized to parties in growth as immature leaves and fruits (Wood and Parish, 2003).

The concentration of nutrients in the petiole sap indicates the amount of this circulating in the plant at the time and so the present levels both in the plant as being absorbed. The analysis of the sap, for diagnostic purposes, seeks to determine, in real time, the

momentary concentration of K in the plant or NO_3 , and usually has a significant correlation with the concentration found in the leaf determined by the conventional method and the plant production, such as checked Alcantar et al. (2002) for the garlic crop.

After comparing the two methods by polynomial regression to the second degree was reached a relationship where reading with digestion, which is the conventional method used in laboratories, is 9.6857 times

Table 7. K content in g kg^{-1} in the sap of the leaf petiole without digestion (SLP) and in the leaf with digestion (LD) just above the first, second and third bunches in accordance with the increase of K doses of experiment 2.

K in g kg^{-1}		Doses of K (mg dm^{-3})**					CV(%)	p>F*
		60	120	180	240	300		
1 st bunch	SLP	4.39	4.94	5.07	5.47	6.31	18.58	*
	LD	46.39	49.19	62.53	63.06	64.92	18.04	*
2 nd bunch	SLP	2.95	5.34	6.35	6.67	6.91	23.42	*
	LD	43.52	66.11	71.35	73.39	75.50	16.07	*
3 th bunch	SLP	3.33	5.45	7.53	8.15	8.47	12.46	*
	LD	25.87	54.05	63.81	69.17	72.15	14.21	*

*: Significant data to 5% by regression analysis. **: Treatments with five doses of K (60.120.180.240 and 300 mg dm^{-3}) in nutrient solution.

ns: not significant data for 5% by regression analysis.

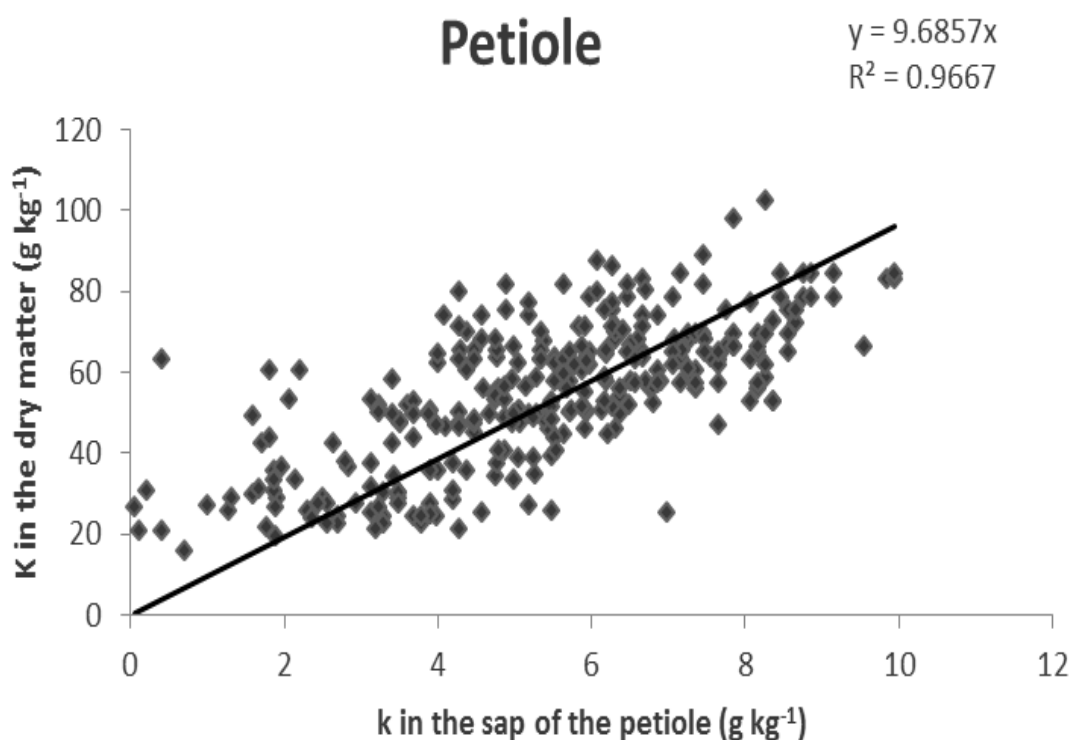


Figure 2. Regression between readings with and without digestion for the two experiments on leaves in the three bunches. According to five levels of K.

higher than the figures given for reading without digestion that is, $y=9,6857x$, where y is the value obtained by reading the sample after undergoing digestion exo value obtained without prior digestion, as shown in Figure 2.

Moreira and Vidigal (2009) found relationship between NO_3 content in the fourth potato leaf petiole sap with increasing doses of N. For K close relationship between its content in the sap of the petiole and leaf dry weight

were reported by Qian et al. (1995) for the culture of canola and Kallenbach (2000) for alfalfa, agreeing with the observed in this study.

The K values obtained by both the methodology for direct reading without digestion, as the methodology with digestion could be compared using regression and correlation to find a relationship between the methodologies and nutritional aspects of the two varieties

Table 8. Macronutrient in g kg⁻¹ in index leaf of the first and second experiment. collected just above the second bunch.

	N	P	K	Ca	Mg	S	
	Mg dm⁻³		g kg⁻¹				
E1	60	33.83	9.77	30.97	34.67	3.00	11.87
	120	32.17	10.63	35.01	36.54	3.05	14.95
	180	29.82	10.68	37.69	30.12	2.71	14.41
	240	32.28	10.55	48.84	19.27	2.93	16.43
	300	34.30	9.57	48.41	18.76	2.73	14.65
	CV(%)	14.32	13.76	25.17	32.66	21.21	23.42
p>F*	ns	ns	*	*	ns	ns	
E2	60	43.00	12.44	43.52	33.51	2.71	6.66
	120	37.49	10.02	65.55	36.50	3.01	12.42
	180	27.20	11.27	71.35	31.90	2.35	12.26
	240	32.34	10.21	73.39	20.41	2.77	17.70
	300	31.89	9.68	75.50	18.74	2.88	18.57
	CV(%)	10.70	27.78	16.50	32.88	26.38	24.06
p>F*	*	ns	*	*	ns	*	

*: Significant data to 5% by regression analysis. ns: not significant data for 5% by regression analysis.

of tomato. Comparing the K in the sap of the petiole and leaf nutritional index, which in this study was considered just above the second cluster (Table 8) and the Convention as K values being obtained by reading with digestion, in tomato E1 Pizzadoro we came to a positive correlation of 0.924 (Figure 3A) and significant relationship verified through regression. For Carina conducted in tomato E2, also we had significant positive results by regression and correlation of 0.967 as shown in Figure 3B.

For variety Pizzadoro E1 compared by means of regression, the K content in fruit (Tables 4 and 5) without prior digestion with the content in the sap of the petiole (Tables 6 and 7), also without digestion was obtained a CV 33.62% with 5% significance and a correlation of 0.977, according to 3C.

The variety Carina E2 showed similar behavior when comparing the same parameters, where he met significance by regression analysis between K in the sap of the fruit without digestion and petiole of the leaves, also for reading without digestion. Was met a CV of 25.89% and correlation of 0.98 as a 3D figure.

The relationship between the K content found in the sap fruit without digestion with the leaf above its respective bunch, again reveals significant values for both experiments with positive correlation for the 0.885 tomato Pizzadoro (E1) (Figure 3E) and for the 0.903 tomato Carina (E2) (Figure 3F). When compared to the K content in the sap of the leaf petiole with the tomato have significant results for the two experiments, when subjected to regression of 0.375 and a positive correlation for E1 (Figure 3G) and 0.996 to E2 (Figure 3H).

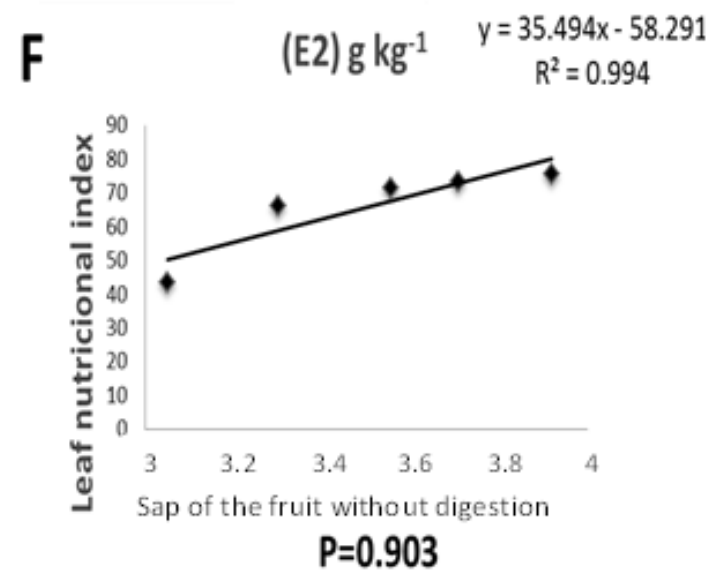
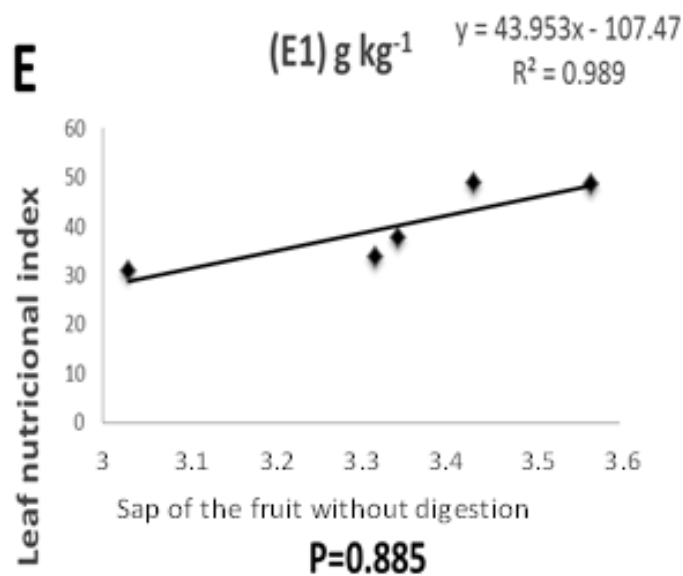
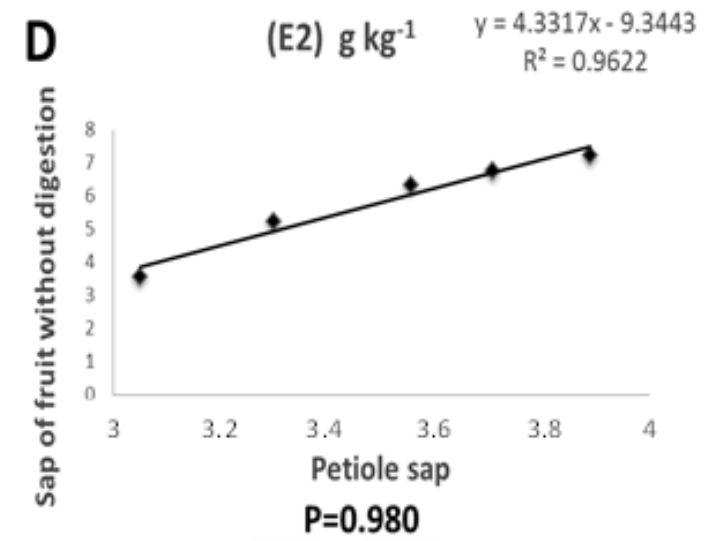
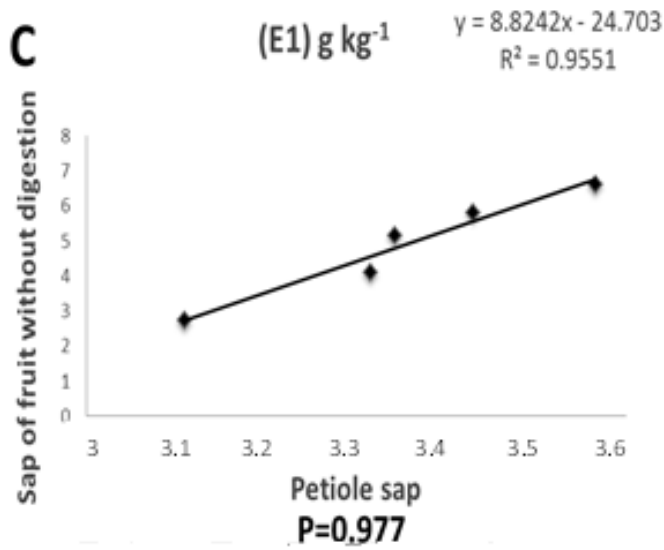
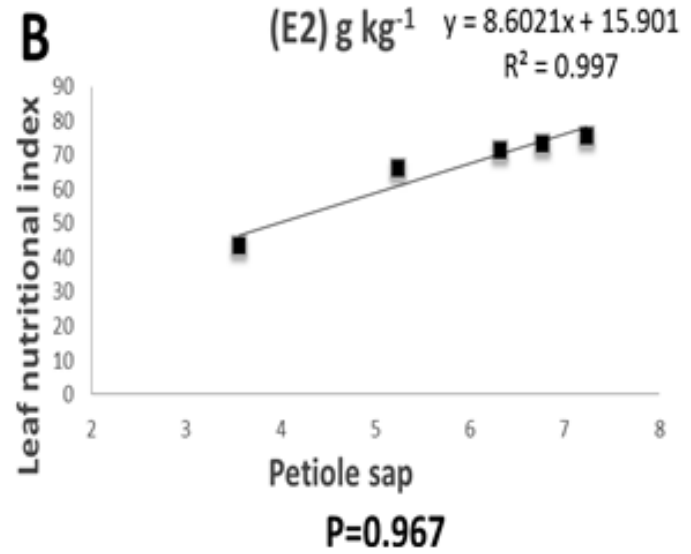
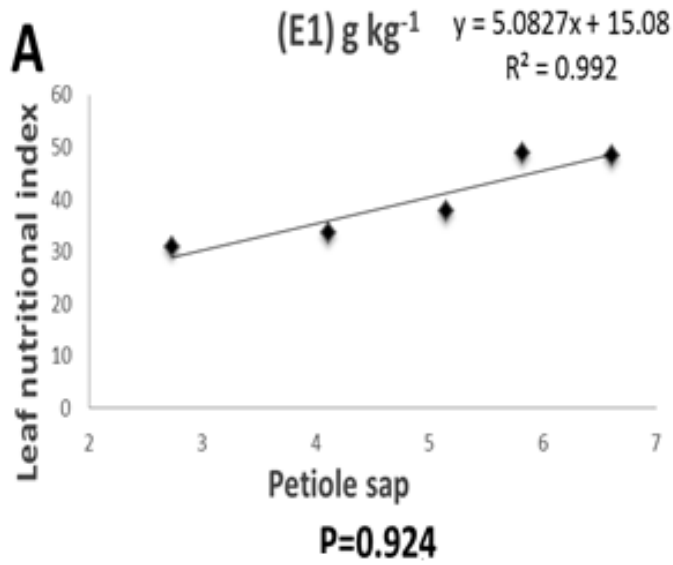
The production compared with the K content in the

leaves is significant in the regression analysis for the two varieties used, having a positive correlation of 0.113 in E1 (Figure 3I) and 0.983 in E2 (Figure 3J).

When comparing the K content in the fruit after digestion with the production correlation found was positive 0.431 (Figure 3K) and no significant results after regression analysis for E1 and 0.981 positive and significant correlation values after regression analysis of E2 (Figure 3L). This fact is mainly the difference between varieties, where the Pizzadoro tomato (E1) had an increase in its output until the dose of 180 mg dm⁻³ of K and then a beginning of competition for nutrients and a decrease in productivity tend.

Already variety Carina (E2) responded better to increased K levels with significant results in production until the dose of 300 mg dm⁻³, showing the behavior of each variety with respect to increasing doses and its response in production as can be seen in Figure 3K and L.

According to the results presented above with the correlations between: the K content in the sap of the fruit without digestion with the content in the petiole sap, the sap of the petiole and the nutritional leaf index, the sap without digestion fruit and leaves, sap petiole and the production and ultimately production and leaves, we can observe the close relationship between potassium uptake with increasing doses and their interaction with production. From the results it gives importance to the quadratic increase in production for the E1 and E2 linear for showing the influence of genotype on the absorption of nutrients and nutritional requirement as verified by Singh et al. (2000), Youssef et al. (2001), Ravinder-Singh et al. (1999) and Warner et al. (2004). About correlations can be said that the higher the K content in the leaf, the



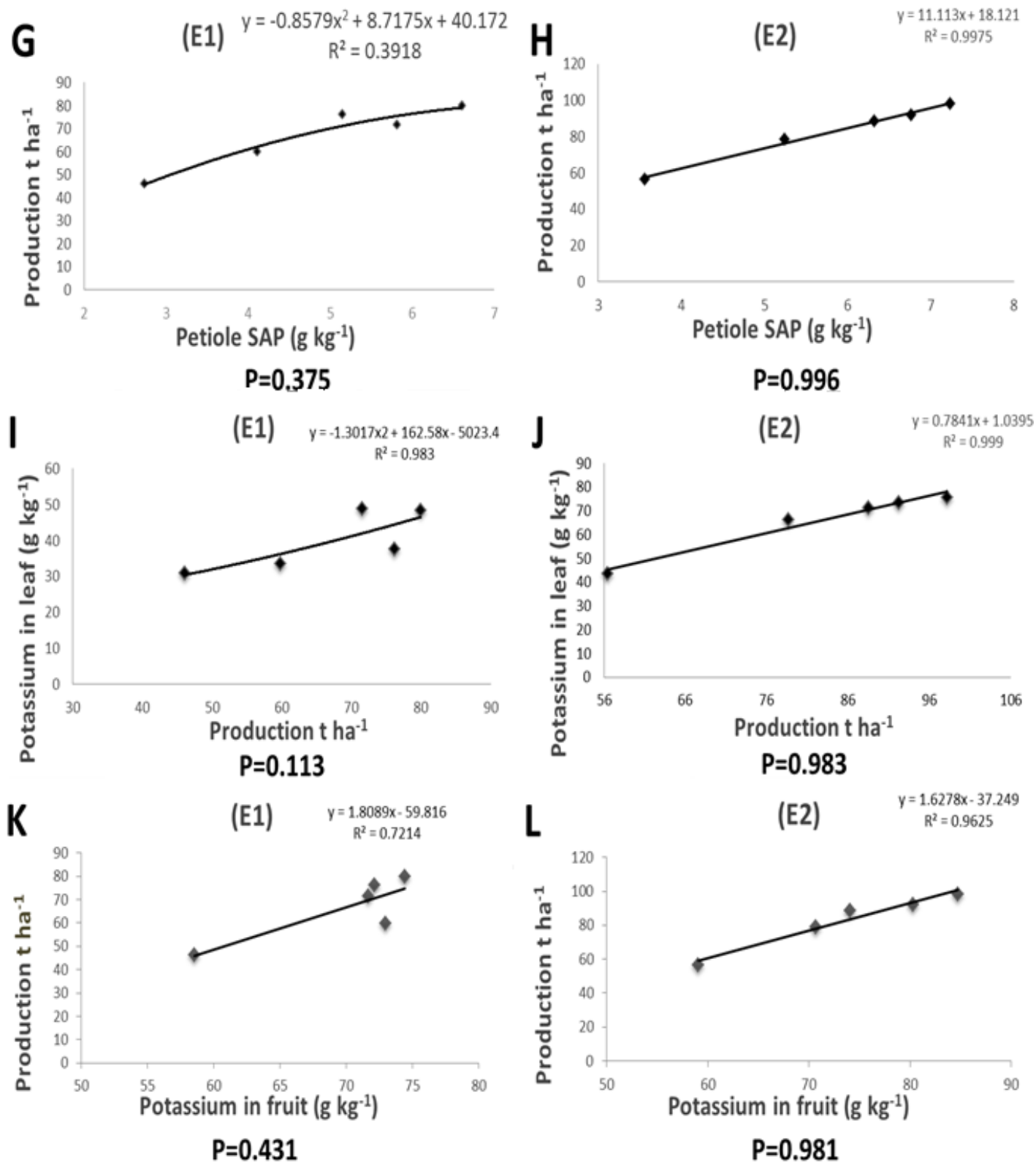


Figure 3. A and B-Levels of potassium found in the sap of the petiole and nutritional leaf index of experiments 1 and 2. C and D-Potassium Levels found in the sap of the fruit without digestion and in the petiole sap from leaves without digestion of experiments 1 and 2. E and F Relationship between potassium levels found in the sap of the fruit without digestion and dry matter of the leaves in the experiments 1 and 2. G and H Relationship between potassium levels found in the petiole sap without digestion with production in experiments 1 and 2. I and J- Relationship between K concentration found in the leaf with production in $t\ ha^{-1}$ for the experiments 1 and 2. K and G- Ratio between potassium levels found in fruit with production in $t\ ha^{-1}$ in the experiments 1 and 2.

higher also in developing fruit and this nutrient has strong positive correlation with production, mainly because it is the nutrient most required by culture and also since it is experiments under fertirrigation where all nutrients are readily available to the plant, making the increase of K doses the main factor for the increase of production.

Therefore both the direct analysis of the K content in the sap of the fruit, in the petiole sap from the leaves and the leaves reflect the increase in production as verified by Coltman and Riede (1992) working with the cultivar grown under Celebrity fertirrigation home conditions vegetation with five levels of K by monitoring the concentration of K in the sap of the petiole with colorimetric paper strip.

The authors noted that the methodology was tested sensitive to different doses of K and K levels of the petiole sap, and fruit production a quadratic increase, where the maximum yield was obtained in the range 190 to 200 mg per liter of K the external solution and 5.9 mg of K per ml of the petiole sap.

Conclusions

The methodology developed for determining potassium in the sap of the fruit and leaf petiole of tomato is effective to evaluate the potassium content and the plant nutritional status of practical and economical way. The direct analysis is recommended to determine potassium in tomato. The recommended dose of potassium is 180 mg dm⁻³ to pizzadoro tomato and 300 mg dm⁻³ to carina tomato. Increased potassium levels significantly influence the illuviated electrical conductivity, productivity and nutritional content in the plant tissue.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviation

E1, Experiment 1; **E2**, Experiment 2; **EC**, Electrical conductivity; **SFWD**, sap fruit without digestion ; **FRD**, fruit with digestion; **SLP**, sap of leaf petiole without digestion; **LD**, leaf with digestion.

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Full Length Research Paper

Willingness to participate in the market for crop drought index insurance among farmers in Ghana

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The study, conducted among maize farmers in the Nanumba North District of the Northern Region of Ghana, examined the willingness of smallholder farmers to participate in the market for drought index crop insurance. A total of 100 farmers participated in the study. The study employed the logistic regression analysis to predict decision to participate in crop insurance. Quantitative and qualitative data collection methods were employed to allow for triangulation. These included questionnaires, focus group discussions and key informant interviews. The results demonstrate that access to credit, education and experience of other forms of insurance are the most important determinants of farmers' willingness to participate in crop insurance. Total damage incurred also increased the probability of decision to participate, whereas return period of disaster event in the past and number of non-nature dependent income sources reduce the probability of decision to participate in crop insurance. The results emphasize the need to integrate crop insurance into micro-finance to enhance buy-in by farmers. Mass education via Radio and television are keys to improving access to information on crop insurance by farmers. However, significant investment in education in rural areas is critical, in the long term, to ensure the adoption of crop insurance.

Keywords: Crop insurance, climate change, participation, weather-indexed insurance, agriculture, climate change, adaptation, maize, farmers.

INTRODUCTION

It is widely predicted that agriculture will be most affected of all sectors by climate change in Africa (Nelson et al., 2009). The resultant higher temperatures, higher incidence of droughts and floods as well as other weather related events pose production risks to farmers. The strong dependence of agriculture on the natural environment, especially rainfall, makes it risk prone rendering large sections of the agricultural population vulnerable to climate change (Kurukulasuriya et al., 2006). Parry et al.

(1999) estimate that climate change will put an estimated 55 to 70 million extra people at risk of hunger in Africa by 2080. The most affected regions in Africa include Western and Central Africa where agricultural output is expected to reduce by 2 to 4% (Mendelsohn et al., 2000). The impact of climate change on agriculture in Ghana is expected to be even more severe in the northern Savannah zones where annual droughts are already a problem and affect the livelihoods of the population which

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is mostly rural and dependent on agriculture. The area is characterized by a single rainfall season and most dependent on rainfall for agricultural production. It is expected that the mean daily temperature will increase by 3°C while rainfall declines between 9 and 27% by the year 2100.

The rapidly escalating climate change related risk to agriculture has challenged the ability of the natural system to cope. This has brought new and unprecedented pressure to bear on the natural system as has been observed elsewhere by Ziervogel et al. (2008). In the face of such challenges public intervention in mitigating the effects of climate change has been called for and justified on the grounds that agriculture accounts for a major source of livelihoods. Therefore, such covariate risks as posed by climate change will exacerbate the poverty and food security, especially, in most rural areas. The role of adaptation measures in managing the negative consequences of climate change is undeniable. However, climate change adaptation, just like any other strategy, requires a conducive policy framework to be effective. Khan et al. (2009) affirm the need for multi-sectorial interventions to address food security and poverty. A key challenge in Africa, in this regards, is the fact the region has seen adequate investment in agriculture over a long period of time. This has resulted in poorly developed agricultural and supporting infrastructure as well as poorly developed agricultural markets. One would therefore, expect that Africa lags behind in climate change related disaster preparedness has a long way to go in putting in place the necessary measures to off-set the effects of climate change to any appreciable extent in the near future.

It is obvious that the most debilitating and obvious effect of climate change on food security, so far, has been increasing incidence of drought and irregular distribution of rainfall (Vermeulen et al., 2010). In this regard, irrigation agriculture is expected to play a significant role in the fight against the negative consequence of climate change. This is supported by Valipour (2014a) who indicates that, globally, 46% of all agricultural land is not suitable for agriculture due to climate change. The situation, as would be expected, would be more severe for the global south where irrigation agriculture is poorly developed and a large section of the agricultural population still depends on smallholder rain-fed agriculture. Valipour (2014b) shows that, globally, irrigated area has expanded by less than 1% since 1975. This is against the background that current estimated potential crop yields are less than 30% for sub-Saharan Africa (Valipour, 2014a). Again, Valipour (2014a) shows that the value of irrigation-equipped area as a percentage of total agricultural area is only 5.8% for Africa. This is the case in Ghana where irrigation agriculture accounts for only 1% of agricultural area (Ghana Irrigation Development Authority, 2012). It is important to note that apart from the fact that Africa and Sub-Saharan Africa, in particular, is

is lagging behind in irrigation development, existing irrigation schemes and infrastructure have suffered the consequences of poor policies and institutions. Valipour (2014b) cites poor macroeconomic policies that render irrigation agriculture unprofitable and the poor performance of many irrigation projects as some of the challenges that have countered the expected poverty reduction effects of irrigation development. This is supported by Hanjira et al. (2009) who argue that human capital and access to markets are critical in ensuring that irrigation development achieves the intended poverty alleviation impact. This situation is typical of Ghana and is amply demonstrated in the Tono irrigation scheme in Ghana where Dinye (2013) reveals that Ghanaian traders prefer to purchase tomato from neighbouring Burkina Faso despite the fact that farmers produce large quantities of tomato that go to waste annually. This is the result of poor policies that have rendered irrigation agriculture in Ghana non-competitive. According to the irrigation development Authority of Ghana (2012), a number of factors account for the inadequacies within the irrigation sector including organisational and institutional weaknesses, as well as, lack of clarity of institutional mandate. Thus, irrigation development will continue to be slow unless the right policy and institutional frameworks exist to provide adequate support to the sector.

An agricultural system, like any other system, has a measure of inbuilt adaptation capacity (Ziervogel et al., 2008). However, the current rapid rate of climate change will impose new and potentially overwhelming pressures on existing adaptation capacity. It has been argued above that the growing climate change risks and the inability of existing agricultural systems in Ghana and elsewhere, as argued above, to cope with its effects has brought forth the need to consider contingency plans against possible widespread food insecurity and famine. One of such interventions is crop insurance. Interest has grown over the years in the introduction of weather-based crop index insurance as one way to address climate related risks to farmers. A notable example, in this case, is China where the agricultural insurance market has grown rapidly over the years. Many countries in Africa have investigated the feasibility of agricultural insurance, and some have implemented pilot programs with support from international donors notably the World Bank. In Ghana, the project "Innovative Insurance Products for the Adaptation to Climate Change" (IIPACC) funded by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety implemented a pilot weather-indexed crop insurance (WII) scheme between 2009 and 2013. It was jointly implemented by the National Insurance Commission of Ghana (NIC) and German International Cooperation (GIZ) with support from Swiss Re. The first WII product for drought cover of maize was sold to four institutions in northern Ghana in May 2011 and covered over 3000 smallholder farmers under the Ghana Agricultural Insurance Program (GAIP).

In 2012 the first payouts were made to 136 farmers. Currently crop drought index insurance is offered in six regions, namely: Northern, Upper East, Upper West, Brong Ahafo, Ashanti and Eastern regions. Currently, weather indexed crop insurance is offered by a consortium of private insurers to farmers across Ghana.

While proponents of crop insurance see it as a viable risk coping mechanism that has the potential to help farmers in developing countries to cope with weather related risk others hold the view that crop insurance is unsustainable citing high and unaffordable premiums (Skees et al., 1999). The latter view is also supported by Hazell et al. (1986) and Gurenko and Mahul (2004) who indicate that most crops insurance, globally, fail to earn enough premiums to cover pay-outs and administrative costs because farmers are unwilling to pay the full cost of insurance. This school of thought argue that the case for public subsidy on crop insurance tenable on these grounds. Although the decision to buy insurance is purely an economic one public subsidy on crop insurance is justified on the basis that weather related agricultural constraints such as climate change pose a covariate risk that has a wider implication for sustainable livelihoods and food security for large sections of the people vulnerable to climate change. It has been argued that the absence of a purely private agricultural insurance product is the result of market failures resulting from systemic risk associated with correlated yield losses among. However, this argument appears to be untenable as others (Wright and Hewitt, 1994; Goodwin and Smith, 2009) argue that systemic risk is much more severe in markets for other types of insurance that are offered by the private sector. The real challenge inducing low uptake of crop insurance by farmers appears to be the fact that premiums are more than what farmers are willing to pay as argued above. Much as it is undeniable that the ability of farmers to pay premiums is important in determining farmers' decision to participate in crops insurance narrowing the debate to this makes it somehow simplistic. The study rather sought to assess farmers' willingness to participate in the market for crop insurance. This way, it is possible to examine a wider array of factors, including the ability to pay for crop insurance, regarding uptake of crop insurance.

MATERIALS AND METHODS

The Study area

Northern Ghana is situated between 8° and 11° N latitude and 0° to 3° W longitude. Administratively it comprises of the Upper West Region (UWR), Upper East Region (UER) and Northern Region (NR). The area falls within the dry land Savannah zone occupying an estimated 40% of the country. The rainfall pattern is mono-modal. The rainy season permits a growing season of 150 to 160 days in the Upper East Region and 180 to 200 days in the two other regions. Mean total annual rainfall varies from 1,000 mm in the Upper East Region to 1,200 in the south eastern part of the Northern

Northern Region. The rainfall shows wide variations from year to year, both as regards the amount and the time when it occurs. Food shortages are a common feature of the dry season. According to the 2010 population and housing census the 3 northern regions together account for 17.3% of the total population of Ghana. Northern region accounts for the largest share (10.2%), followed by Upper East region (4.3%) and the Upper West region (2.8%). The Northern Region, despite being the largest, in terms of land mass, is the least populated among the 3 administrative regions of northern Ghana with a population density of 35 persons per kilometre square. The Upper East region has the highest population density of 118 km² while the Upper West region has a population density of 38 persons per square kilometre. The study area, Nanumba North District, is located in the eastern corridor of the Northern Region of Ghana between latitude 8.5 N and 9.25 N and longitude 0.57 E and 0.5 W. It shares boundary with Yendi District to the north, Nanumba South District to the southeast, East Gonja District to the west and south west and Nanumba South District to the south and the east. The District covers an area of 1,986 km² with an estimated population of 101,760 people. Annual rainfall averages 1268 mm with most of it falling within six (6) months. Agriculture is the main source of livelihood of the people engaging about 85% of the population. Crops grown include root and tubers, cereals, legumes and tree crops such as teak and cashew nuts. Animal rearing including poultry keeping is an integral part of every household.

Sampling and data collection

The study was conducted among maize farmers. The rationale for selecting maize is based on the fact that maize is a major staple, as well as, a cash crop in the study area. Thus, the importance of maize in terms of food and cash crops makes it a very important crop. Ten communities were randomly selected for the study. From each of the ten villages selected, 10 farmers were selected to form a sample size of 100 farmers using simple random sampling technique. Quantitative and qualitative approaches are adopted in collecting data for the study to allow for statistically reliable information by way of triangulation.

The questionnaire used for the survey consisted of around 40 questions and divided into different sections. In the first section respondents are asked about their age, occupation, educational background, family size, sources of income, assets, standard of living, type of crop cultivated and so forth. The second section comprises questions related to households' experience of catastrophic events where respondents are first asked whether or not they suffer from weather disasters (drought). The third section of the questionnaire introduced the respondent to a hypothetical 'Drought Index Insurance' that will effectively help to spread the risk of damage caused by drought. Since a Drought Based Index Insurance has been already been introduced in Ghana, a hypothetical market similar to the existing product was explained to the farmer and farmers asked whether they want to buy the hypothetically designed insurance product. After this description of the proposed insurance scheme, respondents were asked whether or not they would be willing to participate in such an insurance scheme in order to reduce the damage risk they are exposed to at that point in time.

Respondents who reply in a positive way are then subsequently asked in a follow-up question, how frequently they would like to pay for the insurance and whom they prefer as the provider of the insurance scheme (Government, micro-credit organizations, insurance companies, and local co-operatives). Respondents who do not agree to participate in the proposed drought insurance scheme were asked for their reasons for not buying insurance in a follow-up question.

Table 1. Description of variables and expected signs of coefficients.

Variable	Definition	Hypothesized sign
Exogenous risk exposure indicators		
R_Period	Return period of disaster event in the past (once every.....year)	+
Damage	Total damage incurred during the last disaster event in monetary terms	+
Endogenous risk exposure indicators		
I_Sources	Number of non-nature dependent income sources	-
Relief	Availability and access to post disaster relief (Yes=1, No=0)	-
Budget constraint		
Income	Yearly income	+
Credit	Access to credit (yes=1, No=0)	+
Socio-demographic characteristics		
Education	Respondent Education (High school and above=1, Otherwise=0)	+
Insurance	Access 1 or more insurance (yes=1, No=0)	+

Theoretical model

The following theoretical model was constructed for drought index insurance participation:

$$D_i = F(R_i, L_i, A_i(Y_i, C_i), S_i) \quad (1)$$

Equation (1) represents the decision of an individual to participate in drought index insurance (D_i) which is expected to depend on the level of risk exposure (R_i), the level of damage caused by an event (L_i), the ability to pay the insurance premium (A_i), which is determined by the flow of income (Y_i) and in part access to and availability of credit (C_i), and relevant socio-economic and demographic characteristics of farmers (S_i).

Conventional risk theory disaggregates risk exposure into exogenous and endogenous components (Shogren and Crocker, 1991; Smith, 1992). Following others (Faber and Proops, 1990) and Funtowicz and Ravetz, 1992), exogenous risk exposure may be further disaggregated into: the likelihood of being struck by disaster and the consequence of risk exposure. The probability of being exposed is measured by way of the return period of natural disasters based on experiences in the past considered while consequence of risk exposure is measured through the economic damage to the individual farmer. The more frequent a disaster occurs the more likely a farmer is to invest in insurance and the higher the economic cost of the damage the more likely a farmer is to take insurance cover. Endogenous component of risk include diversification of income sources (Rosenzweig and Stark's, 1989; Brouwer et al., 2007). Consequently, the 'number of non-nature dependent income sources' that a farmer has is expected to have a negative effect on the tendency of a farmer to invest in crop insurance. Following Lewis and Nickerson (1989), the availability and access to disaster mitigating measures such as disaster relief, is used as a proxy for ex-poste exposure to endogenous risk and is expected to have a negative effect on a farmer's willingness to invest in crop insurance.

Budgetary considerations also play an important role in determining the risk behaviour of a farmer. As would be expected farmer's ability to pay for crop insurance is influenced by the extent of cash liquidity. Consequently, the nature of a farmer's income as determined flow of a farmers direct income and access to credit, are both expected to affect a farmer's willingness to pay for crop insurance positively. The higher a farmer's income the more likely it

is that they are more able to pay a higher risk premium. Although the nature of the relationship between access to micro-credit and willingness to pay for insurance has been questioned (Adger, 1999) it is still included as a proxy for income since access to credit plays an important role in income generation (Khadaker, 2005). Quite apart from endogenous and exogenous factors, as explained above, social and demographic characteristics of farmers are expected to affect farmers' decision to invest or not to invest in crop insurance. Key among these is education and access to other forms of insurance. Gine et al. (2008) find that non-insurance purchasers are less likely to invest in insurance because they do not understand the nature of insurance and how it will help them mitigate risk. In the specific case of this study, participation in health insurance is considered as a proxy of farmers' experience of insurance. Considering the wide spread patronage of the National Health Insurance Scheme, the experience of its benefit or of any other form of insurance is expected to positively influence the demand for crop insurance. Education also enhances respondents' ability to understand the product even if they have very little or no prior experience with it. Therefore, respondent's level of education is expected to positively influence insurance participation. The variables and their a priori expectations are as indicated in Table 1.

Preliminary examination of the data showed that 59 respondents were willing to participate in the market for drought indexed crop insurance while 41 were not willing to participate. Consequently, a logit probability model was used to examine factors affecting crop drought index insurance participation due to the disproportionate sample sizes. The logit model is based on the cumulative distribution function. Consequently, it yields results that are not sensitive to the distribution of sample attributes when estimated by maximum likelihood since it only affects that constant term and not the estimated coefficients (Maddala, 1992).

The mathematical form of the model used in this study is:

$$\log \frac{P(D)}{1-P(D)} = \beta_0 + \sum_{j=1}^K \beta_j X_j + \beta_K X_K$$

Where D is the probability of the i th farmer willing to participate and X_k the k th explanatory variable. The dependent variable, $\log(P(D)/(1-P(D)))$, in Equation (1) is the log-odds ratio in favour of decision to participate in crop insurance market (Gujarati, 1995).

A logit model is specified as:

Table 2. Demographic and socio-economic characteristics of respondents.

Characteristics of respondents	Mean	Range	Percentage
Age	41 year	16-78	
Sex			
Male	-	-	98
Female	-	-	2
Average household size	10 persons	3-40	
Education	-		
JHS & above		-	27
Non educated			73
Household yearly income (Ghc)	Ghc3,085.63	Ghc100–hc12307.9	
Farm size (acres)	4.5	1-5 acres 6-10 acres 11-14 acres	74 20 6
Yield loss per acre (100 kg)	3.5 bags (100 kg)	0-1 1-2 2-3 3-4 4-5 5-6	11 12 11 30 19 17
Access to credit	-	Access No access	22 78
Number of non- nature income sources	.31	1-3 1-22	
Return period of drought (years)	2 years	Once every year .Once every 2 year Once every 3 year Once every 5 year Once every 21 year	50 14 33 2 1
Access to other forms of insurance		1 or more None	56 44
Decision to participate			
Yes			59
No			41
Relief			
Yes			0
No			100

Source: Field data.

$$\text{Logit } (D_i^1 - D_i) = \beta_0 + \beta_1 R_Period + \beta_2 Damage + \beta_3 L_Sources + \beta_4 Relief + \beta_5 Income + \beta_6 Credit + \beta_7 Educ + \beta_8 Insurance + \dots e \quad (2)$$

RESULTS

Table 2 summarizes the demographic and socio-

economic characteristics of respondents included in the study. The respondents included Ninety-eight were males and two females. This is usual of the study area. Females have limited access to land as men control access traditionally. Moreover, females help their husbands on the family land and have little time to engage in crop

Table 3. Significance of the predictors.

Predictor	Chi - square	Df	Significance
Step	60.536	6	0.00
Block	60.536	6	0.00
Model	60.536	6	0.00

Table 4. Model summary.

-2 Log likelihood	Cox & Snell R square	Nagelkerke R square
74.836	.454	.612

cultivation in any significant way. The few exceptions are female household heads. The average age of the respondents was 41 years and fell within the range of 16 and 78 years. The average age of 41 years means that most of the farmers are within the economically active age. Older farmers were found to be less active on the farmer leaving the management of the farm to their eldest son in most cases. Seventy-three per cent of the respondents had no formal education. This is not unexpected since illiteracy rates in rural areas and among crop farmers in Ghana is generally high (GLSS, 2010). Each household consisted of an average of 10 family members. Crop farming is the primary occupation of 85% of the sampled farmers. Almost all sampled farmers (94%) owned the farmland where they cultivated their crops. Land, in the study area, is typically owned outright by members of a household. Land is traditionally owned by households and held in trust for the members by the household head. As members come of age they are apportioned sections of the land to cultivate to feed their families and to provide for other needs. The average farm size is 1.6 ha. This is typical of farms in the study area and reflects the general situation in Ghana as most farmers are subsistence farmers. Average yearly crop income accounted for 80% of yearly household income. Average annual household income was about Ghc 3,085.6 with majority of respondents falling within the range of Ghc 2,000 and Ghc 10,000. Seventy-eight percent of respondents had no access to formal credit. In Ghana most financial service providers have poorly developed network in the rural areas and this has constrained access to credit. The relatively high risk posed by agriculture has further constrained access to credit by small holder farmers. Fifty-nine percent of respondents are either registered with the National Health Insurance Scheme or a motor insurance scheme. This is expected and is rather low since each district operates a health insurance scheme. Estimated average yield loss per acre as a result of drought is about 350kg per acre translating into about Ghc 350.00. The average return period of drought was 2years with 48% of

respondents reporting a return period of 1 year. In all, 41% of respondents declined to participate in the proposed crop insurance scheme either because they do not have sufficient income to pay the premium or simply did not believe that they will actually be paid any claim in case of crop failure.

Empirical results

A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between acceptors and decliners of the offer to participate in crop insurance or not (Table 3).

Nagelkerke's R^2 of 0.612 indicates a moderately strong relationship between prediction and grouping (Table 4). Prediction success was 75.5% for decline and 83.1% for acceptance with an overall prediction success of 80%.

Access to credit, education and participation in other forms of insurance contributed significantly to willingness to pay for crop insurance at 5% significance level ($p = 0.010$, 0.039 and 0.002 respectively). Number of non-nature income sources and damage made significant contribution to willingness to pay for crop insurance at 10% significance level ($p = 0.094$ and 0.052 , respectively). Frequency of return period of drought was not a significant predictor and income had no influence on participation. Access to relief was excluded because of collinearity and farm size was also excluded because of its strong correlation with damage. Number of non-nature income sources and frequency of return period of drought were negatively related to participation (Table 5).

The log odds ratio (EXP (B)) is 46.9 times as large for respondents who had access to credit than those who did not. Therefore farmers who have access to credit are 46.9 more likely to participate in crop insurance than those who do not have access to credit. This is as predicted given the existence of evidence to the effect that micro-credit plays an important role in promoting income generation activities among beneficiaries (Khadaker, 2005).

Table 5. Results of the regression.

Variable	B	SE	Wald	Df	Sig	Exp (B)
Credit(1)	3.848	1.498	6.600	1	0.010	46.916
Educ(1)	2.492	1.204	4.282	1	0.039	12.082
H_INS(1)	1.850	0.596	9.647	1	0.002	6.360
I_Sources	-1.106	0.660	2.804	1	0.094	0.331
Damage	0.057	0.029	3.789	1	0.052	1.059
R_Period	-0.493	0.327	2.274	1	0.132	0.611
HH_I	0.055	0.033	3.67	1	0.122	1.00
Constant	-1.002	0.861	1.356	1	0.244	0.367

Consequently, farmers' access to credit increases their disposal income making them more likely to buy insurance cover. The odds ratio of those who have education is 12.1 times as large. Therefore, farmers with some level of formal education are 12 times more likely to participate in a crop insurance market than those who do not have formal education. This met the priori expectation that a respondent's level of formal education affects demand for insurance positively and significantly since education enhances the respondents' ability to understand the product offered even if they have very little or no prior experience of it. The odds ratio of those who participate in one or more forms of insurance is 6.4 times as large indicating that they are 6 times more likely to participate in crop insurance than those who do not participate in any other form of insurance. Experience of from one or more forms of insurance was predicted to have a significant positive effect on a respondent's decision to participate in the market for crop insurance. The effect of income on willingness to participate in the crop insurance market did not meet the a priori expectation that the higher the household income the more willing a farmer will be to participate in crop insurance. The odd ratio of 1.00 means that a unit increase in income will have no effect on willingness to participate. The log odd ratio for number of non-nature dependent income sources is 0.331. This implies that a unit (an additional source of income) increase in number of non-nature dependent income sources decreases the willingness of respondent to participate in crop insurance by 0.331 times. This outcome, although marginal, is as predicted. The higher the number of 'non-nature dependent income sources' the lower a respondent is exposed to endogenous risk. This is expected to influence the decision to participate in crop insurance negatively. The log odd ration for the return period of drought is 0.611. This implies that when the return period of drought increases by one year farmers are 0.611 times less likely to participate in crop insurance. This is expected as it was hypothesized that increase in the return period of drought influences insurance participation decision negatively. When damage increase by one unit (100kg) the odds ratio is 1.059 times as large and

therefore farmers are 1.059 more times likely to participate in crop insurance. This meets the a priori expectation that, the higher the loss caused by catastrophic events the more willing farmers are willing to participate in the market for crop insurance.

DISCUSSION

It is revealing to note that as many as 41% of respondents were not willing to participate in the market for crop insurance. These farmers viewed insurance as unnecessary and additional burden. This is not surprising since knowledge and access to insurance among the rural populace in Ghana is generally poor. This appears to be supported by the fact that 44% of respondents are not insured. The only form of insurance that appears to be working in the rural areas is the national health insurance which is subsidized and does not reflect reality of as far as premiums are concerned. Culture and, to some extent, religion appears to influence the decision to participate in insurance generally. This is because it is not considered normal to anticipate disaster as it would mean wishing disaster for oneself. This belief is quite strong among the more traditional societies. This phenomenon is common not only in rural areas but is experienced in the cities as well. The results show that access to credit, education and experience of other forms of insurance are the most important determinants of farmers' willingness to participate in crop insurance. The indeterminate effect of a farmer's direct income on the willingness to participate in the market for crop insurance is surprising viewed against the significant and positive contribution of credit to willingness to participate in crop insurance. Perhaps, this may be attributed to the fact that farmers consider it too risky to mortgage their household income for future and unknown benefit. Generally, direct household from crop farming which is the major source of livelihood in the area is low. Under such circumstances it is natural that people are more likely to protect their meagre incomes than gamble it against future benefits. However, access to credit is perceived as a major incentive to obtain extra income through income generating

generating activity and hence, improves a farmer's disposable income. The positive relationship between farmers' access to credit and insurance participation extending and strengthening credit facilities in rural areas can play an important role in increasing the up-take of crop insurance schemes. Unfortunately, experience in the study area shows that access to credit by farmers is limited and, when it is available, farmers are not willing to access it due to high interest rates. Currently, the average interest rate charged by micro-finance institutions is about 40%. Access to banks is even more limited and micro-finance institutions are unwilling to operate in remote rural areas due to higher cost of administering credit and risk. One potential strategy to encourage financial institutions to extend credit to poor farmers in such remote rural areas is to incorporate crop insurance into credit products by insuring against potential risk. This reduces lender risk and encourages insurance up-take in rural areas although it will increase the cost of credit to farmers who are already not happy with the current regime of high cost of credit. However, an innovative credit product that ensures high returns on investment in spite of the relatively higher interest rates and the benefit of insurance is likely to motivate farmers to take up credit. For instance, a combination of pre-financing farmers' cash needs during critical periods such as the period immediately preceding harvest when prices are lower and providing insurance cover for farmers' harvest against price fluctuation later will ensure that farmers benefit from higher prices for their products subsequently. However, the poor presence of micro-finance institutions in rural areas is a major limitation in this regard. Access to education and experience of other encourages insurance uptake since it enables easier comprehension of such a novel concept as crop insurance. Given the high illiteracy rate in the area and the substantial numbers of farmers who have not experienced other forms of insurance in rural areas it is expected that the introduction in rural areas will be slow and extra effort is required to promote insurance up-take. Therefore, there is the need that the promotion of crop insurance be preceded by extensive education and awareness creation drive in the short-term. In the longer term, it is expected that improved access to education, as a key poverty alleviation strategy by the government, will simultaneously improve insurance up-take. In this regards, public support in promoting crop insurance is critical.

Conclusion

The positive relationship between farmers' access to credit and insurance participation suggests that micro credit and micro-insurance are complementary products. Therefore, extending and strengthening credit facilities in rural areas can play an important role in increasing the take-up of crop insurance schemes. This will address the fear of loan default among farmers which, hitherto, had

rendered them reluctant to access loans. A major challenge in this regard is that most financial service providers in Ghana have poorly developed network in the rural areas resulting in limited access to the majority of smallholder farmers. Similarly, high interest rates, averaging, 48% per annum has deterred most smallholder farmers from accessing loans. On the other hand financial service providers consider extending credit to smallholder farmers more risky and expensive due to distance and the scattered nature of smallholder farmers. To address the challenges on both sides and ensure increased uptake of crop insurance therefore, financial service providers must insure their loan portfolios. This way, farmers are relieved from the burden of bearing the cost in the case of widespread losses while financial service providers are able to recover their losses. Given the high illiteracy rate in the area, reducing illiteracy and improving education levels in the long term will ensure substantial uptake of crop insurance. Therefore, government's drive to improve education in rural areas, as expected, has important implication for rural poverty reduction, in general, and crop insurance uptake, in particular, as it would result in increased access to information and improved understanding of the crop insurance concept and the need to seek protection.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Blood survey of *Babesia spp* and *Theileria spp* in Mono's cattle, Benin

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A cross-section study was carried out in the Mono district in Benin to establish the different proportions of *Babesia* and *Theileria* in ticks and blood. Blood samples were collected between October and November, 2008, a period of relative abundance of ticks. The investigation covered nine herds (villages), including the farm of Kpinnou, in an agro-ecological zone where the people adhere to the same pastoral-farming tradition. In total, 756 ticks and 36 drops of blood on filter paper were taken from 36 bovines at a rate of 4 per village. Using morphological identification, identified 166 *Rhipicephalus* spp., 185 *Amblyomma* spp. and 405 *Rhipicephalus (Boophilus) microplus* individuals were collected, corresponding to 53.57% of the vectors. Molecular diagnosis confirmed the results of the morphological identification. Molecular methods were also used for detecting *Babesia* and *Theileria* in the bovine blood samples. Different parasites have been identified in varying prevalences: *Babesia bigemina* (13.89%), *Babesia bovis* (5.56%), *Theileria mutans* (22.22%), *Theileria ovis* (5.56%) and other *Babesia* species (22.22%). No data exists so far for the district, but the results of this study are very similar to those obtained in the neighboring districts.

Key words: Cattle, ticks, *Rhipicephalus microplus*, *Babesia bigemina*, *Babesia bovis*, Benin.

INTRODUCTION

Tick-borne diseases (TBD) such as babesiosis and theileriosis (Uilenberg, 1995) infect domestic livestock

and wild animals in most regions of the world, causing problems of veterinary, medical, and economic

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Table 1. Geo-reference of the sampling points.

S/N	District	Villages	Breeders' names	Longitude	Latitude
1	Lokossa	Adjacome	Nompke Herbert	1.730194	6.646250
2	Houeyogbe	Zoungbonou	Amadou Hamidou	1.814611	6.556194
3	Come	Akodéha	Abe Come	1.911611	6.455278
4	Athieme	Sazouekpa	Kpachavi Sessouvi	1.743111	6.593833
5	Lokossa	Ouèdemè	Gnindokponou Ferdinand	1.679222	6.706083
6	Athieme	Awame`	Hanz Dalmeda	1.672139	6.609250
7	Come	Tovi	Hamidou Soumanou	1.850944	6.420194
8	Houeyogbe	Salahoue´	Désire Lakossa	1.808250	6.599611
9	Athieme	Kpinnou	Proj. of Liv. Dev3.(PDE3)	1.788083	6.566972

importance (de la Fuente et al., 2008). Although there have been a number of studies on the prevalence of tick-borne pathogens in vertebrate hosts and tick vectors in Benin, little information is available about the frequency of ixodid tick species and the prevalence of TBD in most areas of the country (Madder et al., 2012; De Clercq et al., 2012). In Benin, as in the majority of the African countries, the economic losses due to TBD and tick infestations are not known since the incidence/prevalence of such diseases has not been quantified. The control of TBD and tick rests on human, technical and especially financial potentialities that enable it possible to make a choice of a method of fight. Unfortunately methods incomplete or non-existent of control remain a practice in the majority of breedings. Few stockbreeders use acaricides, and the few stockbreeders get to use it badly, creating problems of resistance of the vectors (Achukwi et al., 2001). This attitude reinforces the presence of the ticks and thus diseases which they transmit.

To make an attempt to improve local breeds through the importation of highly productive dairy cattle have often resulted in high mortality from TBD (Loria et al., 1999). For the development and implementation of control strategies, it is important to know and understand the epidemiology of these pathogens in the respective geographical regions. The fight against this scourge requires a thorough knowledge of the various blood parasites and their vectors encountered in animals. The impact of these diseases on animals such as abortions, falling weight and reduced milk production is reported by many authors (Stachurski et al., 1988; Teglas et al., 2005).

The objective of this study is to establish the different proportions of *Babesia* and *Theileria* in ticks and in the blood of animals in the district of Mono in Benin.

MATERIALS AND METHODS

Study area

The study took place between October and November, 2008. Eight

villages (Table 1) were randomly selected in Mono District: Salahoue, Zoungbonou, Adjacome, Ouedeme, Awame, Sazouekpa, Akodeha and Tovi (Figure 1). This area of district of Mono which houses one of the four state farms (Ferme d'Elevage de Kpinnou, FEK), is one of the twelve districts of Benin, with an area of 1396 km². It is a small farming area (less than 0.7% of cattle) and bounded on the north by the district of Couffo, west along the Togolese border, to the east by the district of Zou and south by the Atlantic Ocean. The sampling area is marked by four seasons and identical agro-ecological characteristics: a long dry season from January to March, a long rainy season which runs from April to July, a short rainy season from August to October, and a short dry season from November to December, during which a dry wind blows from the interior of the continent to the ocean (called "harmattan"). The annual change of seasons ensures the availability of natural pasture; even the artificial pasture provides good nutrition for animals throughout the year.

Sampling strategy

Four animals per farm were sampled. Samples of ticks and blood were made during the period of October-November, 2008 since the relative abundance of ticks is highest during this period (Farougou et al., 2007). A GPS GARMIN eTrex Venture was used to record the geographic position of the sampled farm.

Ticks

Ticks were removed from the cattle by simple extraction with forceps after restraint of the animal. To standardize the sampling, collection was done by the same person. Ticks were immediately placed into a flask containing alcohol 70°, one flask for each animal and labelled with number of the animal, age, health status, name of the village and others.

Blood

Blood sampling was performed on the same animals immediately following tick sampling. Blood was taken from the jugular vein in labelled tubes containing 5 ml Venoject *ethylene diamine tetra acetic acid (EDTA)* in a vacuum. Two drops of fresh blood of approximately 50 µl was also collected in a Whatman filter paper No.3 labelled. Blood drops were *dried* under a mosquito net at room temperature, protected from flies and dust. After half a day of drying, the filter papers were carefully superimposed, always interspersed with a clean filter paper No.4. They were stapled and put into a plastic bag containing blue silica gel grains to reduce the

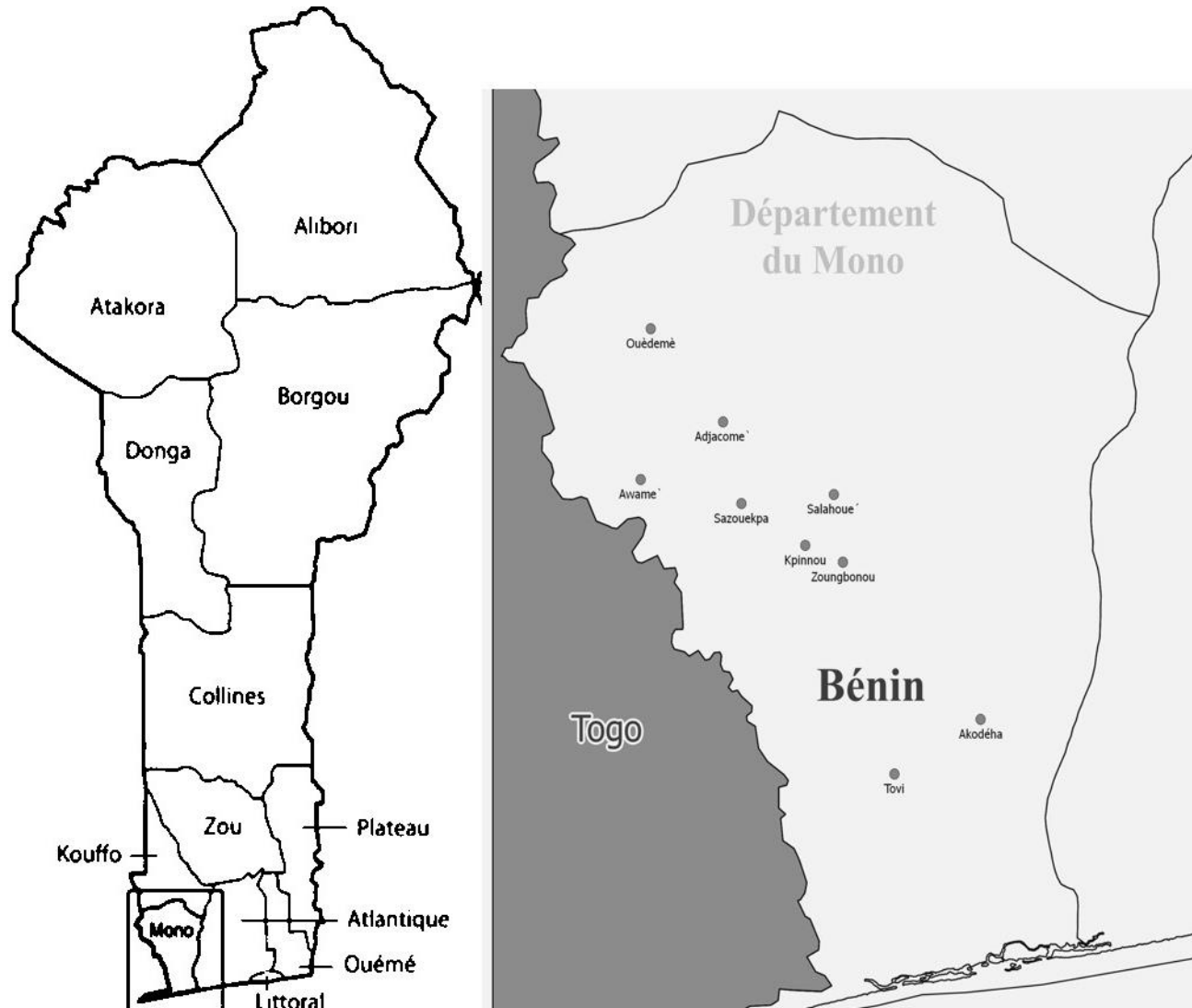


Figure 1. Left the 12 departments of Benin, right the location of the collection sites in Mono.

moisture absorption by the filter paper. Samples on filter paper were stored at -20°C .

Routing samples

At the end of each day of sampling, the samples were transported in a cooler to the Veterinary Diagnostic Laboratory of Bohicon (LADiVet) for storage. At the end of the field mission, all samples were gathered and sent to the laboratory of the Institute of Tropical Medicine in Antwerp, Belgium, accompanied by a Health certificate.

Laboratory analysis

Tick identification

The collected ticks were first identified up to genus level using a Zeiss Stemi 2000 stereomicroscope. Only *Rhipicephalus* (blue) ticks were further identified up to species level with a microscope

(Zeiss Axioscope, at magnification 1009). The main characteristics used for identification were the hypostome dentition, presence or absence of the ventro-internal protuberance bearing setae, shape of the adanal plates and their internal and external spurs and the presence of scalelike external spurs on coxae 2 and 3 of the female ticks. Several identification keys were used (Walker et al., 2000; Madder et al., 2012). For confirmation of the identification of *Rhipicephalus* (*Boophilus*) ticks the traditional polymerase chain reaction (PCR) was used.

Pathogen detection

Two DNA analysis were made. First, bovine blood drops on filter paper were screened for *Babesia* and *Theileria* and secondly, DNA was extracted from the ticks to confirm microscope identification on the one hand, and to screen them for pathogens on the other hand. Two methods were used, being the PCR and the *restriction fragments length polymorphism* (RFLP). For DNA extraction of the ticks of the species *Rhipicephalus* (*Boophilus*), the technique of

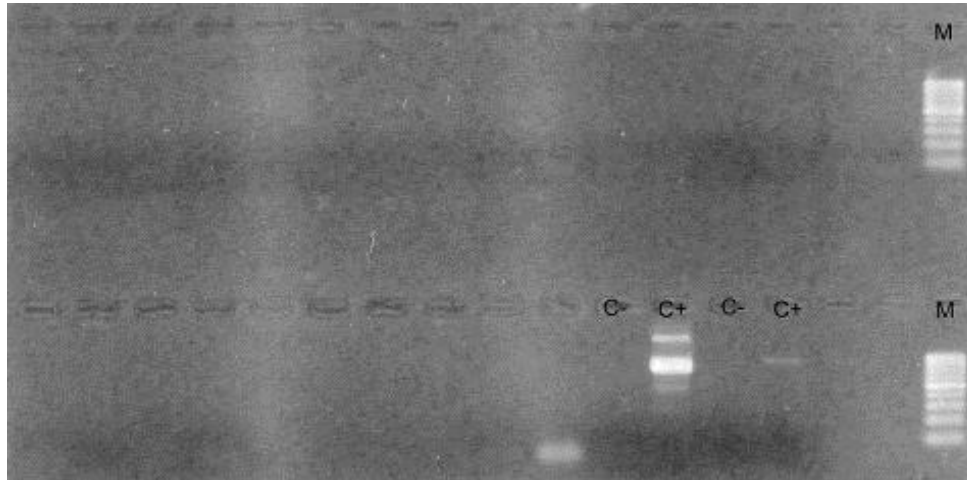


Figure 2. PCR product from the extraction of *Rhipicephalus* (*B*) *microplus* on 2% agarose gel.

Boom is used (Boom et al., 1990). For the DNA extraction of the blood drops, the technique used was the saponin-PBS 0.5% extraction. For pathogen detection a semi nested PCR or PCR 2nd round was performed, which yields a good test specificity. The pairs of primers BabF3/BabR2 and BabF3/BabR3 were used in each amplification of haemoparasites. The pair of primers BoopITS2F/BoopITS2R was used in the reaction of amplification (First-round PCR) of *Rhipicephalus* (*Boophilus*) DNA (Lempereur, 2008). The positive samples were tested again with the RFLP with a specific enzyme of restriction which makes it possible to make a cleavage (cut) in amplicons obtained with the PCR during a digestion at a temperature indicated. The reading of the results was made with UV. BseDI, MspI, DdeI and AluI are the enzymes used during various RFLP. The remaining of the samples not identified were cloned and sequenced at the University of Antwerp in Belgium.

Data analysis

Data analyses were performed with Excel 2003. Maps were made using Quantum GIS software (1.8.0).

RESULTS

Tick identification

In total, 756 ticks were collected from 36 cattle animals in nine different villages in the Mono district. Two genera were identified morphologically: *Amblyomma* spp. (24.47%) and *Rhipicephalus* spp. (75.53%). Among them, all blue ticks collected were identified as *Rhipicephalus microplus* (53.57%), and the results of the morphological identification are similar to those obtained with PCR concerning the confirmation of the identification of *Rhipicephalus* (*Boophilus*) ticks. This sampled tick species are those well known to the zone taking into account the national inventory of the species of ticks taken in 19 localities of Benin and the District of Mono (Vercruyssen et al., 1982).

Babesia species identification in blood

Babesia spp. was detected in 25 from the 36 sampled bovines. restriction fragment length polymorphism (RFLP) was performed in the positive samples. This freezing shows profiles which are identical with controls of *Babesia bigemina*, which is very similar to parasites like *Theileria* (Figure 2). The results obtained with the enzyme of DdeI restriction present identical profiles for all samples. This enzyme does not allow one to distinguish the various species of *Babesia* or *Theileria* (Gragnon, 2005). This was also the case for the enzyme of AluI restriction. With the enzyme of MspI restriction, the results showed four groups of similar samples (Figure 3), of which *B. bigemina* and *Babesia bovis* are quite distinct. The remaining of the samples not identified was cloned and sequenced at the University of Antwerp in Belgium. After cloning and sequencing, these samples showed different profile of either *Theileria ovis* or *Theileria mutans*. After them, a representative of the non-specific samples of eight samples was made and tested with the enzyme BseDI. This complementary test made it possible to identify samples similar to *Babesia* but different from *B. bigemina* and from *B. bovis*. In all things considered, *B. bigemina* (5), *B. bovis* (2), *T. mutans* (2), *T. ovis* (8) and *Babesia* spp (8) were identified. The relative prevalence of parasites is shown in Figure 4. The present study shows a prevalence of 5.56% for *B. bovis*; 13.89% for *B. bigemina*; 5.56% for *T. mutans*, 22.22% for *T. ovis* and 22.22% for *Babesia* spp.

DISCUSSION

Ticks identification

All the collected tick species were already reported in the analyzed geographical region (Vercruyssen et al., 1982).

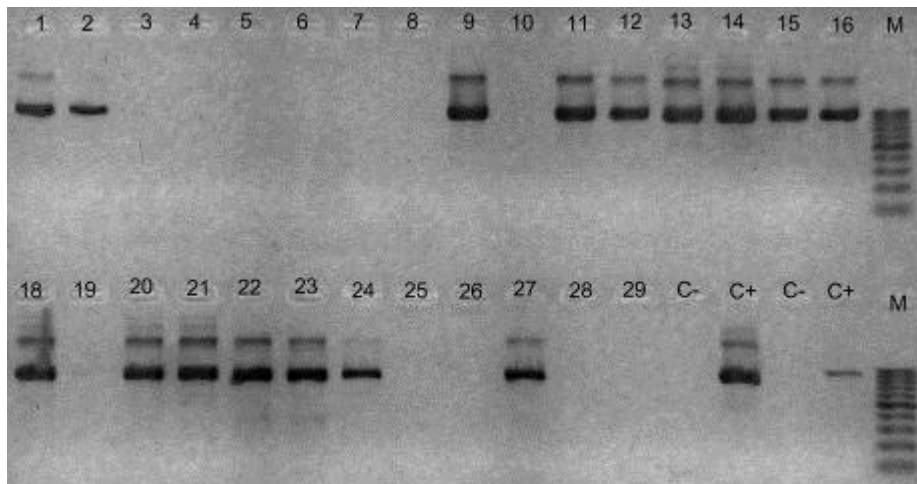


Figure 3. PCR products of bovine blood after migration on agarose gel 2%.

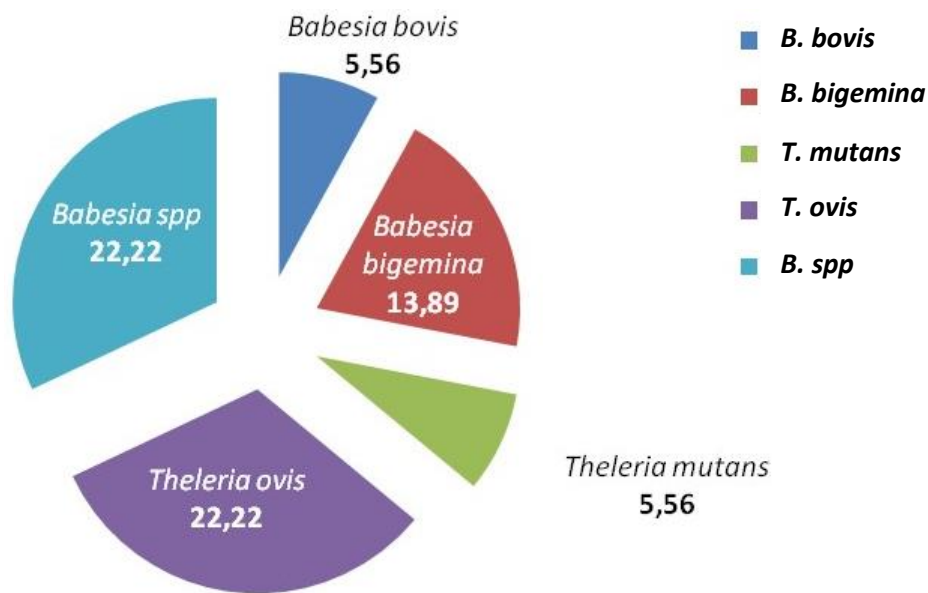


Figure 4. Respective prevalence of different haemoparasites in the blood samples.

Authors classified the species by numerical order of importance: *Rhipicephalus (Boophilus) microplus*, *Amblyomma variegatum*, *Rhipicephalus (Boophilus) geigyi*, *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus senegalensis*. This classification was confirmed in a recent study of ticks (Farougou et al., 2007; Madder et al., 2012). The resistance of animals with respect to the ticks is a very significant factor, because the tick must fix itself on the host during 3 days at least before *Babesia* pathogens be transmitted. At the same moment, tick can be rejected or not *in order* to be able to nourish itself. Another significant factor which influences the level of infection of the ticks is the

parasitemy of the host. During latent infections, the parasitemy is tiny and a very small proportion of the ticks is infected (Marcotty, 2009). During strong parasitemies, much of *Boophilus* females die because of the intestinal lesions caused by the parasite, which decreases also the rate of infection.

Babesia pathogen detection in tick

The high rate of *R. microplus* recorded on animals should give hope for an increase of blood pathogens in ticks. But unfortunately the polymerase chain reaction (PCR) which

is a significant and specific diagnosis test (Devos, 2001), judicious to detect the parasites even in weak proportion, did not give any results, all samples were negative. However the same ticks DNA extracts were used to highlight the species of *Rhipicephalus (Boophilus)*. This shows that the DNA extractions of the tick samples do contain DNA strings. The negative results can be explained in several ways. The periodic treatment of breedings against trypanosomes in programs of disease prevention constitutes an obvious reason of the very weak parasitemy and absence of the blood pathogens DNA in PCR samples. The vector concerned here has a monoxen cycle and both *Babesia* species are transmitted by various manners, on the level of the vectors as well as on the level of the hosts. *B. bovis* is transmitted by the larval stage and adults to animals considered as sinks on which they are obligatory reinfected. Actually, the parasitemy is weak in the endemic zones and the probability of the adults infecting themselves is even weaker. This low level of infection justifies the result obtained. In the case of *B. bigemina*, the transmission is not only transovarial as at *B. bovis*, but also transstadial and it is only the adults and the nymphs that transmit parasites to animals. In this case, if the females ticks are lesser infected, then the probability of having the infected larvae and other stages is very weak. The result is identical to the precedent. The method of DNA extraction of the ticks is not effective for obtaining the DNA of haemoparasites. Parasites are as small and numerically weak as one needs a method of extraction that is much more specific. It is as possible as trypanocides the almost quarterly treatments of the bovines in the zone of sampling hustled the cycle of haemoparasites returning not infected the parasites.

Babesia pathogen detection in host

The PCR/RFLP was used to highlight the various parasites of blood (Devos, 2001). The various parasites observed are well-known in the zone of collection and the animals which are there profit from a stable endemicity compared to the affections which they cause (Farougou et al., 2007; Pangui and Salifou, 1992). The proportions described for these parasites are almost similar to those obtained for our collection. That of *B. bovis* seems to be a little low (5.56%) compared to that of 14.58% observed by Pangui and Salifou (1992) using microscopy which is less sensitive and less specific than the molecular method. The weak rate obtained for *B. bovis* can be explained by the fact that the blood test was done in the chin-strap and not in the blood capillaries, place of concentration of the parasite, contrary to *B. bigemina* which prefers young red blood corpuscles in general blood circulation (Figueroa and Camus, 2003).

The originality of the data of this study is explained by the fact that no data on the haemoparasites existed for

the area of collection. All the investigations former on these haemoparasites were interested in other districts of Benin and almost never to that of Mono. The primers used for the amplification of the *Babesia* revealed the presence of *Theileria (Gragnon, 2005)*. The cloning-sequencing of not identified samples showed profiles specific with the cleavage of the enzymes of restriction used during the RFLP. This made it possible to highlight *Theileria*. It is of *Theileria mutans* and a strong specific proportion of *Theileria*: one suspects a mutant form of *T. mutans* and of *T. ovis* in the blood of the sampled bovines.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth and physiological responses of sunflower grown under levels of water replacement and potassium fertilization

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Water deficit is one of the limiting factors of agricultural production, especially in semi-arid regions. In this sense, the aim of this study was to evaluate the growth and the physiological characteristics of sunflower cv. Hélio 251 at different levels of water replacement and potassium doses in an experiment conducted in the greenhouse of the Center for Technology and Natural Resources of UFCG, Campina Grande, PB. The experiment was laid out in randomized complete block design, by studying five levels of water replacement (40, 60, 80, 100 and 120% of actual evapotranspiration - ETr) associated with potassium fertilizer levels (50; 75; 100; 125 and 150% of the indication for assays). The increase of water replacement levels promoted increase in plant height, stem diameter, number of leaves per plant, leaf area, and dry biomass of leaves, and dry biomass of the stem. The level of 75.25% of ETr provided the highest leaf dry weight (0.88 g). The increase in water replacement from 51.33% of ETr provided a reduction in the SPAD index. Water replacement lower than 100% ETr affected gas exchange of sunflower plants, reducing rate of the rate of the photosynthesis by 66% by the water deficit in the soil. The potassium doses had no effect on sunflower growth at 45 DAS neither they altered gas exchange in sunflower plants in the grain filling stage.

Key words: *Helianthus annuus* L., water stress, SPAD index, gas exchange, rate of the photosynthesis.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a very important crop for the Brazilian semiarid region because of its broad climatic adaptability, high drought tolerance and yield (Prado and Leal, 2006), providing a greater competitive advantage over other crops such as soybeans, because

it has higher yield per hectare in oil production (Zobiole et al., 2010).

In this sense, sunflower can be used to meet the noble edible oils market, feeding birds, silage production, meal and cake for animal feed, ornamental production as well

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as the possibility of export of grain (Lima et al., 2013). Sunflower accounts for about 13% of all vegetable oil produced in the world, with in recent years, increase in cultivated area (Nobre et al., 2011).

The Brazilian production of sunflower in the 2012/2013 harvest was 110 thousand tons, and the largest producer was the state of Mato Grosso with nearly 85 thousand tons, which corresponds to 64.9% of national production and an average productivity of 1671 kg ha⁻¹. In the Northeast, the highlights are the states of Ceará and Bahia with production of 200 tons and an average yield of 422 kg ha⁻¹ (Conab, 2014).

The water needs of the sunflower crop are still not clearly defined. In most cases 400 to 500 mm of water, well distributed throughout the cycle, resulting in yields close to the maximum potential (Castro et al., 2005). The range between 500 and 700 mm water well distributed throughout the cycle has resulted yields close to the maximum (Acosta, 2009). According to Silva et al. (2007), the total depth of 522.14 mm provides higher grain yield, oil and greater plant height. In this sense, the study of different irrigation levels constitutes a way to determine the water needs of a species in different regions (Azevedo and Bezerra, 2008).

Sunflower is very demanding in potassium, exceeding crops such as corn and soybeans (Uchôa et al., 2011). Following phosphorus and nitrogen, potassium is the element that most influences the growth and production of sunflower dry biomass (Prado and Leal, 2006). The increase in agricultural productivity, resulting from the addition of potassium fertilizers to the soil, mainly varies with the amount of available K and soil fertility (Feitosa et al., 2013). However, the semiarid region is characterized by low natural soil fertility (Menezes and Oliveira, 2008). Thus, the use of supplementary fertilization is indispensable for obtaining good crop yields.

Potassium is a key element for most biological processes in a plant and when it is not available at the lowest dose, it can reduce the development of the crop and consequently its productivity (Malavolta et al., 1997; Castro and Oliveira, 2005). Therefore, it can be said that the quantity of the element present in the soil were sufficient for the nutritional requirements of the culture, and there was no optimization of the cultivation as according to the increased levels of this macronutrient.

The chlorophyll pigments are responsible for the conversion of light radiation energy in the form of ATP and NADPH; therefore, they are closely related to the photosynthetic efficiency of the plants and, consequently, their growth and adaptability to different environments. Present in higher plants under the "a" and "b" forms, the chlorophylls are constantly synthesized and destroyed, whose processes are influenced by internal and external factors to plants. Among the external factors, mineral nutrients stand, by integrating the molecular structure of plants but also by acting in some stage of the reactions leading to the synthesis of these pigments (Taiz and

Zeiger, 2013).

The application of indirect chlorophyll meter Minolta SPAD-502 (Soil Plant Analysis Development) Minolta (1989) has been studied for several cultures and with satisfactory results on the evaluation of the nutritional state of N (Zotarelli et al., 2002). However, it is necessary to its calibration for each crop and in every situation. Several studies have shown that SPAD-502 can be used to indirectly assess the nutritional status of N and consequently infer about the need for fertilization of many cultures (Fox et al., 1994). In addition to N, other elements such as S, Mn and Fe cause chlorosis of the leaves, when they are at the adequate levels in plants, which highlight its importance in chlorophyll synthesis (Malavolta et al., 1997). SPAD readings provide a correlation with chlorophyll content in leaf. The values are calculated by the differential reading of the amount of light transmitted through a sheet in two wavelength regions (650 to 940 nm), and light absorption by chlorophyll occurs at the first wavelength (Swiader and Moore, 2002).

Several physiological indices are related to the use of water by plants. In this sense, rate of the photosynthesis and stomatal conductance are highlighted because an osmotic adjustment, such as stomatal closure, allows plants to escape from dehydration and turgor loss for maintaining the water content in the cells (Roza, 2010). Investigations concerning physiological characteristics responses of sunflower crop to water stress conditions are less conclusive (Silva et al., 2013). One way to find out whether the culture is under adequate growing conditions is related to the gas exchange of the plant because the plant under stress tends to reduce your cell water potential, performing the closing of the stomata and the formation of photoassimilates (Taiz and Zeiger, 2013).

Considering these facts, the objective of this work was to evaluate the growth, the physiological characteristics behavior through gas exchange and SPAD (Soil Plant Analysis Development) index, and the production of sunflower cv. Hélio 251 at different levels of water replacement and potassium doses.

MATERIALS AND METHODS

Location of the experiment

The experiment was carried out from November 2013 to January 2014 under greenhouse conditions at the Agricultural Engineering Department of the Federal University of Campina Grande, Paraíba State, Brazil located in the municipality of Campina Grande, Paraíba State with geographic coordinates 7°13'11" S, 35°53'31" W and altitude of 547.56 m.

Experimental design and treatments

The treatments were carried out in a randomized block design, in a 5 × 5 factorial experiment (five water replacement levels and five

Table 1. Mean values of plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), dry biomass of leaves (DBL), dry biomass of the stem (DBS), and SPAD index of sunflower due to the water replacement levels (%Etr) and doses of potassium (DK%).

%Etr	PH (cm)	SD (cm)	NL	LA (cm ²)	DBL (g)	DBS (g)	SPAD (%)
40	58.28	1.135	19.07	2785.71	0.76	0.29	50.28
60	61.27	1.281	20.47	3404.98	0.87	0.29	50.13
80	66.87	1.352	21.73	4032.77	1.29	0.33	50.48
100	69.22	1.439	22.80	4882.55	1.24	0.36	48.89
120	71.50	1.489	23.40	4871.48	1.16	0.33	48.15
DK%							
50	63.42	1.29	20.47	3665.75	1.09	0.34	49.00
75	65.01	1.35	21.67	3989.96	1.02	0.32	50.70
100	66.20	1.38	22.00	4155.49	1.22	0.31	50.17
125	66.49	1.34	21.53	4069.97	1.03	0.32	49.30
150	65.99	1.32	21.80	4069.32	0.94	0.30	48.75

potassium fertilization doses) with three repetitions, total of 75 experimental units. The treatments were water replacement levels corresponding to 40; 60; 80; 100 and 120% of actual evapotranspiration (ETr) and five potassium doses corresponding to 50; 75; 100; 125 and 150% of the indication for potassium application according to Novais et al. (1991), whereas 100% corresponds to 150 mg K kg⁻¹ of soil.

The real crop evapotranspiration (ETr) was estimated from the drainage lysimeter as described by Bernardo et al. (2008). Therefore, consumption of water by plants was determined from the control treatment (ETr 100%), obtained from the difference between the applied volume and the anterior irrigation drained volume, resulting in consumed volume, when multiplied by the factors 0.4; 0.6; 0.8; 1.0 and 1.2, obtaining irrigation of 40, 60, 80, 100 and 120% of ETr, respectively. Furthermore, the application of water replacement levels began at 16 days after sowing.

Conduct of the study

Each experimental unit consisted of a plastic vase filled with 14 kg of soil with the following chemical characteristics according to the methodology of Embrapa (2011): pH (H₂O) = 5.8; Ca = 2.37 cmol_c kg⁻¹; Mg = 3.09 cmol_c kg⁻¹; Na = 0.37 cmol_c kg⁻¹; K = 0.18 cmol_c kg⁻¹; H + Al = 1.78 cmol_c kg⁻¹; OM = 21.20 g kg⁻¹; P = 53.60 mg kg⁻¹.

Six sunflower seeds (cultivar Hélio 251) were sown on November 11, 2013 directly in the pots at a 2 cm depth. A simple hybrid with achenes striated color presents an average cycle of 100 days, average plant height of 1.87 m, and average yield under irrigation conditions of 4631 kg ha⁻¹ (Aquino et al., 2013). Sixteen days after sowing (DAS), seedlings were thinned to two plants per pot.

In foundation, fertilizers were applied per pot: 8.07 g of monoammonium phosphate and urea 3.11 g, as indicated by Novais et al. (1991). The soil material after conditioning in the experimental units (pots) was moistened at field capacity. Potassium fertilization was divided into three times and applied via fertigation at intervals of seven days from 24 DAS, being applied per pot to treat 100% recommendation 23.2 g of potassium chloride.

Analyzed variables

When the plants reached the harvest stage (45 DAS) were evaluated the following components: plant height (PH) in cm; stem diameter (SD) in cm; number of leaves per plant (NL); leaf area

(LA) in cm², measured by non-destructive method Maldaner et al. (2009) according to Equation 1; dry biomass of leaves (DBL), and dry biomass of the stem (DBS) (g) by drying in air forced circulation stove at a temperature of 65°C until constant weight; the relative chlorophyll content (SPAD index) with the use of a chlorophyll meter SPAD-502, in the second fully expanded leaf from the apex to the plant base.

$$LA = 0.1328 \times L^{2.5569} \quad (1)$$

Where, LA = leaf area (cm²); L = length of midrib of the leaves of each plant (cm)

Gas exchange was measured at 60 DAS on the third leaf from the apex, using portable equipment analysis through infrared (IRGA) and the following physiological characteristics variables were determined: Internal CO₂ concentration (Ci) (μmol m⁻² s⁻¹), stomatal conductance (gs) (H₂O mol m⁻² s⁻¹), transpiration (E) (H₂O mmol m⁻² s⁻¹), and CO₂ assimilation rate (rate of the photosynthesis) (A) (μmol m⁻² s⁻¹). Based on these data, it was determined the water use efficiency (WUE) (A/E) [(μmol m⁻² s⁻¹) (mmol H₂O m⁻² s⁻¹)⁻¹] (Carneiro, 2011; Silva et al., 2013).

Statistical analysis

The experimental data were analyzed by ANOVA. The data were subjected to analysis of variance using F test at 5% significance level for all analyzes. In case of significant effect, it was proceeded regressions (linear and quadratic). All analyses were performed using statistical software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Variance of analysis

The means of the variables analyzed in this study are presented in Table 1. It can be observed that the higher values of % ETr (100 and 120) presented the highest mean values for PH, SD, NL, LA, DBL and DBS; on the other hand, the lowest values of % ETr (40, 60 and 80) presented the highest values for SPAD (%). However, based on the results presented in Table 2, it can be observed that the increase in water replenishment levels

Table 2. Test result of 'F' for plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), dry biomass of leaves (DBL), dry biomass of the stem (DBS), and SPAD index of sunflower due to the water replacement and potassium levels.

Source	F test						
	PH	SD	NL	LA	DBL	DBS	SPAD
Water replacement (RH)	**	**	**	**	**	**	*
Linear regression	**	**	**	**	**	**	**
Quadratic regression	ns	ns	ns	ns	*	ns	*
Doses of K (DK)	ns	ns	ns	ns	ns	ns	ns
Interaction (RH x DK)	ns	ns	ns	ns	ns	ns	ns
Block	ns	ns	ns	**	*	**	*
CV (%)	6.48	6.59	8.44	12.50	35.0	17.91	4.53

(**), (*); (ns) significant at ($p \leq 0.01$) and ($p \leq 0.05$); no significant, respectively, for the F test.

Table 3. Summary of the analysis of variance for the gas exchange variables: internal CO₂ concentration (Ci) (CO₂ mmol m⁻²), stomatal conductance (gs) (mol H₂O m⁻² s⁻¹), transpiration (E) (mmol H₂O m⁻² s⁻¹), rate of the photosynthesis (A) (CO₂ μmol m⁻² s⁻¹) and instantaneous water use efficiency (WUE) [(μmol m⁻² s⁻¹) (mmol H₂O m⁻² s⁻¹)⁻¹] at 60 DAS on the basis of irrigation levels and potassium fertilization.

Source	F test				
	Ci	gs	E	A	WUE
Water replacement (RH)	**	**	**	**	**
Linear regression	**	**	**	**	**
Quadratic regression	ns	ns	ns	ns	ns
Doses of K (DK)	ns	ns	ns	ns	ns
Interaction (RH x DK)	ns	ns	ns	ns	ns
Block	ns	ns	ns	ns	ns
CV (%)	23.43	24.49	15.31	29.84	37.19

(**), (*); (ns) significant at ($p \leq 0.01$) and ($p \leq 0.05$); no significant, respectively, for the F test.

(HR) had a significant effect on all variables for linear regression. On the other hand, there was no significant effect due to applied of potassium. For significant data, regression analyses were used with adjustment of the greatest determination coefficients ($p \leq 0.05$). All analyses were performed using statistical software SISVAR (Ferreira, 2011): fertilizer levels (DK) and the interaction between HR x DK factors for any variable analyzed.

This result demonstrated that the doses of K behaved similarly in the different levels of irrigation used in this experiment, used, which may be related to low nutritional demand of sunflower early in the growing season, especially in the first 30 days after emergence (DAE) accordingly (Castro and Oliveira, 2005). However, Uchôa et al. (2011) studied the three production components of sunflower under different potassium doses in coverage and they found significant effect of potassium application on growth of the analyzed variables and production of sunflower. Corroborating Zobiolo et al. (2010) mentioned that the greater absorption of potassium by sunflower occurs after 74 DAE. However, Uchôa et al. (2011) studying the three sunflower cultivars producing

components subjected to various doses of potassium coverage, observed significant effect of potassium application on the growth and production of sunflower. The omission of K significantly reduced plant height, stem diameter, leaf area and dry biomass sunflower, as observed by Prado and Leal (2006).

The summary of the analyzes of variance of physiological characteristics sunflower responses regarding the effects of treatments for the results with the data of internal CO₂ concentration (Ci), stomatal conductance (gs), transpiration (E), rate of the photosynthesis (A), water use efficiency (WUE) and intrinsic water use efficiency are shown in Table 3, where there is the F test indicated that all physiologic variables were changed significantly ($p < 0.01$) only by a factor water replacement. Thus, the potassium doses and the interaction did not affect gas exchange sunflower at 60 DAS.

Plant height (cm) and stem diameter (cm)

The increase in water replacement levels promoted linear

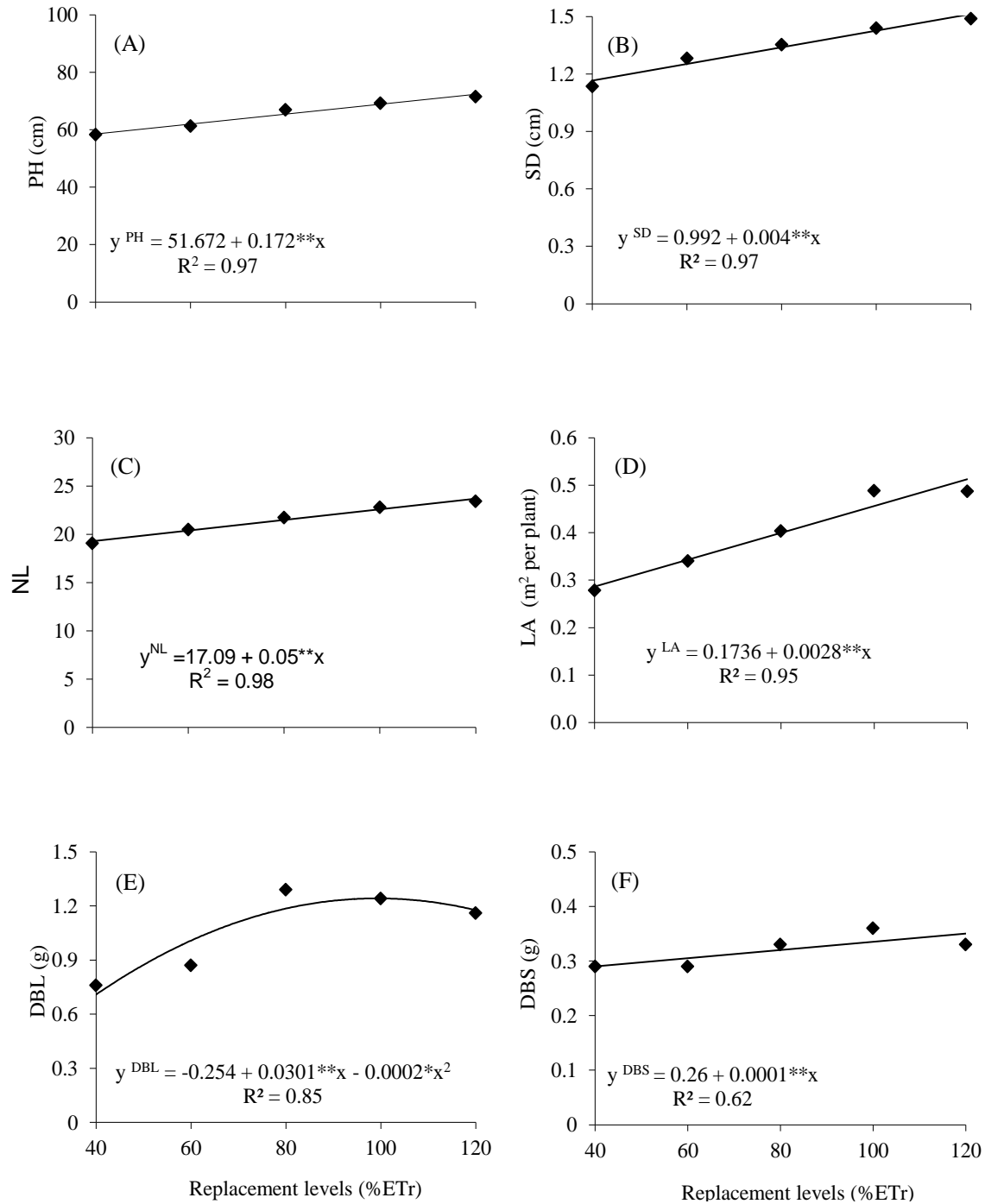


Figure 1. Plant height - PH (A), stem diameter - SD (B), number of leaves – NL (C), leaf area - LA (C), dry biomass of leaves - DBL (D) and dry biomass of stem - DBS (E) of sunflower as a function of water replacement levels - ETr%.

increase in plant height (PH) and stem diameter (SD) in sunflower plants (Figure 1A and B), corroborating by Viana et al. (2012) who found a linear increase on PH and SD sunflower plants cv. Catissol-01 with increasing water depth. The largest PH and SD values were 72.31 and 1.47 cm, respectively, with the level of ETr 120%,

which corresponded to an increase respectively, 19.03 and 21.73% in relation to irrigation with 40% of ETr. Such results may be related to increased water potential of the cells, favoring larger cell elongation and hence increased growth. Accordingly, it was found that plant growth depends directly on the water absorption. The water inlet

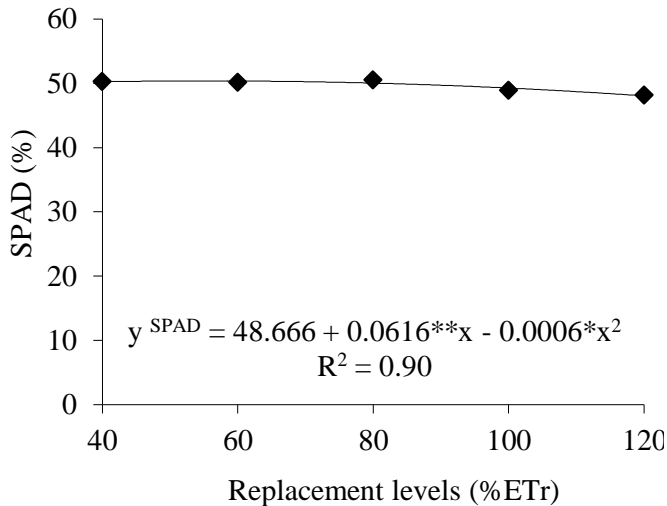


Figure 2. Sunflower SPAD index as a function of water replacement levels - ETr%.

in the cell produces an internal pressure, known as pressure or turgor potential (P or Ψ_p), which expands the protoplast from the cell wall may also increase the cell size (Taiz and Zeiger, 2013).

According to Nezami et al. (2008) water deficiency leads to reductions in water potential of stem cells composing until reaching a water potential level below the minimum necessary to trigger the cell elongation process of internodes resulting reductions in height and stem diameter of the plants. However, these values are lower than reported by Biscaro et al. (2008), who obtained at 45 days after seeding (DAS) the values of 1.84 and 114.7 cm to the PH and SD respectively in sunflower plants genotype Hélio358. Studying the same genotype, Gomes et al. (2010) obtained at 40 DAS values for PH and SD respectively, 101.22 and 1.97 cm.

Number of leaves and leaf area (cm^2)

The increase in irrigation water depths promoted linear increase in the number of leaves per plant (NL) and leaf area (LA), and the highest values obtained with the level of 120% of ETr, which corresponded to an increase respectively, 18.33 and 44.09% compared to the level of 40% of ETr (Figure 1C and D).

In this sense, it was found close connection between the NL and LA of sunflower plants. According to Taiz and Zeiger (2013) the water stress affects plant leaf expansion, which is dependent on the cell turgidity state. These results are consistent with those reported by Dutra et al. (2012), who observed an increase in NL and LA sunflower cv. Embrapa 122/V-2000 when subjected to higher water availability.

Despite the increase observed in the NL (23.57 leaves

per plant) and LA (5120.8 cm^2 per plant), these values are lower than reported by Silva et al. (2012) who obtained at the 52 DAS 29.09 leaves per plant and LA 73121.6 cm^2 per plant of sunflower cv. Multissol grown without water stress.

Dry biomass of leaves and stems (g)

According to the regression equation (Figure 1E), it can be observed the adjustment in the quadratic model for dry biomass of leaves (DBL), which was observed an increase until the level of 75.25% ETr (0.88 g). From this value, there is little reduction in DBL. However, with regard to the dry biomass of the stem (DBS) (Figure 1F), it was increased linearly with increasing water replacement levels, with higher values obtained when the plants were submitted to irrigation with the level of 120% of ETr (0.27 g), corresponding to an increase of 3.7% in relation to the application of 40% of ETr. According to Andrade and Abreu (2007) the production of dry biomass in sunflower is adversely affected by water deficit conditions due to adaptive physiological characteristics mechanisms, anticipation of leaf senescence that leads to restriction of leaf area, and consequently the surface area exposed to losses due to transpiration. In this sense, it is observed that the leaf expansion is very sensitive to water stress, being completely inhibited under moderate stress levels, a fact that severely affects the photosynthetic rates and, consequently, the biomass production of shoots. Similar results were reported by Dutra et al. (2012) which obtained an adjustment to the quadratic model for dry biomass and leaf and stem with the increase of the water supply. Oliveira et al. (2012) observed linear increase of dry weight of leaves and stems of sunflower cv. Embrapa 122 / V-2000 with the increase in levels of available water in the soil. Despite the dry biomass increase obtained with increasing water depths, the values are lower than those reported by Gomes et al. (2010), who obtained dry biomass values greater than 11 g in water stress conditions at 40 DAS.

SPAD Index

Regarding the SPAD index (Figure 2), there is better fit to the data for the quadratic regression model, where it was observed an increase until the level of 51.33% of ETr (50.24) corresponding to an increase of 0.15% compared to the level 40% of ETr. From this point, it was observed that there was a reduction of 5.63% in relation to the application of 120% of ETr. According to Dutra et al. (2012), the excessive increase of the water content in the soil can cause increased leaf senescence by elevating the concentration of abscisic acid and ethylene, and induces increase of chlorosis due to chlorophyll degradation. The Nezami et al. (2008) stated that water

stress causes an increase in sunflower SPAD index. However, the water restriction on reduction in chlorophyll content has also been reported in sunflower plants (Kiani et al., 2008).

The results obtained in this study for sunflower growth at 45 days after emergence point to the need to apply irrigation depths with values above the soil field capacity (100% ETr). However, the study in a protected environment prevents, in part, that the data obtained in field conditions are represented accurately, since there are alterations in climate conditions such as temperature, solar radiation, wind, humidity and atmospheric composition, interfering directly on evapotranspiration (ET). Inside the greenhouse, usually the ET is less than the one observed externally, which is justified by the partial opacity of the plastic cover to solar radiation and by reducing the action of the winds. According to Rosenberg et al. (1989), evapotranspiration inside a greenhouse is around 60-80% lower than the one observed externally.

Gas exchange

The drought conditions decreased the amounts of gas exchange. Similar results have been reported by Cechin et al. (2010), who observed reduction in physiological characteristics variables such as rate of the photosynthesis, stomatal conductance and transpiration in sunflower leaves as a result of water stress. This contrasts the findings of Silva et al. (2013), which stated that the sunflower crop can be irrigated at 50% ET in all phenological phases without damage to the photosynthetic process, when they assessed gas exchange sunflower subjected to drought regimes at different growth stages.

Studies carried out by Silva et al. (2011) analyzed the influence of six irrigation levels on productive performance of two sunflower cultivars (Catissol-01 and Embrapa 122V-2000), and it was noted that most irrigation depth (150% evaporation of the class A pan) provided the greatest potential for producing achenes in both cultivars.

The regression analysis of means of variables related to gas exchange for the isolated effect of irrigation factor is presented in Figure 3. The linear model fitted the values of all variables, notably the change in internal CO₂ concentration (Figure 3A). Plants grown under 60% of the blade ETr maintained a higher carbon content in the internal substomatal camera with 299.73 $\mu\text{mol m}^{-2} \text{s}^{-1}$, decreasing 41.1% for water conditions imposed by the application 120% irrigation replacement. Possibly, these results were influenced by the CO₂ assimilation rate (rate of the photosynthesis -Figure 3D).

Plants with larger water supplies in the soil increased linearly the photosynthetic rate, increasing 7.36% as a unit in rate of the photosynthesis to each percentage of

ETr applied in relation to lower water replacement. Thus, an increase in the demand for CO₂ was observed in plants irrigated with larger water replacement, reducing the Ci. Although significant, there is low variation in stomatal conductance and transpiration, as shown in Figure 3B and C, respectively. In this sense, the carbon input diffusion process for substomatal camera depends on the opening of the stomata, which reflects the transpiration (Machado et al., 2011; Taiz and Zeiger, 2013).

Since the gas exchanges are regulated through the stomatal movement, the external CO₂ absorption promotes increased transpiration; likewise, reduction of transpiration limits the carbon entering the camera substomatal (Shimazaki et al., 2007). Thus, it is necessary that the plants have higher water use efficiency, that is, the maximum absorbing CO₂ with minimal water loss (Jaimez et al., 2005; Taiz and Zeiger, 2013).

By analyzing the effect of applied irrigation water, it is verified that the reduction in water availability reduced the WUE (Figure 3E), the behavior was linear, with greater use in the assimilation of CO₂ per unit of water lost observed in 120% of ETr. Similar results were obtained by Brito et al. (2012) when evaluated the physiological characteristics behavior of citrus under water stress; these authors point out that achieving better physiological characteristics aspects enables greater formation of biomass plants.

In this sense, these results are consistent with those reported by Soares et al. (2015) to assess the biomass of sunflower cv. 'Hélio 251' on the basis of irrigation with different levels of fluid replacement and potassium fertilization, where it was observed that the fluid replacement levels up to 120% of ETr linearly increased the mass of the total seeds and mass of hundred sunflower seeds.

Conclusions

Under experimental conditions, the application of potassium in the soil did not influence the growth and production of sunflower cv. Hélio 251. The growth and production of sunflower cv. Hélio 251 was significantly influenced by increasing water replacement levels. The level of 75.25% of ETr provided the highest DBL (0.88 g). Increasing water replacement from 51.33% ETr provided a reduction in the SPAD index. Potassium fertilization as the conditions of this study does not alter gas exchange in sunflower plants in the grain filling stage. Water replacement lower than 100% ETr affected gas exchange of sunflower plants, reducing rate of the photosynthesis by 66% by the water deficit in the soil. The largest values of rate of the photosynthesis and instantaneous water use efficiency was 17.181 CO₂ $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 6.00 $\mu\text{mol m}^{-2} \text{s}^{-1} / \text{mmol m}^{-2} \text{s}^{-1} \text{H}_2\text{O}$ as a result of 120% ETr

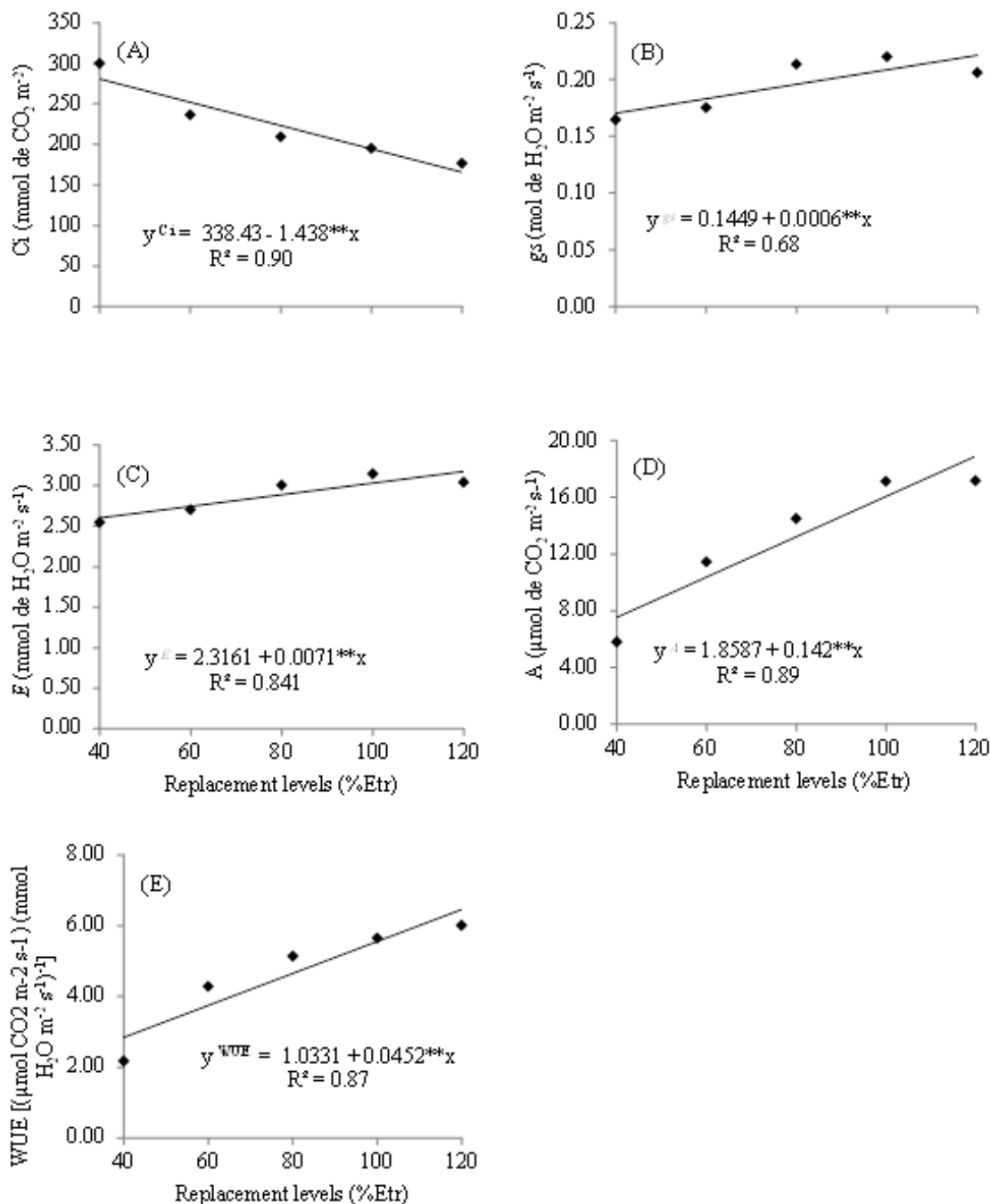


Figure 3. Regression analysis for values of variables: (A) internal concentration (Ci) of CO₂ (CO₂mmol m⁻³) (B) stomatal conductance (gs) (H₂O mol m⁻² s⁻¹) (C) transpiration (E) (H₂O mmol m⁻² s⁻¹), (D) rate of the photosynthesis (A) (CO₂ μmol m⁻² s⁻¹) and (E) water use efficiency (WUE) [(μmol m⁻² s⁻¹) (H₂O mmol m⁻² s⁻¹)⁻¹] at 60 as a function of water replacement (%Etr).

water replacement.

Abbreviations

PH, Plant height; **SD**, stem diameter; **NL**, number of

leaves per plant; **LA**, leaf area; **DBL**, dry biomass of leaves; **DBS**, dry biomass of the stem; **ETr**, real crop evapotranspiration; **SPAD** index, relative chlorophyll content; **DAS**, days after sowing; **Ci**, CO₂ concentration; **gs**, stomatal conductance; **E**, transpiration; **A**, CO₂ assimilation rate; **WUE**, water use efficiency.

Conflict of Interests

The authors have not declared any conflict of interest.

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