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Full Length Research Paper

Irrigation water planning for crops in the central highlands of Ethiopia, aided by FAO CROP WAT MODEL

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Information on crop water requirement of crops is vital for irrigation water planning. In the central parts of Ethiopia, agriculture has solely been dependent on rain-fed until recent time that irrigation for vegetable production is becoming one activity for crop production. However, irrigation practice in terms of the amount of water to be used and frequency of application has lacked proper knowledge. The purpose of this study is therefore to deliver the preliminary information on seasonal water requirement of different crops based on the widely used FAO crop wat model. The lowest values of reference evapotranspiration were observed for Akaki, followed by Debere Zeit, Alemtena and Modjo. Accordingly, the results showed Onion requires frequent application of irrigation followed by tomato while wheat needs longer interval that in all the sites. The seasonal net irrigation application for Onion (60%field efficiency) is 2890, 2920, 3870, 3840 m^3 for Debre Zeit, Akaki, Modjo, and Alem-Tena with their respective orders. Tomato requires net application of irrigation amount, 4650, 4030, and 5560, 4720 m^3/ha for the sites, Debre Zeit, Akaki, Modjo and Alemtena respectively. Similarly, at Debre Zeit, Chickpea needs 3000 cubic meter per hectare while wheat requires 3670 m^3 of irrigation water.

Key words: Crop water requirement, field efficiency, irrigation frequency, FAO CROP WAT 8.1

INTRODUCTION

Water for agriculture is becoming increasingly scarce in the light of growing water demands from different sectors (IWMI 2010). Water supply matters in the world that will soon have to grow food for billions more people as world population is projected to peak at 9.3 billion in 2050, an increase of 28%. Analysis showed that the total crop water requirement of all major crops increased with the rising temperature thereby increasing the simulated irrigation water demand (Kijne, 2010; Surendran et al., 2014). In the future, food and livelihood security may be challenged due to global environmental changes, particularly global climatic changes that evidence has

gradually shown to be appearing (Aggarwal and Singh, 2010). Developments in irrigation are often instrumental in achieving high rates of agricultural goals but proper water management must be given due weightage in order to effectively manage water resources. Better management of existing irrigated areas is required for growing the extra food to fulfill the demand of increasing population (Hari Prasad et al., 1996).

Irrigation water management is a crucial component of any irrigation project. Wise use of water resources is becoming the important element in agriculture as the demand for the resource is dramatically increasing because of population pressure and hence feeding the world is a priority issue. Knowledge of crop water requirements is therefore quite helpful for planning a sound irrigation scheduling where water can be used efficiently and effectively.

Over last two decades, considerable progresses have been made to understand key factors controlling crop water requirement and consequently led to development of new techniques of evapotranspiration (ET) estimation (Patel et al. 2005) but operational applications of ET estimates yet heavily rely on the FAO-56 model because of minimum requirement of phonological and standard meteorological inputs (Evett et al., 1995; Kite and Droogers, 2000; Allen, 2000; Eitzinger et al., 2002). In FAO-56 approach, actual ET is calculated by combining reference evapotranspiration (ETo) and Kc. The Food and Agriculture of the United Nations has been extensively working on models that are capable of estimating crop water requirement and exercising irrigation scheduling of crops for any irrigation project for the last thirty years. The models have been widely used in the in the research, academia and developments sectors.

Understanding crop water needs is essential for irrigation scheduling and water efficient use in an arid region (Parry et al., 2005). Further, with increasing scarcity and growing competition for water, judicious use of water in agricultural sector will be necessary (Ali, 1991). Predicting water needs for irrigation is necessary for the development of an adequate water supply and the proper size of equipment. In our study area consistent information on irrigation water use is still lacking. The objective of this study was to estimate irrigation water requirement of rice (*Oryza sativa*) using the Cowpat model. Cowpat is a FAO model for irrigation management designed by Smith (1991) which integrates data on climate, crop and soil to assess reference evapotranspiration (ETo), crop evapotranspiration (ETc) and irrigation water requirements.

The CROPWAT model developed by the FAO Land and Water Development Division includes a simple water balance model that allows the simulation of crop water stress conditions and estimations of yield reductions based on well-established methodologies for determination of crop evapotranspiration (FAO, 1998)

and yield responses to water (Doorenbos and Kassam, 1979). CROPWAT is a decision support system developed by the Land and Water Development Division of FAO for planning and management of irrigation. CROPWAT is meant as a practical tool to carry out standard calculations for reference evapotranspiration, crop water requirements and crop irrigation requirements, and more specifically the design and management of irrigation schemes. It allows the development of recommendations for improved irrigation practices, the planning of irrigation schedules under varying water supply conditions, and the assessment of production under rain-fed conditions or deficit irrigation (Doorenbos and Kassam, 1979).

In Ethiopia, the major portion of irrigation water management is cultural where farmers are irrigating as long as the water is available, without considering whether it is above or below the optimum of the crop water requirement. For large dams, the information of crop water requirement of the proposed crops is usually used for design purposes and it is not exercised on the real duty of irrigation operation, however. Moreover, in areas where, farmers are cultivating on small scale, the same information is critically limiting and more water is believed to be wasted. The aim of this research is therefore to estimate the crop water requirement of some crops in the central highlands of Ethiopia where information can be available for small irrigators so that it can be applicable for sound irrigation water management.

MATERIALS AND METHODS

The study was undertaken in the Central part of Ethiopia, and the sites were, Debre Zeit, Akaki, Mojdo, and Alemtena. The soils for Akaki and Debre Zeit black vertisols, where Modjo and Alemtena have light texture soils. The map of the study sites is as shown in Figure 1.

The crops included in this study were: Onions, Tomato, and Wheat. The reference evapotranspiration values (ET0) for each of the sites were calculated from the long term meteorological variables (Monthly Minimum and Maximum temperature, wind speed, sunshine hours and relative humidity) using the crop wat vewrsion 8.1, based on the Pen man-Moeinth formula. From the long term ETo data, the best estimate was obtained by the fitting the values in to different probabilistic functions of using the Easy Fit computer model. The soils physical properties of the sites (Texture, Bulk density, basic infiltration rate, Water holding capacity of the soils) has been determined using the standard soil lab procedures. The Kc values have been adopted from the FAO 33 and 56 of the irrigation and drainage papers. FAO crop wat computer model has finally been employed to obtain the crop water requirements of the crop and exercising irrigation scheduling for each of the sites.

RESULTS AND DISCUSSION

The reference evapotranspiration (ETo) values of the different sites have been presented in Table 1. As can be seen from the table, the lowest values of reference evapotranspiration were recorded for Akaki, followed by
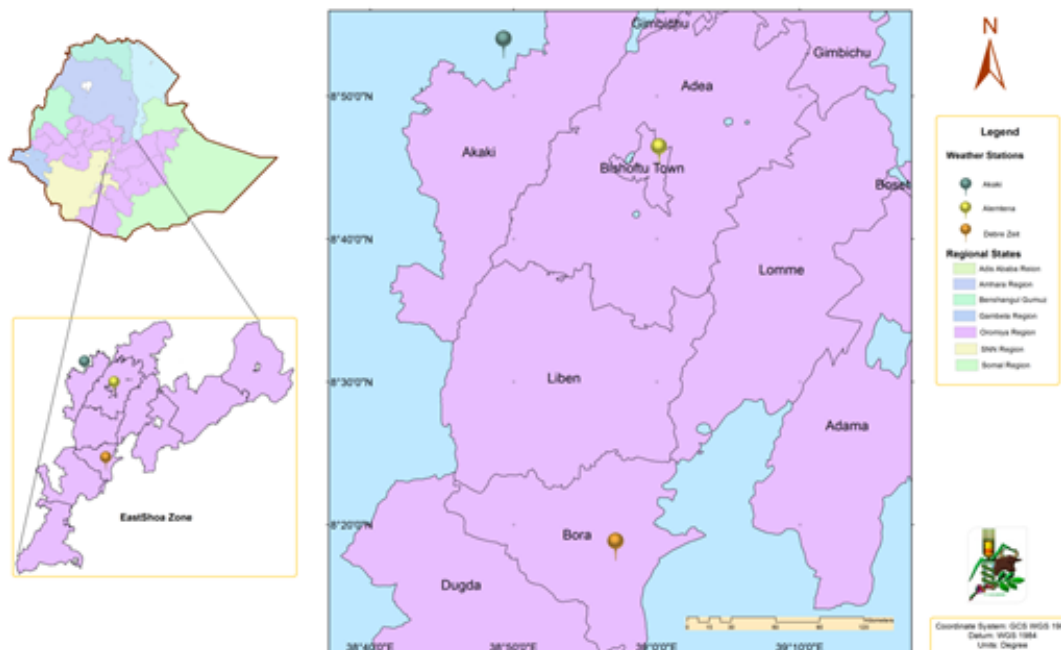
Figure 1. Location map of the study sites.

Table 1. Reference evapotranspiration (mm/day) values of the different sites.

Months	Akakai	Sites		
		Alem-Tema	Modjo	Debre Zeit
January	4.87	4.70	4.99	4.73
February	4.71	5.14	5.41	5.40
March	4.87	5.56	5.93	5.37
April	5.24	5.48	5.87	5.60
May	4.68	5.60	5.86	5.76
June	4.79	5.33	5.57	5.76
July	4.01	4.53	4.86	3.80
August	4.07	4.59	4.83	3.75
September	4.58	4.81	5.21	4.13
October	4.84	4.96	5.28	5.10
November	4.09	4.82	5.02	4.64
December	4.76	4.54	4.82	4.49

Debere Zeit, Alemtena and Modjo. The highest monthly ET0 for Akaki was observed in April (5.24 mm/day), while the lowest was detected in July (4.01 mm/day which corresponds to the mid of the main growing season in the area. Similarly, at Alemtena, the highest was in May (5.6 mm/day and the lowest value, 4.53 mm/days was for July. The highest value, 5.93 mm/day was observed in March while the lowest in December around Modjo. August had the lowest (3.75 mm/day) reference evapotranspiration value around Debre Zeit but the highest, 5.76 mm/day was seen both in May and June for this particular site.

The probable irrigation season for Debre Zeit and Akaki may start as early as November where the evapotranspiration rates are relatively low until the crops will have full maturity and hence planting during those periods will have two advantages; using the soil moisture reserve that could have been stored from that recedes in late September or early October. Secondly, planting crops at times of low evapotranspiration is implicated that the demand of the crops for water is also low. Therefore, irrigation water saving is more practical for early planning

Table 2. Crop Water and Irrigation Requirement of Different Crops at Debre Zeit (m^3/ha).

Crop	Planting Date	Growing period	Seasonal Etc/m^3/ha)	Irrigation requirement (m^3/ha)
Onion	15-Jan	95	4364	3900
Tomato	15-Jan	145	7127	6211
Chickpea	15-Jan	110	5780	5044
Wheat	15-Jan	130	4899	4317

Table 3. Crop water and irrigation requirement of different crops Akaki (m^3/ha).

Crop	Planting date	Growing period	Seasonal Etc/m^3/ha)	Irrigation requirement (m^3/ha)
Onion	15-Jan	95	4017	3472
Tomato	15-Jan	145	6354	5296
Chickpea	15-Jan	110	4500	3781
Wheat	15-Jan	130	5225	4295

Table 4. Crop water and irrigation requirement of different crops Modjo (m^3/ha).

Crop	Planting Date	Growing period	Seasonal Etc/m^3/ha)	Irrigation requirement (m^3/ha)
Onion	15-Jan	95	4624	4226
Tomato	15-Jan	145	7421	6649
Chickpea	15-Jan	110	5181	4669
Wheat	15-Jan	130	6083	5445

in November. Similarly for areas in Modo and Alemtena, October panting could favor water conservation as rainfall recedes early in these areas as compared to Debre Zeit and Akaki. However, the soils in Alemtena is relatively light textured that, unlike that of the other sites (Vertisols), soil water stored from main rain season could not be significant. Thus proper planning in irrigation operation in the later areas is more crucial.

For planting in mid-January at Debre Zeit, onion needs a seasonal net irrigation water requirement of 4364 m^3, while tomato, Chickpea, and Wheat require, 7127, 5780 and 4899 m^3 reactively (Table 2). At times when irrigation water is critical, crops like wheat and onion can be a choice for this particular area. Alternatively water application limiting to more water sensitive stages can also be an advantage. For instance, wheat is more sensitive to water stress during anthesis and heading. Thus, by irrigating water below the demand during to the rest of the crop growth stages can help farmers to save irrigation water.

At Akaki, the crop water requirement for onion, Tomatoes, Chickpea, and Wheat is in the order of 4017, 6354, 4500, and 5225 m^3/ha (Table 3). The demand of the crops for water in this area is irrelatively lower than at Debre Zeit. This is duet to Akaki is more in the highland than Debre Zeit where temperature could also be lower and correspondingly the crop water demand is reduced in

relative terms. Interestingly, chickpea and onion are observed to demand less water. This is congruent with the current practice of crop production in the central highlands of Ethiopia where chickpea is grown on residual moisture after the main rainy season.

Similarly, the crop water requirements of the crops at Modjo, Onion, Tomato, Chickpea and Wheat are 4624, 7421, 5181 and 6083 m^3/ha, respectively (Table 4). The result obtained for this particular area also showed that onion and chickpea are the least water demanding as compared to tomato and wheat. Again, at times when water may be limiting, the choice for these two crops may be advised. However, as this study has not consider the economic return of irrigation practice for crops, caution should be taken in selecting crops, unless, water is so critical that crop production under irrigation may be under severe stress.

The crop water demand of the crops at Alemtena is presented in Table 5. Accordingly, growing tomato under irrigation is observed to require the highest (7004 m^3/ha) followed by wheat (5721 m^3/ha), chickpea (4870 m^3/ha) and onion (4347 m^3/ha).

From the analysis, onion is observed to require frequent irrigation application at al study sites. At Debre Zeit, onion the average irrigation frequency is about 12 days while tomato required to be irrigated every 18 day while chickpea and wheat 20 and 28 days respectively

Table 5. Crop water and irrigation requirement of different crops Alem-Tena (m³/ha).

Crop	Planting Date	Growing period	Seasonal Etc/m³/ha)	Effective rainfall	Irrigation requirement (m³/ha)
Onion	15-Jan	95	4347	32.8	4016
Tomato	15-Jan	145	7004	97.8	5977
Chickpea	15-Jan	110	4870	40.1	4465
Wheat	15-Jan	130	5721	54.1	5161

Table 6. Irrigation schedule of different crops at Debre Zeit.

Crops		Seasonal net irrigation (m³/ha)	No.
Onion	Interval	13,13,13,11,11,11,11,11	2890
	Depth (mm)	28,28,28, 41,41,41,41,41	
Tomato	Interval (days)	23,23,23,15,15,15,15,15	4650
	Depth (mm)	50,50,50,63,63,63,63,63,	
Chickpea	Interval (days)	24,24,16,16,16	3000
	Depth (mm)	54,54,64,64,64	
Wheat	Interval (days)	54,22,22,15	3670
	Depth (mm)	87,94,94, 92	

Table 7. Irrigation schedule of different crops at Akakai.

Crops		Seasonal net irrigation (m³/ha)	No.
Onion	Interval	13,13,13,13,11,11,11,11,	2920
	Depth (mm)	32,32,32,32,41,41,41,41,	
Tomato	Interval (days)	19,25,25,17,17,17,24	4030
	Depth (mm)	39,56,56,63,63,63,63	
Chickpea	Interval (days)	26,21,21,21	2360
	Depth (mm)	47,63,63,63	
Wheat	Interval (days)	55,26,26	2710
	Depth (mm)	89,91,91	

after the first irrigation with full field capacity (Table 6). Similarly the average depth of water required is 35, 56, 58, 92 mm for onion, tomato, chickpea and wheat in their respective orders.

At Akaki, onion the average irrigation frequency is about 12 days similar with what observed at Debre Zeit while tomato required to be irrigated every 21 days, chickpea and wheat 22, and 35 days respectively after the first irrigation with full field capacity (Table 7). The implication is that frequency of irrigation in the highland is relatively higher that could be attributed to the lower evaporative demand due to low temperature.

Accordingly, the average depth of water required for onion ranged from 32 mm in the first irrigation (during germination and developmental crop growth stages) and should reach up to 41 mm during the critical growth stages, may be fruit setting. For wheat, the average irrigation depth is around 90 mm that can be served in three irrigation cycles, unlike that the rest of the crop that have significant variation in irrigation frequency in their crop growth stages. For instance, tomato requires 25 days of irrigation interval in its mid stage while in the subsequent stages the frequency of irrigation can be reduced to up to 17 days and again in ripening stage, the

Table 8. Irrigation schedule of different crops at Alemtena.

Crops		Seasonal net irrigation(m^3/ha)	No.
Onion	Interval	10,10,10,10,7,7,7,7,7,7,7,7,7,7	3840
	Depth(mm)	21,21,21,21,30,30,30,30,30,30,30,30,30,30	
Tomato	Interval(days)	16,16,16,13,13,9,9,10,10,10,10	4720
	Depth(mm)	34,34,34,45,45,44,48,48,48,48	
Chickpea	Interval(days)	18,13,11,11,11,11,11	3180
	Depth(mm)	40,48,46	
Wheat	Interval(days)	42,20,14,14,14,17	4050
	Depth(mm)	56,69,71,71,71,67	

interval goes higher to 24 days.

At Alemtena, the irrigation interval for all crops is relatively more frequent than Debre Zeit and Akaki. Due to the fact that the evaporative demand is relatively higher and also the soils in this area is light textured and storage of water in the soil depth can be smaller. According to the results, the irrigation interval for onion ranges from 10 (in the first growth stages) and 7 days during the later growth stags (Table 8). The irrigation depth during irrigation application should not be below 21 mm in the earlier stages and 30 mm in the advanced stages for onion. Tomato requires less frequent irrigation during the earlier stages (16 days) and more frequent irrigation application is required at later stages and the depth of irrigation should be kept at 48 mm. Chickpea and wheat are also requiring less frequent irrigation during the earlier stages, 13- 8 days for chickpea, and 20 -40 days for wheat after the firs irrigation with field capacity.

Similarly, At Modjo, the irrigation interval for all crops is relatively more frequent than Debre Zeit and Akaki. Due to the fact that the evaporative demand is relatively higher. The interval for irrigation application for onion ranges from 7-11 while it requires less frequent in the latter stages. The irrigation depth for each irrigation cycle progressively develops from 18 mm at the earlier sage to 33 mm in the middle of the crop growth stages and ultimately drops to 30 mm further in the advanced stages. Likewise, the interval for irrigation application is more frequent in the middle of the crop growth stages for tomato (10 days) and relatively less frequent in the latter stages. The depth of irrigation should be kept 35 mm in the earlier days and goes up to as high as 47 mm in the latter stages. Chickpea and wheat require more frequent irrigation during the mid and latter crop growth stages, 11 days for chickpea and 15 for wheat. The depth of irrigation develops 35 to 48 mm for chickpea while wheat need to irrigated to the minimum 69 mm for most of the crop growth stages, with exception for its initial stage that requires 54 mm.

In general, the water consummation level of the sites for growing those crops is in the order of Akaki, Debre Zeit, Alemtena, and Modjo respectively, Akakai consumes the lowest. To summarize, in all the sites, Onion requires frequent application of irrigation followed by tomato while wheat needs longer interval. The seasonal net irrigation application for Onion is as shown in Tables 8 and 9.

Conclusions

Understanding crop water needs is believed assist decision making in irrigation water management. The objective of this study was to estimate crop water need and seasonal crop water demand of crops in the central highlands of Ethiopia. FAO CROP WAT model was employed for the purpose and the results showed that the seasonal net irrigation application for Onion (60% field efficiency) is 2890, 2920, 3870, 3840 m^3 for Debre Zeit, Akaki, Modjo, and Alem-Tena with their respective orders. Tomato requires net application of irrigation amount, 4650, 4030, 5560, 4720 m^3/ha for the sites, Debre Zeit, Akaki,Modjo and Alemtena respectively. Similarly, at Debre Zeit, Chickpea needs 3000 m^3/ha while wheat requires 3670 m^3 of irrigation water. The information generated can be helpful for small scale irrigators in the study sites where the same is critically limiting. Farmers are culturally irrigating their fields only based on the availability of water regardless of the crop water need, too much and too low is equally affect the yield of irrigated crops. Thus, up until, the analysis of this result can be verified in the field, farmers can be advised to apply the information generated from this study and the follow up of this work is now undertaken to make the information complete.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Table 9. Irrigation schedule of different crops at Modjo.

Crops		Seasonal net irrigation (m^3/ha)	No.
Onion	Interval	8,11,11,11,7,7,7,7,7,7,7,7,7	3870
	Depth(mm)	18,33,33,33,30,30,30,30,30,30,30,30,30	
Tomato	Interval(days)	14,14,14,10,10,10,10,10,10,10,12,12,13	5560
	Depth(mm)	35,35,35,46,46,46,46,46,46,46,47,47	
Chickpea	Interval(days)	17,17,11,11,11,11,11,11,	358
	Depth(mm)	35,35,48,48,48,48,48,48	
Wheat	Interval(days)	39,19,15,15,15,15	3990
	Depth(mm)	54,69,69,69,69,69	

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Full Length Research Paper

White clover tolerance to herbicides applied at different rates and phenological stages

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Glyphosate, 2,4-D, imazethapyr and paraquat + diuron were studied in greenhouse experiments at four application rates in order to evaluate their effects on white clover (*Trifolium repens* L.) growth and aerial shoot injury at three different phenological stages. Herbicide-induced foliar injury on white clover ranged from no symptoms to early chlorosis, leaf necrosis, stunted growth and death depending on herbicide rate and white clover phenological stage. Imazethapyr showed the highest selectivity at the three-leaf trifoliate stage. Seedlings treated with glyphosate at 1080 g a.i. ha^{-1}, however, were dead at 21 days after application (DAA). White clover herbicide tolerance increased with plant age, and selectivity was found to be affected as herbicide rate increased. Paraquat + diuron caused the highest levels of white clover phytotoxicity and plant death. The herbicides studied showed potential to be used as selective products on white clover depending on their rates, as well as the timing of their application onto white clover depending furthermore, on the specificity weeds infesting white clover fields.

Key words: Glyphosate, 2,4-D, Paraquat + diuron, Imazethapyr, *Trifolium repens*.

INTRODUCTION

Emphasis on reducing nitrogen fertilizer requirement, better quality forage and cover crops for multiple purpose with perennial plants for living mulch has stimulated interest in white clover (*Trifolium repens* L.).

White clover is an important perennial forage legume that is grown in Southern Brazil. However, it is not as important in Brazil as it is in other countries with similar climate such as New Zealand and Australia where clover is the most widely-used legume. White clover is also an important forage legume in tropical and subtropical regions of the world (Carvalho et al., 2010). Among the reasons for its low adoption, is the lack of registered herbicides available for growers, as chemical weed control in white clover is currently a critical part of agricultural management to maximize the crop´s uses and yield. In many cases, weeds are a major problem

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limiting white clover establishment (Rolston and Archie, 1999; Schuster et al., 2013).

Despite the fact that herbicide tolerance is the basis for the success of chemical weed control, the results available on white clover tolerance to herbicides are controversial given that some authors have reported that yield and persistence of the legume were markedly reduced (Griffin et al., 1984; Evers et al., 1993), whereas others have shown that although some herbicides can have initial phytotoxic effects, seedlings usually recover without severe damage (Machado et al., 2013)

Selective post emergence herbicides are needed as emerging clover seedlings development is slow and not competitive with faster growing weeds (Schuster et al., 2013). Moreover, on crop livestock farms, corn may be cultivated using clover as living mulch or over its dead biomass, however, herbicide tolerance needs to be known for improved clover management. Furthermore, white clover might become a weed in summer crops such as soybean (Schuster et al., 2015), and thus, herbicide management to control white clover need to be determined.

Once weed competition problems are overcome, white clover adoption might face a positive scenario, greatly due to its advantages such as biological nitrogen fixation, high nutritional value and excellent adaptability to edaphoclimatic conditions in Southern Brazil. In this context, the study aimed to identify white clover tolerance to herbicides and rates at different phenological stages.

MATERIALS AND METHODS

Experiments were conducted in greenhouses and designed as randomized complete blocks (RCBD) in a 3x4 factorial scheme with four replications, and aimed at evaluating white clover tolerance to different herbicide at different rates and crop phenological stages. Three herbicides were applied at four rates each onto 40, 105, or 125 day old white clover seedlings, which correspond to seedlings at the three-leaf trifoliate stage, full development, and full bloom, respectively. At the first assay, 40-day old plants (three-leaf trifoliate stage) were sprayed with glyphosate (Zapp Qi), 2,4-D (Aminol), or imazethapyr (Pivot) at the rates of 0, 540, 1080, 1620; 0, 200, 400, 600 g and 0, 100, 150, 200 g i.a ha^{-1}, respectively. This application simulated the time period at which weeds are controlled in white clover fields.

At the second assay, white clover plants at full development (105 days after emergence) were sprayed with glyphosate (Zapp Qi), 2,4-D (Aminol) and Paraquat + Diuron (Gramocil) at the rates of 0, 540, 1080, 1620; 0, 400, 600, 800 and 0, 300, 600 and 900 g i.a ha^{-1} respectively, in order to simulate herbicide tolerance of white clover at intermediate cycle development.

In the third assay, white clover plants at full bloom (125 days after emergence) were sprayed with glyphosate (Zapp Qi), 2,4-D (Aminol) and Paraquat + Diuron (Gramocil) at the rates of 0, 720, 1440, 2160; 0, 800, 1600, 2400 and 0, 300, 600, 900 g i.a ha^{-1} respectively. This period simulates a possible desiccation of white clover or its suppression for living mulch cultivation with summer cash crops. White clover cv. Zapican was used in all experiments. Individual experimental units consisted of single 20 cm diameter plastic pots (10 liters capacity) filled with soil with the following chemical properties: pH (CaCl$_2$) 5.0; V% 64; organic matter 63 g

dm^{-3}; P-Mehlich 9.5 mg dm³; Ca, Mg, K and Al of 6.6; 1.9; 0.30 and 0.0 cmol$_c$dm^{-3} respectively and soil cation exchange capacity of 13.81 cmol$_c$dm^{-3}. Soil fertilization was not performed because of the good soil parameters (CQFS, 2004). White clover seeds were hand sowed to the 1 cm top soil layer at a rate of 15 seeds per pot. Pots were watered throughout the course of the experiments to ensure adequate soil moisture.

Herbicides were sprayed with a CO$_2$-pressurized backpack sprayer (XR110.02 spray tips) calibrated to deliver 200 L ha^{-1} maintained at constant pressure (2.07 bar). At the time of applications, the plant surface was dry, relative humidity was above 60% and temperatures were between 25 and 30°C. The amount of herbicide to be applied was calculated considering the concentration of active ingredient of the herbicide trademarks used.

The assessment of phytotoxicity to white clover plants was carried out weekly at 07, 14, 21, 28, 35, 42 and 49 days after herbicide application (DAA) by assigning percentage grades from 0 (e.g. absence of phytotoxicity) to 100% (complete death of plants), according to the methodology proposed by SBCPD (1995). Phytotoxicity-induced plant symptoms included chlorosis, leaf necrosis and stunted growth.

White clover plants were harvested 50 days after herbicide application, and oven-dried with forced air at 55°C for five days, after which dry-biomass weights were determined for each replication. The results were analyzed for homogeneity of variance (Bartlett) and normality (Lilliefors) tests. Data were then subjected to ANOVA and means separations performed using the Tukey test at 5% probability with the Statigraphic 4.1 program. No data transformations were required since assumptions for ANOVA were properly met (data not shown).

RESULTS AND DISCUSION

Results differed among herbicides and rates across different phenological stages. Phytotoxicity occurred at all evaluations periods after application (DAA), and its effects on dry matter yield was also evident.

In the first assay, imazethapyr showed the highest selectivity on white clover among the evaluated herbicides (Table 1). Phytotoxicity levels increased as herbicide rate increased and even though there was an initial phytotoxic effect, such weakened with time and seedlings were thus able to recover without further implications. Accordingly, there was no significant difference on growth at 49 DAA with applications of imazethapyr at 100, 150 and 200 g a.i. ha^{-1} relative to the untreated control.

Ferrel and Sellers (2014) reported a slight leaf yellowing and a temporary growth reduction on white clover after imazethapyr (Pivot®) application, similar to the symptoms found in this work. However, they did endorse this herbicide as a good option to control broadleaf weeds in post emergence (seedlings older than the two-trifoliate leaf stage), as well as established clovers. They also reported that grasses such as perennial ryegrass in white clover seed fields can be easily controlled with clethodim or sethoxydim using trademarks products such as Select® or Poast® at rates of 120 and 180 g i.a ha^{-1}, respectively.

According to Ferrel and Sellers (2014), imazethapyr is already registered and recommended for use in the U.S.

Table 1. Herbicide[4] phytotoxic levels (%) on white clover and its dry matter (DM) weight for treatments applied at the three-trifoliate leaf growth stage.

Treatments	Days after application (DAA)							DM
	7	14	21	28	35	42	49	
Control	0.0[f]	0.0[e]	0.0[d]	0.0[e]	0.0[e]	0.0[d]	0.0[c]	8.4[b]
Gly[1] 540	25.0[c]	25.0[c]	35.0[b]	35.0[c]	22.5[c]	12.5[c]	5.0[c]	5.2[d]
Gly 1080	37.5[b]	50.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	0.0[e]
Gly 1620	47.5[a]	55.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	0.0[e]
2,4-D[2] 200	17.5[d]	20.0[c]	17.5[c]	17.5[d]	15.0[d]	10.0[c]	2.5[c]	7.3[c]
2,4-D 400	37.5[b]	40.0[b]	37.5[b]	37.5[c]	35.0[b]	32.5[b]	17.5[b]	5.1[d]
2,4-D 600	40.0[b]	45.0[b]	37.5[b]	42.5[b]	37.5[b]	32.5[b]	22.5[b]	5.0[d]
Imaz[3] 100	0.0[f]	0.0[e]	0.0[d]	0.0[e]	0.0[e]	0.0[d]	0.0[c]	9.1[a]
Imaz 150	10.0[e]	15.0[d]	20.0[c]	20.0[d]	12.5[d]	10.0[c]	0.0[c]	8.3[b]
Imaz 200	10.0[e]	15.0[d]	20.0[c]	20.0[d]	17.5[d]	15.0[c]	5.0[c]	6.9[c]
CV(%)	16.2	15.4	9.0	8.8	14.2	10.5	20.8	8.1

[123]Contracted form of glyphosate (Gly), 2,4-D and Imazethapyr (Imaz) applied at rates of 540, 1080, 1620; 200, 400, 600 and 100, 200 and 300 g i.a ha^{-1} respectively. Means with incolumns that are followed by distinct letters differ by Scott-Knott test (p≤0.05). [4]Products not registered for use in white clover crop.

as a selective herbicide in white clover. One must, however, follow label guidelines concerning herbicide rates and withholding periods, which indicates white clover should not be used as feed, raze, or harvest until 30 days after application.

Grasses are usually not a problem in white clover pastures since they are grazed by the animals, and thus broadleaf weed species are the most troublesome species in this situation. According to Schuster et al. (2013), sow thistle (*Sonchus oleraceus*), wild radish (*Raphanus raphanistrum*), ragweed (*Ambrosia elatior*), sticky nightshade (*Solanum sisymbriifolium*), bristly starbur (*Acanthospermum hispidum*) and arrowleaf sida (*Sida rhombifolia*) are the most important weed species on white clover fields in southern Brazil. Therefore, 2.4-D stands as a good alternative due to its control efficacy over these weeds. Moreover, in Brazil, Mexican fire plant (*Euphorbia heterophylla*) is considered resistant to imazethapyr (Xavier et al., 2013) and is still well controlled by 2,4-D.

It was noticed that clover selectivity to 2,4-D decreased as the rate of the herbicide increased, however, plants seemed to be able to detoxify the herbicidemolecule since phytotoxicity was reduced from 45% at 14 DAA to 22.5% at 49 DAA for the highest rate (600 g a.i ha^{-1}) (Table 1). It is noteworthy that label recommendation for broadleaf control at most crops ranges from 400 to 600 g i.a ha^{-1} and the fact that this rate is effective on broadleaf control and there is no need to use higher rates since they may cause phytotoxicity on the crops. Moreover, the greater the difference between crop and weed tolerance, the greater the security to recommend treatment employing a particular herbicide.

White clover tolerance to 2,4-D and imazethapyr was

also suggested in other experiments (Mccurdy et al., 2013). Machado et al. (2013) assessing the selectivity of imazethapyr (100 g a.i ha^{-1}) and 2,4-D (201 g a.i ha^{-1}) applied to white clover seedlings at the two-leaf trifoliate growth stage reported phytotoxic levels of 1.5 and 9.7%, respectively, at 84 DAA, corroborating with the results of this work. Enloe et al. (2013) studying white clover tolerance to multiple formulations of 2,4-D with imazethapyr reported effective weed control in mixed white clover/grass pastures without affecting white clover populations.

Highest phytotoxicity levels occurred when plants were treated with glyphosate at a rate of 1080 g a.i ha^{-1} (applied as Zapp Qi® herbicide at 1.7 L ha^{-1}), at which plants were dead 21 DAA (Table 1). On the other hand, the lowest glyphosate rate (540 g a.i ha^{-1}) showed intermediate phytotoxicity, reaching values of 35 and 5% at 28 and 49 DAA, respectively. Biomass dry weight showed a slight difference (38% less) as compared to the control. This value was similar to the treatment with 2,4-D at either the 400 and 600 g a.i ha^{-1}.

Moreover, it is important to emphasize that this growth reduction and lower dry matter accumulation may over time be recovered once weeds are controlled. On the other hand, if weed control is not performed, losses can reach as high as 96%, practically making it impossible for grower to cultivate this species (Schuster et al., 2013).

It is also important to observe the herbicide tolerance increases as plant age increases. Machado et al. (2013) evaluating 2,4-D applied at the first (16 days after emergence) and second-leaf trifoliate (27 days after emergence) growth stage reported lower toxicity for older plants. Young plants are more susceptible to herbicides than older plants, mainly because they have more

Table 2. Herbicide[4] phytotoxic levels in white clover and its dry matter (DM) weight applied at full development.

Treatments	Days after application (DAA)							MS
	7	14	21	28	35	42	49	
Control	0.0[g]	0.0[e]	0.0[f]	0.0[e]	0.0[e]	0.0[d]	0.0[e]	35.9[a]
Gly[1] 540	37.5[d]	32.5[d]	32.5[e]	35.0[d]	35.0[d]	30.0[c]	28.0[d]	14.2[c]
Gly 1080	50.0[c]	55.0[c]	42.5[d]	45.0[c]	45.0[c]	47.5[b]	47.5[c]	8.7[d]
Gly 1620	60.0[b]	85.0[b]	90.0[b]	95.0[b]	95.0[b]	95.0[a]	95.0[a]	0.5[f]
2,4-D[2] 400	20.0[f]	5.0[e]	0.0[f]	0.0[e]	0.0[e]	0.0[d]	0.0[e]	36.4[a]
2,4-D 800	30.0[e]	50.0[c]	40.0[d]	40.0[d]	30.0[d]	30.0[c]	30.0[d]	16.8[b]
2,4-D 1200	40.0[d]	57.5[c]	50.0[c]	50.0[c]	42.5[c]	35.0[c]	37.5[d]	8.8[d]
Parq+di[3] 300	80.0[a]	92.5[a]	92.5[b]	92.5[b]	92.5[b]	92.5[a]	92.5[a]	1.7[e]
Parq+di 600	80.0[a]	97.5[a]	100.0[a]	100.0[a]	100.0[a]	97.5[a]	97.5[a]	0.1[f]
Parq+di 900	77.5[a]	92.5[a]	92.5[b]	92.5[b]	92.5[b]	92.5[a]	85.0[b]	2.0[e]
CV(%)	4.7	8.4	5.9	7.0	7.9	8.5	11.1	8.2

[123]Contracted form of glyphosate (Gly), 2,4-D and Paraquat + Diuron (Parq+di) applied at rates of 540, 1080, 1620; 400, 800, 1200 and 300, 600 and 900 g i.a ha^{-1} respectively. Means in the columns followed the distinct letters differ by Scott-Knott test (p≤0.05). [4]Product not registered for use in white clover crop.

meristematic tissues (Evers et al., 1993).

Schuster et al. (2013) while evaluating weed interference in white clover, reported that the critical period of interference occurred between 20th and 62nd days after emergence. Rolston and Archie (1999) also reported that phenological stage affected cultivar sensitivity to the herbicide diflufenican. According to the results, clover growth suppression can occur, but is thought to be due to the clover growth stage at treatment time, rather than reflecting a difference in cultivar tolerance. Plants at the four-trifoliate leaf stage were more tolerant than younger plants. Furthermore, white clover seed crops showed good seed yield tolerance to diflufenican at rates ranging from 25 to 75 g a.i ha^{-1}.

In the second assay, it was noticed, a higher tolerance to herbicides glyphosate and 2,4-D. Phytotoxicity level of glyphosate-treated plants ranged from 28 to 95% at 49 DAA between the lower and highest rate. By day 50, the relative dry weights were 100, 39, 24 and 1.4% for the control, low, middle and high glyphosate rates, respectively (Table 2).

As for the 2,4-D herbicide, phytotoxicity variation between the lowest and highest rate at 49 DAA was lower, ranging from 0 to 37.5% at the rate of 400 and 1200 g a.i ha^{-1}. It caused some phytotoxicity, but the plants were generally not severely damaged and recovered. By the end of experimentation, 2,4-D at the 1X rate did not affect biomass dry weight, but the two (2X) and three times (3X) rates reduced shoot dry weight by 53 and 75%, respectively, as compared to the untreated control treatment.

In general, herbicides affected plant development and caused a delay in plant growth. At the end of the 2nd trial, control plants had already flowered while other treatments were still at vegetative growth stage. It shows that beyond dry matter, these herbicides applied at later stages can affect white clover seed yield.

At both 2nd and 3rd assay (Tables 2 and 3), white clover plants treated with Paraquat + Diuron showed rapid wilting and desiccation of sprayed leaves which, according to Martins (2013) is a consequence of complete loss of photosynthetic electron transport in treated plant material.

Moreover, Paraquat + Diuron caused the highest levels of white clover phytotoxicity throughout the experiment with up to 92 and 97% of white clover plants showing severe foliar injury with the recommended and double rates, respectively at 14 DAA. By the end of evaluation period, treated plant biomass weighed 96 and 99% less than the control for the 1X and 2X rates, respectively. Clark and Mahanty (1991) also reported that paraquat was extremely toxic to white clover plants.

In the 2nd assay, plants treated with Paraquat + Diuron at 600 g a.i ha^{-1} (applied as Gramocil® at 3 liters ha^{-1}) were dead at 21 DAA. The differences among rates might be explained by the mode of action of the herbicide. Due to its contact action, control is achieved only where the herbicide reaches the target and a possible protection of older leaves to the younger ones resulted in differences in phytotoxicity and biomass accumulation among herbicide levels. Non-selective, ystemic herbicides might avoid this problem. Moreover, unfolded leaves which emerged after the herbicide application were undamaged and continued to grow and through that, treatment with 300 g a.i ha^{-1} showed some dry matter accumulation at the end of evaluated period.

In order to develop efficient herbicide use and prevent crop yield loss, information of critical period of weed

Table 3. Herbicides phytotoxic levels of white clover and its dry matter weight (DM) applied at full bloom.

Treatments	Days after application (DAA)							MS
	7	14	21	28	35	42	49	
Control	0.0[f]	0.0[h]	0.0[h]	0.0[h]	0.0[h]	0.0[d]	0.0[e]	38.0[a]
Gly[1] 720	22.5[e]	20.0[g]	20.0[g]	10.0[g]	10.0[g]	0.0[d]	0.0[e]	28.0[b]
Gly 1440	40.0[c]	30.0[f]	30.0[f]	20.0[f]	10.0[g]	2.5[d]	2.5[e]	24.7[c]
Gly 2160	50.0[b]	40.0[d]	55.0[c]	55.0[c]	55.0[d]	55.0[b]	52.5[b]	16.7[e]
2,4-D[2] 800	30.0[d]	35.0[e]	35.0[e]	30.0[e]	25.0[f]	22.5[c]	12.5[d]	27.6[b]
2,4-D 1600	40.0[c]	47.5[c]	40.0[d]	40.0[d]	40.0[e]	30.0[c]	22.5[d]	23.8[c]
2,4-D 2400	40.0[c]	50.0[c]	40.0[d]	40.0[d]	40.0[e]	30.0[c]	20.0[d]	20.4[d]
Parq+di[3] 300	70.0[a]	77.5[b]	80.0[b]	70.0[b]	70.0[c]	50.0[b]	42.5[c]	21.8[d]
Parq+di 600	70.0[a]	87.5[a]	87.5[a]	82.5[a]	82.5[b]	80.0[a]	80.0[a]	3.1[f]
Parq+di 900	70.0[a]	90.0[a]	90.0[a]	87.5[a]	87.5[a]	85.0[a]	85.0[a]	1.4[f]
CV(%)	3.7	6.9	6.3	8.9	8.1	17.4	20.5	6.7

[123]Contracted form of glyphosate (Gly), 2,4-D and Paraquat + Diuron (Parq+di) applied at rates of 720, 1440, 2160; 800, 1600, 2400 and 300, 600 and 900 g i.a ha^{-1} respectively. Means in the columns followed the distinct letters differ by Scott-Knott test (p≤0.05).

control is essential. Moreover, the effect of herbicide on white clover across time is important to determine the right moment to spray it before corn seeding, especially in living mulch production systems.

According to Hall et al. (1992), the critical period of weed control in grain corn starts at the 3 and ends at the 14 leaf stage of development. In accumulated thermal units (ATU), it represents 222 to 416 ATU or approximately 15 to 30 DAE (Bedmar et al, 1999). Following this idea, it is noticed that at 14 DAA, the phytotoxicity levels ranged from 20 to 90% for the lowest and highest rate of Zapp Qi® and Gramocil®, respectively.

It is difficult to estimate the optimal percentage of phytotoxicity or white clover suppression to allow a good corn intercrop development while allowing at the same time a good pasture regrowth in the next growing season. Based on this work, it was estimated, however, that phytotoxicity levels from 20 to 50%, presented by glyphosate and 2,4-D are not sufficient to suppress white clover growth and prevent its competition with corn, which could lead to corn yield losses. Moreover, in spring, days are longer and temperatures are higher than winter, resulting in higher growth rates and more competition potential in white clover.

Differences on biomass accumulation may be explained by leaf injury and also by root injury. Ceballos et al. (2004) detected 2,4-D root injury at the beginning of the experiment. Moreover, Garcia and Jordon (1969) found that nodulation and nitrogen fixation in birdsfoot trefoil (Lotus corniculatus L.) were reduced by 2,4-D. Martensson (1992) reported that, despite the ability of certain Rhizobium trifolii strains to detoxify glyphosate, its use inhibited root nodulation in red clover (Trifolium pratense).

Furthermore, Rolston et al. (1976) reported that paraquat caused a marked decrease in the white clover nitrogen-fixing activity. The equivalent of 70 and 280 g a.i ha^{-1} of paraquat reduced nitrogen-fixing activity per plant by 35 and 70%, respectively. The change was detectable 24 h after spraying and no recovery was detected by day 16. The reduction in nitrogen-fixing activity after paraquat treatment is probably an indirect response, reflecting a decrease in growth and possibly a decline in the supply of photosynthetic assimilate to the nodule. Clark and Mahanty (1991) also reported that paraquat was toxic to Rhizobium trifolii.

Phytotoxicity levels decreased with time and increased as herbicide rates changed from low to high for both glyphosate and 2,4-D treated plants. Plants showed good recovery when glyphosate (720 g a.i ha^{-1}) and 2,4-D (800 g a.i ha^{-1}) were applied showing phytotoxicity levels of 0 and 12% at 49 DAA, respectively, and dry matter weight of 73% as compared to the control.

Schuster (2015), studying glyphosate on clover control reported high tolerance to this herbicide since beyond desiccation, two more glyphosate applications (720 g a.i ha^{-1}) were necessary to reach a percentage of control above 90%. Chakwizira et al. (2011) showed than the higher concentrations of glyphosate (360 g a.i) can reduce seed production and total dry matter yield for white clover.

The herbicides studied show potential to be used on white clover depending on theirrates, phenological stages of spraying, and on the specificity of existing weeds. Furthermore, according to the normative no. 1 of February 23th, 2010 from MAPA, launched in order to regulate the registration of pesticides to cultures with insufficient phytosanitary support, also called minor crops and considering that some of these herbicides are already used in related broadleaf species such as soybean, it is

possible according to the results found in this experiment, to start the herbicides study directing to its use in white clover.

Moreover, this research was conducted under greenhouse conditions and further evaluation under field conditions are needed to verify these findings, since environmental factors may influence the herbicide effects on white clover. Furthermore, other herbicides should also be studied in order to avoid plant resistance.

Conclusions

White clover can be considered tolerant to imazethapyr, glyphosate and 2,4-D at rates of 100, 540 and 200 g a.i ha^{-1}, respectively, meanwhile phytotoxicity increased as 2,4-D and glyphosate rates increased, being the rate of 1080 g a.i ha^{-1} lethal when applied to seedlings at the three-leaf trifoliate stage. Herbicide tolerance increased with plant age at the time of application.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional valorization of ginger lily fiber (*Hedychium gardnerianum*, Sheppard ex Ker-Gawl) for animal feeding

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Animal production in the Azores is conditioned by the cycles of grass production. In a situation when lack of fiber for animal feed occurs, low quality fibrous foods become relevant. For these reasons, *Hedychium gardnerianum* (ginger lily), a well-known and traditional source of fiber used by Azorean farmers have been studied. The plant material was subjected to treatments with 5% dry matter (DM) with urea, 3% DM with NaOH, and the addition of a nitrogen source (soybean meal) to achieve a content of 18% crude protein in DM, in order to study its nutritional value. The treatment with urea and the addition of a nitrogen source showed a significant ($P<0.05$) increase in crude protein (CP), while maintaining the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents, and a variation in the acid detergent lignin (ADL) content. All treatments had a significant effect ($P<0.05$) on ginger lily DM digestibility. It can be concluded that all the treatments performed had a significant effect on the nutritional value of ginger lily.

Key words: *Hedychium gardnerianum,* NaOH treatment*,* urea treatment, roughage.

INTRODUCTION

Animal production in the Azores is conditioned by the cycles of grass production. In the Islands with high altitude impact, for example Pico, S. Jorge and Flores, or in the higher zones of all the islands (more than 300 m of altitude), two lack of grass periods are observed along the year: in summer (namely August and September) and in winter (November, December, January and February).

In the lesser altitude dominated Islands and/or lowlands (less than 150 m of altitude), summer, while being much longer, is the only foraging season. In a situation in which there is a lack of fiber for animal feed, low quality fibrous foods become relevant. However, since these foods are of low food nutritional value, low digestibility and low voluntary intake, they do not satisfy the ruminant's

maintenance needs.

The use of unconventional forages has been studied in several parts of the world, as a mean to combat desertification (Correal and Sanchez-Gomes, 1991; Maestre et al., 2009), and for its nutritional value (Topps, 1992; Chakeredza et al., 2007; Meinerz et al., 2011). In the case of the Azores, we intend to value an invasive species, which, although presenting a low nutritional value, has traditionally been used in animal feed.

In recent years, several studies have been carried out on the nutritive valorization of the ginger lily (*Hedychium gardnerianum*, Sheppard ex Ker-Gawl), of which those carried out by Borba et al. (2015), were emphasized with treatment of the urea content, stating that urea treatment in green forage does not have the same effect as in low quality fibrous forages, especially straw, since the former has a value of crude protein (CP) much superior to that of the latter. The work carried out by Moselhy et al. (2014) who studied the fermentative characteristics of this alternative forage, tested the use of the silage value with different additives, pre-biotic and pro-biotic, with very promising results (Moselhy et al., 2015).

In the work of Borba et al. (2015), the urea treatment was carried out in a green forage, with high water content, with a CP content of 8.05% of DM in non-watertight plastic containers. These constraints may explain the less attractive results found and have laid the groundwork for future trials, in the sense that the treatment should be carried out on dry forage, in watertight containers following one given by Quashie (2014), in addition to testing treatments with sodium hydroxide, to verify if this alkali is more efficient in the degradation of the cell wall of *H. gardenerianum* than urea.

Several attempts have been made to enhance the nutritive value of the fiber of low quality forages, mainly following two main routes, one via complementation with nitrogen (degradable nitrogenous constituents in the reticulum-rumen to give a nitrogen source to the microbial population and non-degradable nitrogenous constituents in the reticulum-rumen) and another with physical, chemical and biological treatments (Jarrige, 1987).

The main objective of this work is to study the nutritive characteristics of *H. gardnerianum*, Sheppard ex Ker-Gawl (ginger lily or Kahili ginger-Conteira or Roca-de-Velha in Portuguese) with the addition of a source of nitrogen treated with urea and NaOH.

MATERIALS AND METHODS

H. gardenerianum Sheppard ex Ker-Gawl is a rhizomatous herbaceous Zinigigeraceae monocotyledon. This species may reach up to 2.4 m in height with broad, long leaves (20 to 60 cm in length and 8 to 18 cm in width) of bright dark green color. Blooms in late summer and its flowers are light yellow with long red stilettos grouped in dense terminal-shaped spiked inflorescences.

Forage collection and preparation

The current study was conducted in the Animal Nutrition Laboratory, Department of Agricultural Sciences, University of the Azores, located in Angra do Heroísmo, Terceira, Azores, Portugal. The whole plant (leaves and pseudostems) was manually harvested in full vegetative state, in Terceira island, in Cinco Ribeiras at 38°40'29.09" N of latitude and 27°18'20.72" W of longitude. This zone is at an altitude of 97 m above sea level. The plant material was chopped to pieces of approximate 2 to 3 cm of length.

Experimental methodology

The following treatments were applied to plant material dried in a forced air oven at 65°C for 72 h: (i) control; (ii) treated with urea (5% of the dry weight); (iii) treated with NaOH (3% of the dry weight) and (iv) addition of a source of nitrogen (soybean meal) to achieve a content of 18% CP in dry matter (DM). Three replicates were used per treatment, in a total of 12 samples to be analyzed. The urea treatment lasted 4 weeks and was performed in a sealed vessel. The application of urea and NaOH was done by spraying a dilution medium.

Sample contents, in triplicate, were sprayed with the NaOH solution in the proportion of 1 L of solution to 1 kg of dry content. The NaOH treatment had a duration of seven days.

Chemical analysis

Dried samples were then ground through a 1-mm screen using a Retsch mill (GmbH, 5657 HAAN, Germany). Ground samples were analyzed for dry matter (DM, method 930.15), crude protein (CP, method 954.01), ether extract (EE, method 920.39) and total ash (method 942.05) according to the standard methods of AOAC (1995). Briefly, the DM content of forage was determined by placing samples in a forced air oven at 105°C for 24 h. Total ash was evaluated by igniting samples in a muffle furnace at 500°C for 12 h. CP was determined by standard micro-Kjeldahl method using digestion equipment (Kjeldatherm System KT 40, Gerhart Laboratory Instruments, Bonn, Germany) and an automated Kjeltec 2300 Auto-analyzer apparatus for distillation and titration (Foss Electric, Copenhagen, Denmark). Ether extract was measured by refluxing forage samples with petroleum ether in a Soxhlet system (Büchi B-810, Switzerland), while neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970). Both NDF and ADF were expressed without residual ash. Dry matter digestibility was determined according to the method of Tilley and Terry (1963) modified by Alexander and McGowan (1966).

Statistical treatment

To evaluate possible differences between treatments on the target variables, analysis of variance (ANOVA) was performed, followed by the Scheffe multiple comparison test whenever significance ($P<0.05$) was detected.

RESULTS

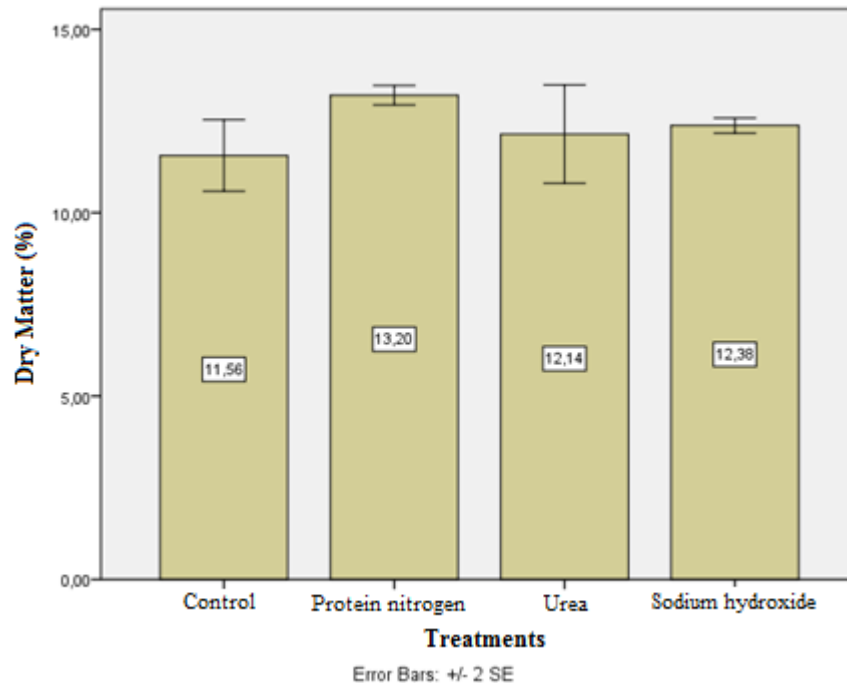In the following figures, the results obtained with the

Figure 1. Mean variation of the Dry Matter of *Hedychium gardnerianum*, with the type of treatment. In the different treatments, there were no statistically significant differences (P <0.05). Error bars represent the standard error.

treatments done to *H. gardenerianum* are presented.

Figure 1 shows a slight increase in DM with the addition of soybean meal, but there were no significant differences (P<0.05) in the control compared to the three treatments. The nitrogen addition treatments showed a significant (P<0.05) increase in CP content, as would be expected (Figure 2).

The trend of the results verified for the NDF (Figure 3) is similar to those verified for the ADF (Figure 4).

In relation to ADL (Figure 5), a significant decrease of this parameter (P<0.05) was observed with the addition of soybean meal.

Regarding the ether extract (Figure 6), a non-significant decrease (P<0.05) was observed in this parameter, especially for NaOH treatment.

The ash content (Figure 7) of the samples treated with NaOH increases significantly (P<0.05), as expected.

Figure 8 shows significant differences (P<0.05) between the DM digestibility of control and the different treatments, being the NaOH treatment presented the best results.

DISCUSSION

H. gardnerianum (ginger lily) is a showy ornamental perennial herb with leafy shoots 1.5 to 2 m t all (Wagner et al., 1999). The leaves are alternate, ovate-elliptic, apex acuminate, 20 to 60 cm long and 8 to 18 cm wide. It displaces native plants, forms vast dense colonies and chokes the understory vegetation. It can also block stream edges, altering water flow (GISD, 2017; Silva and Smith, 2004; Silva et al., 2008). *H. gardnerianum* is native to India and Nepal where it grows on the lower slopes of the Himalayas (1250 m) (Wood et al., 2000). It prefers wet habitats and fertile soils between sea level and 1,700 m (Smith, 1985). Several habitat types have been invaded by these plants, including tropical forests, highland forests, agricultural areas, coastal areas, disturbed sites, natural and planted forests, grasslands, marshes, urban areas and wetlands (PIER, 2004).

H. gardenarium is very abundant in the Azores (Portugal), and is sometimes used as cattle food. However, it is a poor forage of low nutritional value. In order to improve its economic interest regarding animal nutrition, it is important to find a simple and practical method of harvesting this plant as forage and treat it to improve its nutritional value.

Some studies have been carried out with this objective, namely the treatment of green *H.* with urea (Borba et al., 2015). However, with these tests, there were no statistically significant differences in DM digestibility. It is expected that the treatment would have a better effect on dry forage, as in the treatments of cereal straw.

Figure 2. Effect of treatment on the mean Crude Protein content of *Hedychium gardnerianum* forage. Means that display the same index are not significantly different (P <0.05). Error bars represent the standard error.
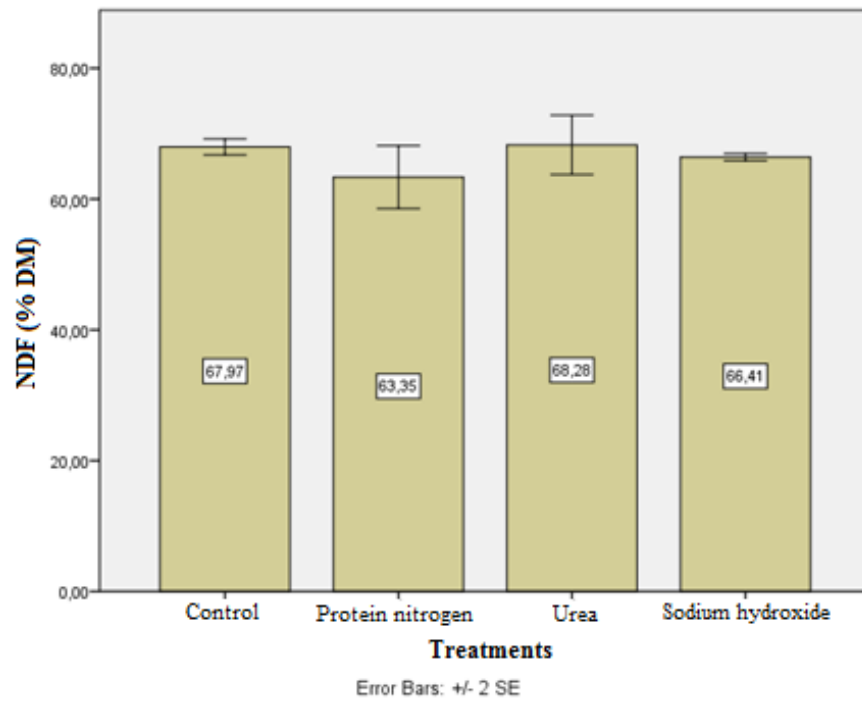
Figure 3. Effect of treatment on the mean NDF content of *Hedychium gardnerianum* forage. In the different treatments, there were no statistically significant differences (P <0.05). Error bars represent the standard error.
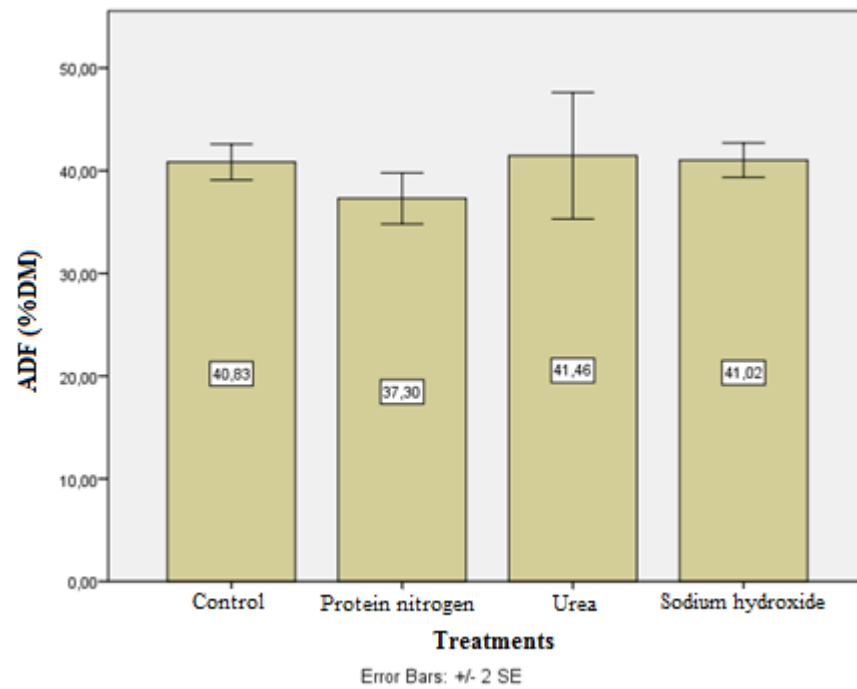
Figure 4. Effect of treatment on the mean ADF content of *Hedychium gardnerianum* forage. In the different treatments, there were no statistically significant differences (P <0.05). Error bars represent the standard error.
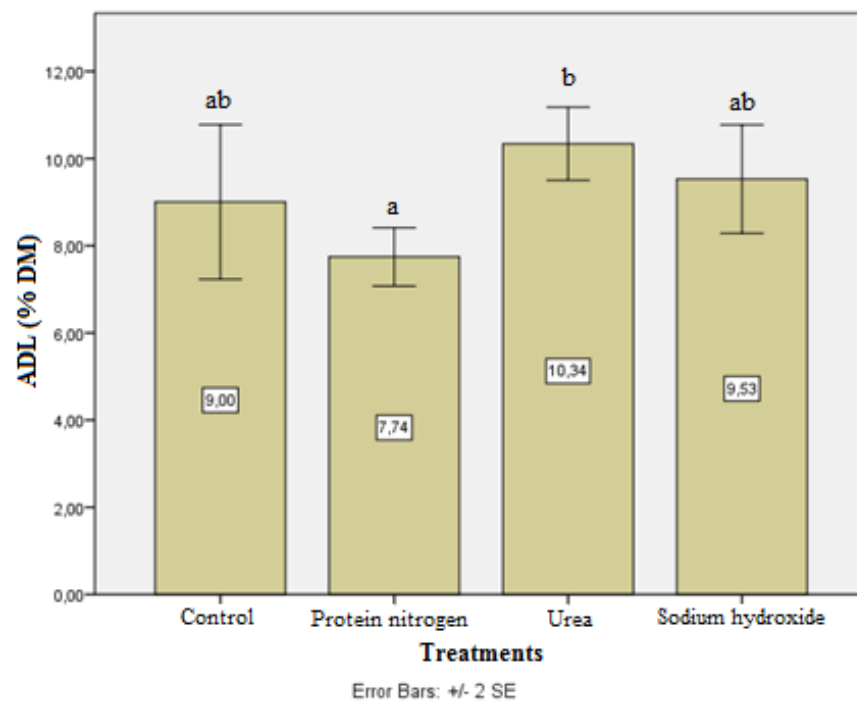
Figure 5. Effect of treatment on the mean ADL content of *Hedychium gardnerianum* forage. Means that display the same index are not significantly different (P <0.05). Error bars represent the standard error.
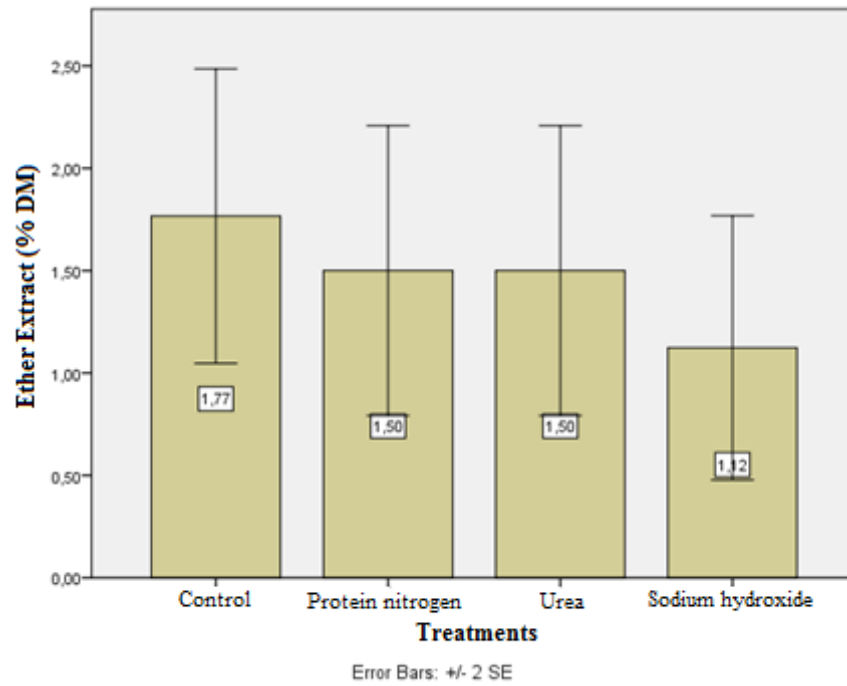
Figure 6. Effect of treatment on the mean Ether Extract of *Hedychium gardnerianum* forage. In the different treatments, there were no statistically significant differences (P<0.05). Error bars represent the standard error.
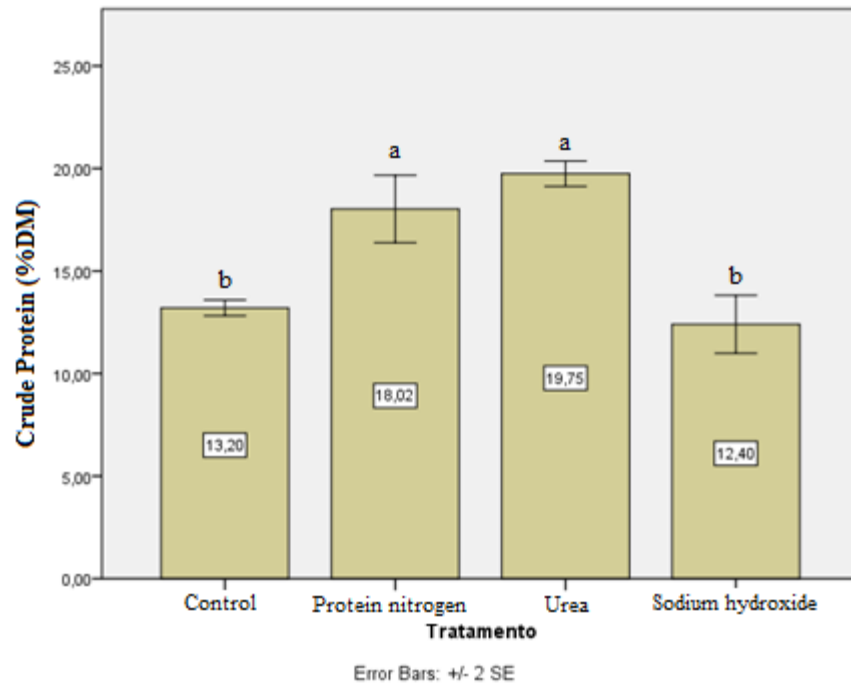

Figure 7. Effect of treatment on the mean Ash content of *Hedychium gardnerianum* forage. Means that display the same index are not significantly different (P <0.05). Error bars represent the standard error.

Figure 8. Effect of treatment on the mean *in vitro* digestibility of Dry Matter of *Hedychium gardnerianum* forage. Means that display the same index are not significantly different (P<0.05). Error bars represent the standard error.
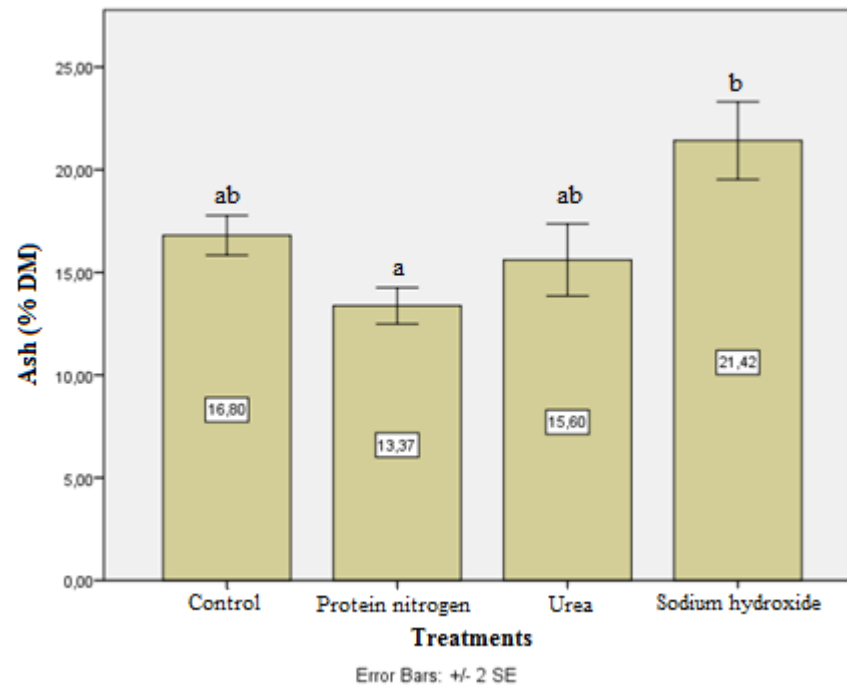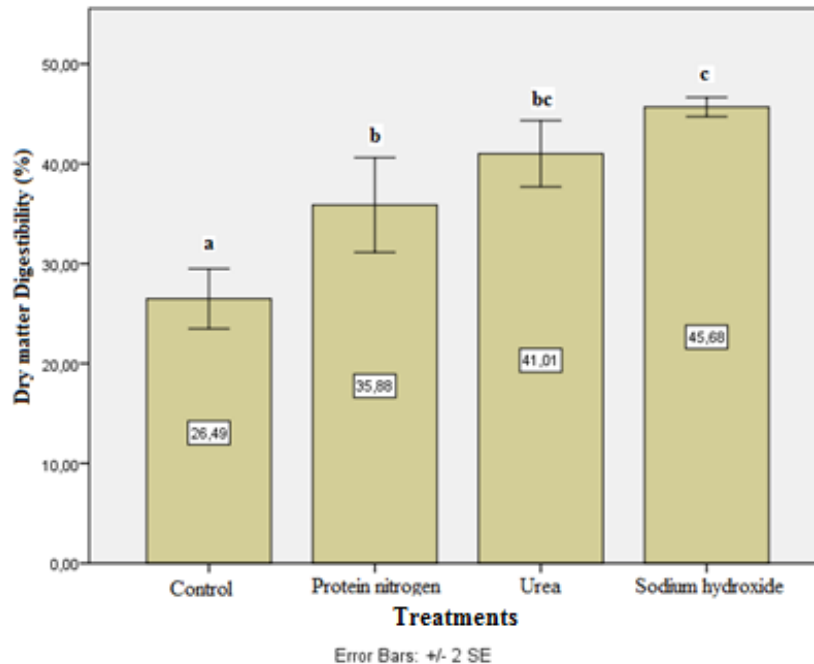
In this study, there was no significant effect (P<0.05) of protein, urea or sodium hydroxide on DM content, contrary to Granzin and Dryden (2003) and Pereira Filho et al. (2003), who observed a significant drop (P<0.05) in the DM content in a NaOH treatment (Figure 1). On the other hand, in nitrogen treatments, as expected, there were significant differences (P<0.05) in the CP content of the samples, with protein and urea treatments, but NaOH treatment had no effect in this parameter (Figure 2).

With the treatments, a decrease of ether extract was observed (Figure 6), although not significant (P<0.05). Utley et al. (1982) reported a decrease in ether extract content in a treatment of coastal bermuda grass forage with sodium hydroxide, but Moradi et al. (2015) did not observe any variation in this parameter when performing treatments with NaOH and with urea.

A significant (P<0.05) increase was found in ash with the treatment with NaOH (Figure 7), a result similar to that reported by Arisoy (1998) in an essay with straw.

The treatments performed did not have a significant effect on the cell wall of the ginger lily, as can be seen from the results (Figures 3, 4 and 5). On the other hand, all treatments had a significant effect (P<0.05) in the DM digestibility (Figure 8). Other authors observed significant increases in DM digestibility with NaOH treatments (Utley et al., 1982; Arisoy, 1998; Granzin and Dryden, 2003; Pereira Filho et al., 2003), to treatments with urea (Ballet

et al., 1997; Candido et al., 1999; Granzin and Dryden, 2003; Hossain et al., 2010; Yadete, 2014). Nianogo et al. (1999) did not find a significant effect of urea treatment on the digestibility of sorghum straws. An explanation for these results may be due to the effect that urea and NaOH have on the breakdown of lignin-carbohydrate cell wall bonds.

In the case of nitrogen treatments, there was a significant enrichment of the CP of the samples, which was reflected in an increase in DM digestibility (P<0.05). This increase in digestibility by nitrogen enrichment may not be the only explanation for the increased digestibility, since untreated forage presents a CP value of 13.20%, which is clearly higher than the 7% reported by Lazzarini et al. (2009) as the minimum limit for normal microbial activity in the rumen.

The treatments with urea and NaOH presented the best DM digestibility, showing no significant differences between treatments (P<0.05), although the NaOH treatment had higher values of DM digestibility.

In our opinion, new treatments must be carried out and this means not only the treatment with urea but also the treatment with sodium hydroxide in variable percentages, with the ultimate goal of finding the right percentage of treatment that will have a significant effect on the degradation of the cell wall of the content, thereby improving its nutritional value.

Conclusions

H. gardenarium (ginger lily) is an infesting plant with great dispersion in the Azores and a threat to the endemic flora of this Archipelago. Ginger lily is a forage commonly used by Azorean farmers in times of grass shortage, especially in summer. Ginger lily is poor forage of low nutritional value, for this reason, the pertinence of finding simple methods for its nutritional valorization was considered.

It can be concluded from this study, which aimed to evaluate the nutritional characteristics of the ginger lily (*H. gardnerianum*, Sheppard ex Ker-Gawl) with the addition of a source of nitrogen and with the treatments with urea and NaOH, that all treatments had a positive and significant effect ($P<0.05$) on DM digestibility of the samples. These results open new perspectives for other works that would determine the best concentration of urea and NaOH to be used in the treatments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Carcass yield, cuts and body components in lambs fed a pineapple by-product silage diet

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The effect of pineapple by-product silage as a substitute for elephant grass on carcass yield, commercial cuts and non-carcass components was evaluated in 25 castrated male Santa Inês feedlot finishing lambs. The lambs had an initial body weight of 18.0±3.4 kg, and they were fed four pineapple by-product silage concentrations (0, 25, 50, 75 and 100%) distributed in a completely randomized design with five treatments and five repetitions. Lambs were slaughtered at a body weight of 30 kg. The hot carcass weight and hot carcass yield were recorded and chilled at 4°C for 24 h. The quantitative parameters of the carcass, the wholesale cuts expressed in kg and percentage, and non-carcass components were determined. Carcasses were divided into seven commercial cuts. The hot and cold carcass yields significantly increased when elephant grass was replaced with pineapple by-product silage in the diets. The weight of the false rib and loin cuts increased linearly when pineapple by-product silage was added to the diets (P< 0.05). No significant differences were observed in the weights of the other cuts (P>0.05). The average weights of the non-carcass components were not affected by the addition of pineapple by-product silage, except for gastrointestinal tract (GIT) content, which decreased linearly, and omental-mesenteric, perirenal and internal fat depots, which increased linearly with the addition of pineapple by-product silage to the diets. The use of pineapple by-product silage as a substitute for elephant grass in growing lamb diets is recommended because it did not negatively affect the carcass characteristics, commercial cut yields or non-carcass components.

Key words: Elephant grass, nutrition, sheep, small ruminant, pineapple.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merril) is extensively produced in all tropical countries, particularly in Brazil, where pineapple production is expanding with 1.56 billion fruits produced in 2015 (IBGE, 2016). Although, much of pineapple production is destined for fresh consumption, processing for production of juices and other products

has achieved great importance. Azevêdo et al. (2011) estimated that 77.5% of the material produced on the farm consists of pineapple leaves, stems, crowns and discarded fruit. After pressing, the total fruit is comprised of 75 to 85 juice and 15 to 25 fruit pulp or meal. Thus, there is a large quantity of byproducts from the pineapple industry that can be used in ruminant feed.

Moreover, the seasonality in forage production has been one of the factors responsible for the reduced productivity of ruminant livestock in Brazil, which combined with frequent fluctuations in cereal prices and protein supplements used in animal feed, has raised interest in the use of alternative foods to reduce production costs (Bezerra et al., 2016; Luz et al., 2017). Among the reasons for the use of byproducts for feeding ruminants is the reduction in production costs and satisfactory food in the dry season.

In the lamb meat production system, quantitative carcass characteristics are essential to the production process as they are directly correlated with the product and help to produce better products that are more competitive in the current market (Lage et al., 2014). Additionally, carcass yield depends on relative cut weight among other factors. Thus, it is essential to understand the effects of nutrition on carcass composition. Besides that, the different cuts that comprise the lamb carcass have different economic values and commercial quality.

The study of non-carcass components can be used as an indirect nutritional assessment in lamb production. The weight of non-carcass components usually corresponds to the animal weight gain, but often to a lower extent. Thus, knowledge of the sources of variation in body organs can help develop strategies to evaluate the effects of nutrition on growth and to optimize the use of alternative foods in ruminant diets (Costa et al., 2013; Cutrim et al., 2016).

Therefore, the aim of the present study was to evaluate the effect of pineapple by-product silage as a substitute for elephant grass on carcass characteristics, commercial cuts and non-carcass components in feedlot lambs.

MATERIALS AND METHODS

The Brazilian Committee for Care and Experimentation approved all procedures involving animals in this study (number 003/2014).

Study location, experimental animals and diet

This study was conducted in the Laboratory of Animal Nutrition in the Department of Animal Science at the Federal Rural University of Amazonia in Parauapebas, State of Pará, Brazil. In total, 25 individuals of various breeds were used in the study with a predominance of Santa Inês individuals. The individuals were castrated males with a mean initial body weight of 18.0±3.4 kg and an average age of four months. Prior to the study, the animals were marked, vaccinated (against ectoparasites and endoparasites) and

given vitamins A, D and E. The animals were kept in individual dirt pens (2.0 x 2.5 m) and had free access to feed and water. The experimental period lasted 76 days with a 14-day adaptation period.

Five diets were formulated to meet maintenance requirements and a 200 g/day weight gain (NRC, 2007). The forage to concentrate ratio was 70:30 with elephant grass (*Pennisetum purpureum* Schum.) and pineapple by-product silage as forage on a dry matter basis. The pineapple by-product silage replaced the elephant grass in the following concentrations: 0, 25, 50, 75 and 100% on a dry matter basis. The concentrate consisted of maize meal, soybean meal, dried molasses, urea, limestone and a complete mineral mixture (8.0 g/kg of DM) (Table 1). Experimental diets were fed *ad libitum* to achieve a refusal rate of 10% (based on dry matter) of that offered. Feed was provided as total mixed ration daily at 8:00 a.m. and 3:00 p.m. The animals had access to water *ad libitum* throughout the experiment.

The pineapple by-product silage was composed of skin, crown and bagasse resulting from fruit processing for pulp production. The skin and crown were triturated in a sieveless (different particle size) stationary disintegrator upon silage preparation and ensiled in cement shackles. The compaction of silage was carried out manually and closed with canvas silos. The silos were opened 30 days after storage immediately before the silage was fed to the animals. The chemistry composition of pineapple by-product silage, proportion of each dietary ingredient and chemical composition of the feeds is shown in Table 1.

The elephant grass (*P. purpureum* Schum) was provenient from an area already established located next to the feedlot where the experiment was undertaken. It has a small slope and every cut was fertilized with 80 kg of nitrogen and 30 kg of potassium chloride per hectare. The elephant grass had a mean age of approximately 70 days and was cut by machine (particle size of 1.5 cm), to produce surface silos. The silo was opened for use 100 days after ensiling, which had the smell and color of a regular silage.

Slaughter and quantitative carcass's measurements

At the end of 76 days, animals were weighed (BW) and fasted for 18 h with free access to water. The animals were then weighed again for determination of body weight at slaughter (BWS) and to assess the weight loss resulting from the imposed fasting (LF), which was calculated as follows: LF (%) = (BW – BWS)/BWS x 100. At slaughter, animals were anesthetised by stunning in the atlanto-occipital region followed by bleeding by sectioning the carotid and jugular veins. Blood was collected in a polyethylene container and weighed per normative instruction No. 0.3 of 01/13/2000 (Technical Regulation of Methods for Humane Slaughtering of Livestock).

After slaughter, the gastrointestinal tract (GIT) content was removed for determination of empty body weight (EBW) to calculate hot carcass yield (HCY) with the following equation:

HCY (%) = hot carcass weight (HCW) / EBW x 100

The HCW was recorded after skinning, evisceration and removal of the head, paws and genitals. After chilling the carcasses for 24 h at 4°C, the cold carcass weight (CCW) was recorded. Chilling loss (CL) was calculated with the following equation:

CL (%) = (HCW – CCW) / HCW x 100

The cold carcass yield was calculated with the following equation:

CCY (%) = (CCW/ BWS) x 100

The carcasses were split in half, and both sides were divided into

Table 1. Chemical composition of experimental diets (dry matter basis).

Ingredient	Proportion of pineapple by-product silage[1]					
	0%	25%	50%	75%	100%	
Proportion of ingredients (%)						
Elephant grass	70.00	52.50	35.00	17.50	-	
Pineapple by-product silage	-	17.50	35.00	52.50	70.00	
Maize meal	22.86	23.13	23.39	23.66	23.90	
Soybean meal	4.70	4.41	4.12	3.83	3.56	
Molasses	0.74	0.76	0.79	0.81	0.84	
Urea	1.20	1.20	1.20	1.20	1.20	
Limestone	0.50	0.50	0.50	0.50	0.50	
Chemical composition (% DM)						PBPS
Dry matter	41.79	41.02	40.25	39.48	38.71	20.95
Organic matter	90.08	90.69	91.31	91.92	92.54	89.18
Ash	8.51	7.92	7.34	6.75	6.16	10.62
Crude protein	15.04	14.55	14.05	13.56	13.08	10.07
Ether extract	2.66	2.43	2.20	1.97	1.74	2.64
ADF	41.42	40.48	39.55	38.62	37.68	-
NDFap [2]	41.97	40.98	39.98	38.99	38.00	55.09
Hemicellulose	8.27	7.89	7.52	7.15	6.78	-
Lignin	4.77	5.37	5.97	6.57	7.17	-
TDN	80.52	82.77	82.26	85.24	86.65	-

[1] 0% Pineapple by-product silage (PBPS) –100% elephant grass as roughage source 25% PBPS – 25% pineapple by-product silage + 75% elephant grass; 50% PBPS – 50% pineapple by-product silage + 50% elephant grass; 75% PBPS – 75% pineapple by-product silage + 25% elephant grass; 100% PBPS – 100% pineapple by-product silage as roughage source. [2] ADF = acid detergent fibre, NDFap = neutral detergent fibre corrected for ash and nitrogenous compounds.

the following seven anatomical regions: neck (seven cervical vertebrae; oblique incision between the seventh cervical and first thoracic vertebrae); shoulder (scapula, humerus, ulna, radio and carpal bone); true rib (first five thoracic vertebrae in addition to the upper half of the corresponding ribs); false ribs (last eight thoracic vertebrae in addition to the upper half of the corresponding ribs); flank (straight line from the dorsal end of the abdomen to the tip of the sternum); loin (six lumbar vertebrae perpendicular to the back between the thirteenth thoracic and last lumbar vertebrae); and leg (gluteal, femoral, and leg regions; tarsal bone, tibia, femur, ischium, pubis and ileum separated by a cross-sectional cut to the back between the last two lumbar vertebrae).

All cuts were weighed separately to calculate the yield based on the cut weight/CCW relationship expressed as a percentage. In addition, subcutaneous fat thickness (SFT) and loin eye area (LEA) were measured on the left half of the carcass. The SFT was measured using a calliper on the *longissimus dorsi* section between the last dorsal and first lumbar vertebrae as previously described by Osório and Osório (2005). The LEA was drawn on the exposed *longissimus dorsi* muscle using a transparent plastic film in the same region where the SFT was measured.

The non-carcass components, including organs (spleen, heart, liver, lungs and kidneys), by-products (blood, skin, paws, head, omental-mesenteric fat, perirenal fat and internal fat) and viscera (rumen-reticulum, omasum, abomasum, small intestines, and large intestines) were weighed. Then, the viscera were emptied, washed and weighed, separately.

Statistical analysis

The experimental design was completely randomized with five treatments (pineapple by-product silage concentrations of 0, 25, 50, 75 and 100%) and five repetitions per treatment. The initial animal weights were the covariates. Data were analyzed using Statistical Analysis System (SAS) software, and the following analyses were performed: analysis of variance using PROC GLM with orthogonal polynomial contrast to analyze linear, quadratic, and cubic effects by the diets and regression analysis using PROC REG at a 5% significance level.

RESULTS AND DISCUSSION

The similar results for body weight slaughter (BWS), empty body weight (EBW), hot carcass weight (HCW), and cold carcass weight (CCW) among the treatments might have been associated with the relatively similar age of experimental lambs at slaughter. In addition, the results of carcass weight confirmed the data published by Cutrim et al. (2013) in which the final BW and daily weight gain were not affected by pineapple by-product silage feeding. Gowda et al. (2015) also verified the same daily weight gain in lambs fed a pineapple by-product

Table 2. Quantitative parameters of the carcass of feedlot sheep fed elephant grass and/or pineapple by-product silage.

Variables[1]	Proportion of pineapple by-product silage[2]					P Value[3]	
	0%	25%	50%	75%	100%	L	Q
BWS (kg)	27.71±1.61	28.85±1.68	31.31±1.82	25.85±1.68	26.31±1.71	0.845	0.792
EBW (kg)	22.15±1.41	23.71±1.51	26.04±1.65	22.90±1.63	23.14±1.64	0.167	0.887
HCW (kg)	12.05±0.68	12.88±0.73	14.39±0.81	12.16±0.77	12.73±0.80	0.115	0.907
CCW (kg)	11.70±0.65	12.54±0.70	14.08±0.79	11.85±0.74	12.48±0.78	0.086	0.924
HCY (%)[4]	43.44±0.95	44.23±0.97	46.14±1.01	46.92±1.15	48.69±1.19	0.001	0.472
CCY (%)[5]	42.16±0.87	43.02±0.89	45.13±0.93	45.71±1.06	47.70±1.10	0.001	0.416
CL (%)	54.32±9.88	54.09±9.84	55.73±10.14	53.04±10.79	55.81±11.35	0.110	0.867
LEA (cm^2)	11.51±0.74	12.16±0.78	12.65±0.81	13.29±0.95	11.14±0.80	0.709	0.128
SFT (mm)	1.12±0.33	1.80±0.52	1.90±0.55	1.00±0.32	1.38±0.45	0.869	0.444

[1]Body weight at slaughter (BWS), empty body weight (EBW), hot carcass weight (HCW), cold carcass wieght (CCW), hot carcass yield (HCY), cold carcass yield (CCY), chilling loss (CL%) loin eye area (LEA), subcutenea fat thickness (SFT). [2]In regression equations "x" means "proportion of by-product silage". [3]There was no cubic effect for the variables evaluated (P>0.05). [4]$Y_{HCY (\%)} = 43.2510 +0.0527x$ (r2=0.41). [5]$Y_{CCY (\%)} = 41.9978+0.0551x$ (r2=0.45).

silage diet, which, support desired growth rate and did not have any adverse effects on nutrient utilization and general health.

The hot carcass yield (HCY) and cold carcass yield (CCY) significantly increased when elephant grass was replaced with pineapple by-product silage in the diets (P<0.05). The equation indicated that for every 1% increase in pineapple by-product silage content, there was a 0.0527% increase in the HCY and a 0.0551% increase in the CCY. Even though the addition of pineapple by-product silage significantly affected the HCY and CCY, no significant differences were observed in the other items (P>0.05, Table 2). The linear increase in the HCY and CCY was likely caused by lower neutral detergent fibre corrected for ash and protein (NDFap) content after the addition of pineapple by-product silage to the diets (Table 1) because the HCW and CCW did not differ among the treatments (P>0.05; Table 2), and the GIT content decreased with the addition of pineapple by-product silage to the diets, as explained later (P<0.05, Table 4). The different organizational arrangements in the NDF fraction result in different degrees of substrate availability to rumen microorganisms, which may alter the digestion rate and, consequently, the rate of food passage through the rumen (Whetsell et al., 2004). Therefore, NDF constituents contribute significantly to the permanence of food along the GIT, which may affect carcass yield.

Moreover, NDF is a chemical fraction consisting mainly of cellulose, hemicellulose and lignin. These polymers have different physicochemical bond arrangements, which allow their composition to change, and can be found in different proportions in food, particularly in by-products (Costa et al., 2012).

Assessment of carcass cut yields is essential to complement calculations of animal performance during growth and to estimate the commercial value of carcasses (Oliveira et al., 2013). The LEA is used to estimate the amount of muscle in the carcass due to its high correlation with muscle content. Ttherefore, the absence of alteration in the LEA can be considered positive when working with diets containing by-product, because the cost with feeding is reduced without altering the performance of the animals (Pinto et al., 2011).

Subcutaneous fat thickness measured above the *longissimus dorsi* muscle is highly correlated with total carcass fat percentage. There is no standard for minimum fat cover thickness in lamb carcasses. Thus, there is no standard value for carcass classification regarding low or excess fat deposited in Brazil. However, according to the classification of Silva Sobrinho (2001), carcasses in the present study had low fat content (1 to 2 mm in thickness).

The weight of the false rib and loin cuts increased linearly when elephant grass was replaced with pineapple by-product silage in the diets (P<0.05, Table 3). No significant differences were observed in the weights of the other cuts (P>0.05). The results observed in the false rib cut may be associated with dietary energy density, which increased with the replacement of pineapple by-product silage (Tables 1 and 3). Because fat accumulates at a faster rate in the rib (Prado et al., 2015), the increase in dietary energy density may have further accelerated the process of fat deposition in this cut, thereby making the false rib cut to gain weight with the addition of pineapple by-product silage (P<0.05). The same results were observed for absolute loin weight (P<0.05, Table 3). The loin cut has a significant amount of fat in its composition. Therefore, the increase in dietary energy density may also explain the gain in absolute loin weight,

Table 3. Wholesale cuts expressed in kg and percentage of feedlot sheep fed pineapple by-product silage.

Variables	Proportion of pineapple by-product silage [1]					P Value[2]	
	0%	25%	50%	75%	100%	L	Q
Weight of retail cuts (kg)							
Neck	1.17±0.08	1.15±0.08	1.35±0.09	1.08±0.08	1.18±0.09	0.377	0.757
Shoulder	2.18±0.13	2.36±0.14	2.58±0.15	2.20±0.15	2.35±0.16	0.128	0.994
True rib	1.32±0.09	1.42±0.10	1.52±0.11	1.41±0.11	1.42±0.11	0.108	0.831
False rib[3]	1.57±0.10	1.76±0.11	1.99±0.13	1.68±0.12	1.83±0.13	0.016	0.918
Flank	0.71±0.07	0.62±0.06	0.87±0.09	0.60±0.07	0.73±0.08	0.295	0.538
Loin[4]	0.84±0.05	1.00±0.06	1.08±0.07	0.95±0.07	0.99±0.07	0.015	0.644
Leg	3.86±0.22	4.14±0.23	4.50±0.26	3.82±0.24	3.87±0.25	0.384	0.643
Relative share of each commercial cut (%)							
Neck	9.93±0.40	9.28±0.38	9.61±0.39	8.98±0.41	9.62±0.44	0.383	0.402
Shoulder	18.71±0.37	18.92±0.38	18.27±0.36	18.71±0.42	18.780.42	0.609	0.949
True rib	11.26±0.35	11.25±0.35	10.83±0.34	11.80±0.42	11.29±0.40	0.645	0.640
False rib[5]	13.41±0.39	13.86±0.40	14.15±0.41	14.21±0.46	14.50±0.47	0.031	0.807
Flank (%)	6.01±0.41	4.88±0.33	6.15±0.42	4.99±0.38	5.64±0.43	0.920	0.254
Loin	7.19±0.23	7.92±0.26	7.66±0.25	8.02±0.29	7.92±0.29	0.053	0.420
Leg[6]	32.93±0.47	33.07±0.47	31.98±0.45	32.37±0.51	31.30±0.50	0.006	0.234

[1]In regression equations "x" means "proportion of by-product silage". [2]There was no cubic effect for the variables evaluated (P>0.05). [3]y= 1.6735 + 0.0019x (r² = 0.03). [4]y=0.9184 + 0.0011x (r² = 0.41). [5]y= 13.5183 + 00103x (r² = 0.14). [6]y =33.1152 – 0.0159x (r² = 0.19).

which may cause a more rapid fat deposition (Carvalho et al., 2014).

With respect to cuts expressed as a percentage, the leg cut decreased linearly with the addition of pineapple by-product silage to the experimental diets (P<0.05), which may be associated with possible differences in tissue growth, especially in muscle and fat tissues. Conversely, the false rib cut showed the same pattern observed for the absolute cut weight by increasing linearly with the addition of pineapple by-product silage (P<0.05). No significant differences were observed in the other cuts when expressed as percentages (P>0.05, Table 3).

Higher pineapple by-product silage contents resulted in greater dietary energy density (Table 1), and caused by TDN increase in diets. It is possible that fat tissue increased with the addition of pineapple by-product silage even though leg is known for its high muscle mass and low fat content (Stanisz et al., 2015). The weight gain in other carcass parts due to increased fat deposition resulted in a lower relative share of leg with the addition of pineapple by-product silage.

Therefore, the addition of pineapple by-product silage may not have changed the amount of muscle tissue deposited but instead caused changes in fat deposition, which is supported by the evidence mentioned earlier. In addition, the increase in omental-mesenteric, internal, and perirenal fat depots with the pineapple by-product silage inclusion (Table 4) supports the hypothesis proposed.

The average weights of the non-carcass components were not affected (P>0.05) (Table 4) by the addition of pineapple by-product silage, except for GIT content, which decreased linearly with pineapple by-product silage addition to the diets (P<0.05), and omental-mesenteric, perirenal, and internal fat depots (Table 4), which increased linearly with the addition of pineapple by-product silage to the experimental diets (P<0.05). The changes observed in the GIT content may be associated with the pineapple by-product silage fibre fraction. Because pineapple by-product silage is a source of fibre with a lower degree of lignification than forage, pineapple by-product silage has a smaller particle size than the average forage particle size and higher specific gravity, and the combination of these factors directly affects the retention time in the rumen. Regarding the non-carcass components that were affected by the diet (Table 4), there was greater fat deposition in the viscera of experimental animals.

The greater proportions of the components in the carcass result in higher maintenance requirements due to the higher metabolic activity of adipose tissue (Rufino et al., 2013). Moreover, food energy is wasted because omental-mesenteric, internal, and perirenal fat depots are not used for human consumption (Costa et al., 2011). The influence of diet on these results indicated that

Table 4. Non-carcass components of feedlot sheep fed pineapple by-product silage.

Variables (kg)	Proportion of pineapple by-product silage [1]					P Value[2]	
	0%	25%	50%	75%	100%	L	Q
Heart	0.13±0.01	0.12±0.01	0.13±0.01	0.12±0.01	1.18±0.08	0.811	0.561
Liver	0.39±0.03	0.39±0.03	0.44±0.04	0.42±0.04	0.41±0.04	0.185	0.864
Lungs	0.27±0.02	0.27±0.02	0.32±0.03	0.45±0.04	0.31±0.03	0.406	0.963
Kidneys	0.09±0.01	0.07±0.00	0.08±0.01	0.07±0.01	0.07±0.01	0.536	0.239
Blood	1.17±0.10	1.21±0.10	1.31±0.11	1.12±0.10	1.09±0.10	0.963	0.622
Skin	1.77±0.14	1.94±0.15	1.81±0.14	1.94±0.17	1.79±0.15	0.457	0.915
Paws	0.82±0.08	0.73±0.07	0.88±0.09	0.77±0.09	0.76±0.08	0.913	0.852
Head	1.56±0.07	1.63±0.08	1.61±0.08	1.51±0.08	1.52±0.08	0.986	0.905
GIT[3]	1.83±0.11	1.75±0.11	1.86±0.11	1.62±0.11	1.59±0.11	0.252	0.873
Content GIT[4]	5.56±0.40	5.14±0.37	5.27±0.38	2.95±0.24	3.18±0.26	0.001	0.507
Mes+omen fat[5]	0.55±0.07	0.92±0.12	1.00±0.14	0.89±0.14	1.16±0.18	0.001	0.538
Perirenal fat[6]	0.23±0.03	0.34±0.05	0.49±0.07	0.45±0.08	0.50±0.08	0.009	0.920
Internal fat[7]	0.08±0.02	0.16±0.05	0.23±0.07	0.20±0.07	0.21±0.07	0.035	0.629
Rumen+reticulum	0.69±0.07	0.73±0.07	0.81±0.08	0.55±0.06	0.61±0.07	0.444	0.529
Omasum	0.08±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.292	0.210
Abomasum	0.14±0.01	0.15±0.01	0.16±0.01	0.14±0.01	0.14±0.01	0.742	0.754
Small intestine	0.55±0.08	0.43±0.06	0.48±0.06	0.53±0.08	0.47±0.07	0.650	0.634
Large intestine	1.34±0.17	1.19±0.15	1.14±0.14	1.00±0.14	0.88±0.12	0.416	0.777

[1]In regression equations "x" means "proportion of by-product silage". [2]There was no cubic effect for the variables evaluated (P>0.05). [3]Gastrointestinal tract (GIT), [4]$Y_{Content\ GIT}$ = 5.8129 -0.0273x (r^2 = 0.57). [5]Mes+omen = mesentery + omentum. $Y_{Mes+omen}$ = 0.6630 +0.0049x (r^2 = 0.14). [6]Y =0.2679 +0.0027x (r^2 = 0.18). [7]Y = 0.1132 + 0.0013x (r^2 = 0.11).

energy was wasted upon deposition of omental-mesenteric, perirenal, and internal fat in the carcass. Thus, the addition of pineapple by-product silage likely resulted in premature carcass termination.

Therefore, use of pineapple by-product silage can be recommended for meat production in feedlot lambs as experimental animals prematurely reached the time of slaughter. Considering the market preference for young animals with carcass weights ranging from 12 to 14 kg, it is possible to produce animals for commercial slaughter by replacing elephant grass (*P. purpureum* Schum.) with pineapple by-product silage, which has low cost of production.

Conclusion

The pineapple byproduct silage alters some carcass quantitative characteristics, preserving desirable parameters for meat production. Therefore, pineapple byproduct silage can be used as a replacement for elephant grass silage at amounts of 100% without causing negative effects on carcass characteristics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Diversity of predatory arthropod communities in tobacco-garlic eco-system

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Predatory arthropods, especially spiders, play a vital role in the control of insect pests in agro-ecosystems. Accordingly, two year field study was conducted at the Longyan Substation of Fujian Institute of Tobacco Agricultural Sciences in China to determine the effects of garlic and tobacco intercropping system on spiders and predatory arthropods. A total of 545 and 860 (in 2011 and 2012, respectively) individuals of predatory arthropods representing 14 families and 16 species were collected in the fields. The diversity indices of the predatory arthropod communities were obviously higher in tobacco-garlic intercropping system than in tobacco fields. The species richness and species abundance of the predatory arthropods collected in tobacco-garlic fields were significantly higher than that of the predatory arthropods collected in tobacco fields in both study years. Moreover, the values of these indices were obviously higher for spider abundance in tobacco-garlic fields than in tobacco fields during the middle stages of tobacco growth. Intercropping garlic in tobacco fields can increase the abundance of spiders and predatory arthropods, and this approach may be useful to control pests in tobacco fields.

Key words: Tobacco-garlic, intercropping system, spider, predator arthropod, diversity.

INTRODUCTION

The presence, abundance and diversity of the predator community have significant impacts on ecosystem functions (Snyder et al., 2006; Schmitz, 2007; Bruno and Cardinale, 2008; Letourneau et al., 2009). The natural enemy hypothesis predicts that predators are more abundant and more diverse in species-rich plant communities because these communities offer a greater variety of microhabitats as well as a broader spectrum and a more stable temporal availability of prey than low diverse communities (Strong and Southwood, 1984; Srivastava and Lawton, 1998; Jactel and Duelli, 2005). With greater diversity of predators, lower trophic levels such as herbivoures can thus more effectively be regulated allowing producers to also thrive. Many studies

have compared predator diversity and abundance relative to plant diversity in monocultures to mixtures of a few plant species and most found that the polyculture system effectively influenced the pests in those systems (Pierre et al., 2011; Lin et al., 2011; Habib, 2012).

There are examples of predators, and among them predatory arthropods, that can exert strong top-down control on the food web (Bell, 2007; Schuldt et al., 2011). In agricultural ecosystems, they are a major component of the natural enemy community, predating mainly on crop pests (Pang and You, 1996; You et al., 2004; Dwyer et al., 2011). Within the predatory arthropod group, spiders are important in agroecosystems and may be a good example for the natural enemy hypothesis. For instance, Cai and You (2007) found that, throughout the growing season, the abundance of spiders from the family Theridiidae, an important group of predators, was greater in garlic (*Allium sativum*)-Chinese cabbage (*Brassica chinensis*) fields than in Chinese cabbage monocultures (Cai and You, 2007). Wu et al. (2011a) have reported that spider diversity and community stability were higher in rice fields adjacent to flue-cured tobacco fields than in the rice fields or flue-cured tobacco fields alone (Wu et al., 2011a).

These studies tend to support the natural enemy hypothesis. However, other studies have not supported it. Lin et al. (2011) demonstrated that polycultures did not effectively optimise the structure of the spider guild nor increase its diversity in rice fields. Similarly, Chen et al. (2011) found that the use of a cover crop in tea plantations did not change spider communities. In a highly diverse forest ecosystem in subtropical China, Schuldt et al. (2011) reported that spider activities, abundance and species richness in fact decreased with increasing tree species richness.

This high variability in responses of spiders to changes in diversity underlines the need to further test this hypothesis. The main reason for the use of spiders is that they are sensitive to disturbances particularly to pesticide applications. Because of this, they have been considered as good indicators for monitoring ecological change and thus testing the natural enemy hypothesis (Pétillon et al., 2008). They have also been proposed as an ideal group for predicting the extinction debts of other taxa due to habitat destruction or disturbance.
In this study, we studied the effects of tobacco-garlic intercropping systems versus tobacco monocultures on spider and predatory arthropod communities and through this, tested the natural enemy hypothesis for predators or spiders.

MATERIALS AND METHODS

Study sites

Field experiments were conducted at the Longyan Substation of Fujian Institute of Tobacco Agricultural Sciences, Fujian Province, China (25°08'N, 116°59'E, 347.30 m altitude) from March to June in both 2011 and 2012. The climate of this area   was   influenced   by   subtropical monsoon, with an average annual temperature of 18-20°C and annual precipitation of 1600 to 1700 mm. The soil type at the study site was a red soil, and a pH of 5.2.

Experimental design

Field experiments were conducted using flue-cured tobacco (var. K326). White garlic, *Allium sativum* L., (cultivar) was planted one month before transplanting flue-cured tobacco plants. During the experiment, randomized block design was followed where three blocks and within each, four treatments plots of 12 x 11.2 m were selected as per Lai et al. (2011). Each plot was separated from each other by a flue-cured tobacco ridge. The four treatments were as follows: A) tobacco plot with two rows of garlic planted on one edge of a ridge, B) tobacco plot with two rows of garlic planted on each edge of a ridge, C) garlic planted between two individual K326 plants in a ridge, and D) monoculture of K326 tobacco. Tobacco was planted at a density of 1.80 individuals m^{-2} and garlic at a density of 5.85 individuals m^{-2}. Garlic seedlings were transplanted by hand in the tobacco fields on January 25, 2011 and January 27, 2012. After a month (on February 25, 2011 and February 27, 2012), K326 seedlings were transplanted by hand according to the density and treatments described above. Forty-five days (April 11, 2011 and April 13, 2012) after transplanting the tobacco plants, all the garlic plants were harvested by hand to avoid affecting tobacco growth. No pesticides was used during the experiment. The plants were fertilised one day before garlic transplantation and then 25 days after planting K326, using organic and chemical fertilisers for a seasonal total content for N, P, and K of 120, 20.57 and 124.47 kg ha^{-1}, respectively. The plants were furrow-irrigated five to six times during the seasons.

Sampling techniques and species identification

Thirty flue-cured tobacco plants were randomly selected from each plot with a jump spreadsheet parallel sampling method (Wu, 2000; Lai et al., 2011). The sampling started 7 days after transplanting the tobacco plants and continued until the day when all the tobacco leaves were harvested. Spiders and other arthropods from a flue-cured tobacco plant and from the 0.50-m^2 area under the plant were captured with a suction sampler (Liu et al., 1999). Sampling was performed at intervals of 7-15 days. All arthropods collected with the suction sampler were transferred and frozen in a plastic bag before identification under a microscope at the Institute of Applied Ecology, Fujian Agriculture and Forestry University, China.

Data analysis

To perform comparisons and analyses of the predatory arthropods, the diversity index was calculated:

$$H = -\sum_{i=1}^{s} P_i \ln P_i \tag{1}$$

Where, $P_i = n_i/N$, P_i = the proportion of the number of individuals of the ith species to the total number of individuals, n_i = the number of individuals of the ith species, N = the total number of all individuals and s = the number of species. To determine the treatment effects, the dominance index was also calculated:

$$D = {N_{max}}\big/{N} \tag{2}$$

Where, N_{max} = the number of individuals in the most abundant species and N = the total number of all individuals. And the predatory arthropods were classified by the degree of dominance of the species (Liu et al., 2000), where species with $D \geq 0.1$ are considered dominant species, species with $0.05 \leq D < 0.1$ are abundant, species with $0.01 \leq D < 0.05$ are frequent, species with $0.001 \leq D < 0.01$ are occasional and species with $D < 0.001$ are rare.

The predatory arthropod or spider datasets in 2011 and 2012 were analysed separately. Statistical analyses were performed with SPSS 15.0 for Windows (Liu et al., 2008). A univariate analysis of variance was used to analyse predatory arthropod or spider community data. Prior to the univariate analysis, the data were log-transformed [$\log_{10} (x+1)$] or log-transformed ($\log_{10} x$) to meet the assumptions of normality and homogeneity of variance. If the F-statistics indicated significant effects, the means were separated with a Fisher's protected least significant difference (LSD) test with a 5% significance level.

RESULTS

A total of 545 and 860 individuals was recorded during 2011 and 2012, respectively and which represent 14 families of five orders and 16 species collected from the tobacco fields. Micryphantidae and Syrphidae families have the highest number of species (two in the first or second year). One species was collected for each of the other families in each study year. The family Theridiidae has the greatest numbers of individuals (108) in 2011, followed by Micryphantidae (103) and Staphylinidae (67). The Micryphantidae, Chrysopidae and Theridiidae has greater number of individuals, that is,149, 126 and 120, respectively in 2012 (Figure 1).

The diversity indices for the predatory arthropods did not differ significantly between the garlic-tobacco and the tobacco fields during 2011 ($F_{3, 80} = 0.675$, $P = 0.570$) or 2012 ($F_{3, 92} = 1.976$, $P = 0.123$). However, the diversity indices for the predatory arthropods were obviously higher in the garlic-tobacco fields than in the tobacco fields in the two years (Figure 2).

The species richness and species abundance of the predatory arthropods differed significantly between the experimental treatments during 2011 ($F_{3, 80} = 6.560$, $P = 0.001$ and $F_{3, 80} = 3.363$, $P = 0.023$, respectively) and 2012 ($F_{3, 92} = 7.620$, $P<0.001$ and $F_{3, 92} = 5.221$, $P = 0.002$, respectively) (Figures 3 and 4). The species richness of the predatory arthropods in the garlic-tobacco fields was significantly higher than in the tobacco fields (Figure 3). Moreover, the species abundance of the predatory arthropods was also significantly higher in the garlic-tobacco fields (Figure 4).

A total of 16 species of predatory arthropods were found during 2011 and 2012, including dominant species, abundant species, frequent species, occasional species and rare species. The occasional species included *Theridion octomacularum* (Boes et Str.), *Erigonidium graminicola* (Sundvall), *Coccinella septempunctata* (Wesmael), *Pardosa tinsigmita* (Boes et Str.), *Propylaea japonica* (Thungberg), *Epistrophe balteata* (De Geer),

Paederus fuscipes (Curtis), *Misumenops tricuspidatus* (Fabricius), *Oedothorax insecticeps* (Boes et Str.) and *Sycanus croceovittatus* (Dohrn). The rare species included *Syrphus corollae* (Fab.), *Coccinella septempunctata* (Linnaeus), *Aphidoletes aphidimyza*, *Calosoma chinense* (Kirby) and *Tetragnatha maxillosa* (Thorell). In addition, *Nabis sinoferus* (Hsiao) was included in the rare species and occasional species in 2011 and 2012, respectively. However, none of the species cited above as rare or occasional was found to be dominant, abundant or frequent in this study.

Consistent significant results were not found for spider abundance in the garlic-tobacco systems during 2011 and 2012. The spider abundances did not differ significantly in 2011 ($F_{3, 80} = 2.400$, $P = 0.074$) but differed significantly in 2012 ($F_{3, 92} = 3.016$, $P = 0.022$). In both years, the spider abundance was similar in the garlic-tobacco fields and in the tobacco fields at the first and last stages of tobacco growth. However, the spider abundance was obviously overall higher in the garlic-tobacco fields, especially in the middle stages of tobacco growth (Figure 5).

DISCUSSION

Predatory arthropods or spiders are common and abundant in agroecosystems (Pang and You, 1996; You et al., 2004; Schmitz, 2007). Intercropping methods have been used to manipulate pests in many crop fields (Shen et al., 2007; Sohail et al., 2008; Lai et al., 2011; Lai et al., 2017). The successful use of spiders or predators for pest management provides support for the natural enemy hypothesis, which suggests that natural enemies are more abundant and diverse in diversified habitats than in monocultures. Moreover, many previous studies have shown that predatory arthropods or spiders in tobacco fields to be a key natural enemy of tobacco pests (Tao et al., 1996; Wu et al., 2005; Lai et al., 2012).

In the present study, it was found that the richness and abundance of predators and the abundance of spiders were significantly higher in the garlic-tobacco fields than in the tobacco fields (Figures 3, 4 and 5). This result is consistent with the findings of Wu et al. (2011a, b). These authors demonstrated that spider abundances and predator arthropods were higher and the abundance of *Sogatella furcifera* (Horvath) was lower in paddy fields adjacent to flue-cured tobacco fields than in paddy fields (Wu et al., 2011a, b). The natural enemy hypothesis is also clearly supported by the study.

The reason for the results cited may be that plant diversity and the stability of the arthropod communities were enhanced by intercropping garlic in tobacco fields. Intercropping garlic in tobacco fields may affect the environment. Pétillon et al. (2008) obtained results similar to these. Their study found that spiders were a suitable indicator taxon for reflecting ecological change because they were sensitive to soil moisture (Pétillon et al., 2008).
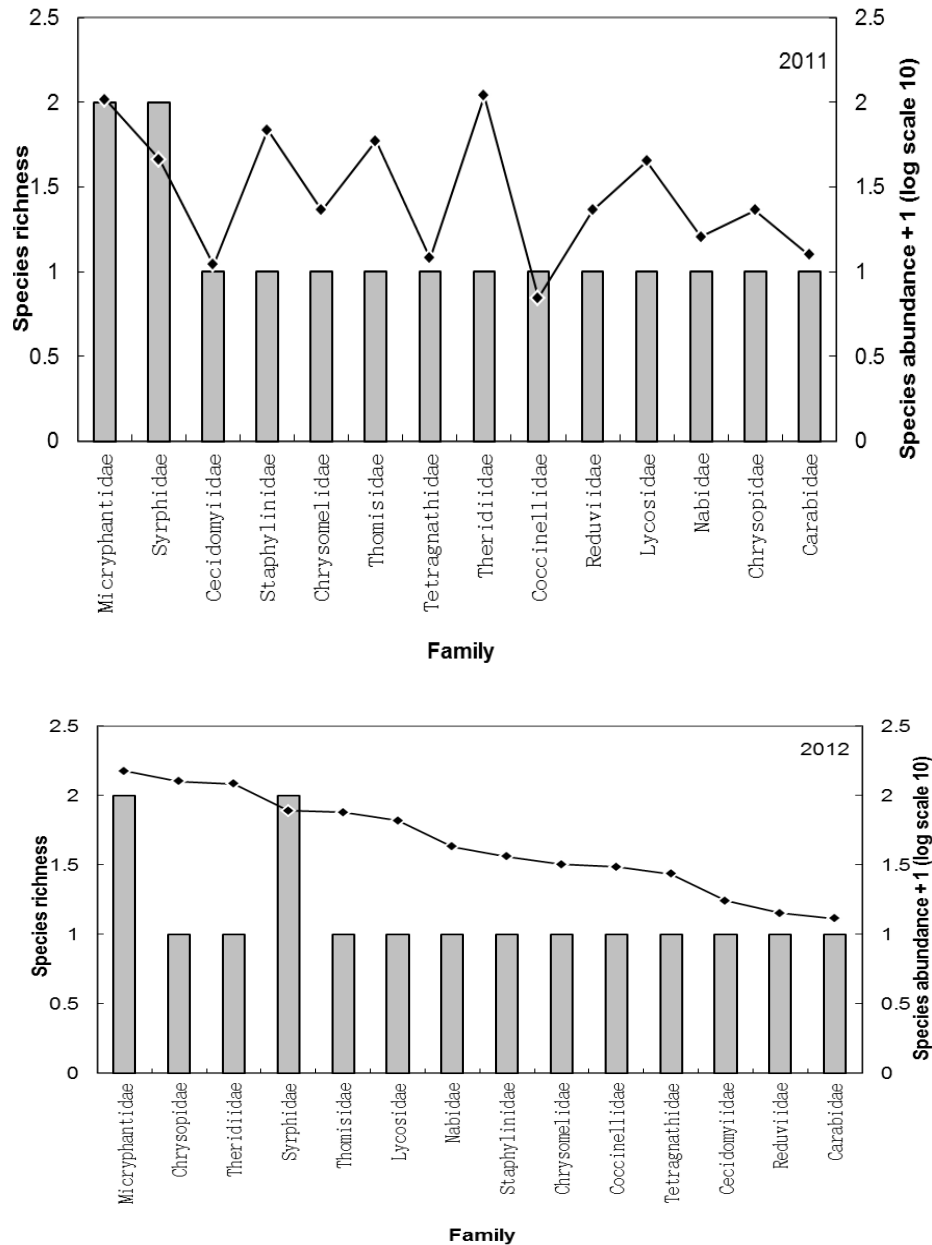
Figure 1. Species richness (bar graphs) and species abundance (line plots) distributes within families for predatory arthropod in garlic-tobacco systems in Longyan during 2011 and 2012.

In addition, Andow (1991) and Cai and Youl (2007) have found that the richness and abundance of natural enemies were higher in intercropping-multiculture fields than in monoculture fields (Andow, 1991; Cai and You, 2007). These findings imply that a higher diversity in tobacco fields may result in higher abundances of spiders or predators. Such results suggest that it may be difficult to control crop pests or to maintain the stability of arthropod community in a monoculture agro-ecosystem.

In this study, it was also found that the abundance of spiders decreased gradually during the middle stage of

tobacco growth in garlic-tobacco fields (Figure 5). Two reasons may help to explain this finding. First, the diversity of the garlic-tobacco ecosystems decreased because all the garlic plants were removed forty-five days after transplanting the tobacco plants (see "experimental design"). Second, the changes in the populations of natural enemies may follow the changes in the populations of the pests (fewer enemies result from fewer pests). The latter reason is consistent with the findings of Shi (2000) and Cai et al. (2007). Shi (2000) have demonstrated that changes in *Myzus persicae* (Sulzer)
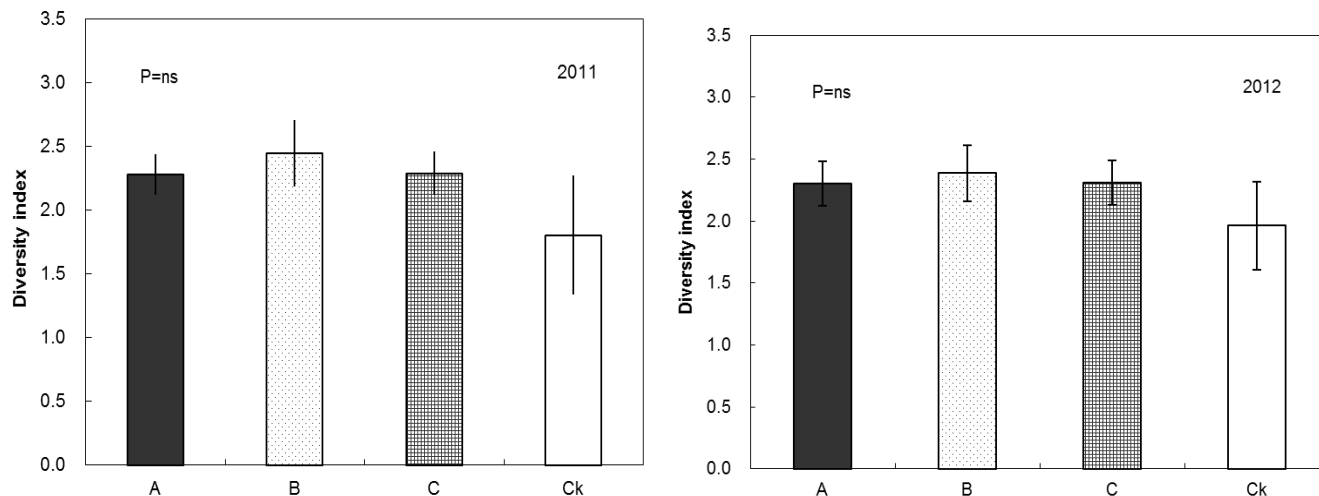
Figure 2. Diversity indexes in predatory arthropod communities in garlic-tobacco systems in Longyan during 2011 and 2012 (Mean±S.E.) Note: Treatment A consisted of two rows of garlic planted on one edge of a ridge; Treatment B consisted of two rows of garlic planted on each edge of a ridge; Treatment C consisted of garlic planted between two individual K326 tobacco plants; and the control treatment (Ck) consisted of all ridges planted with K326 only. ns is not significantly different at 5% level of significance (determined by a Fisher's protected least significant different (LSD) test for means separation) .
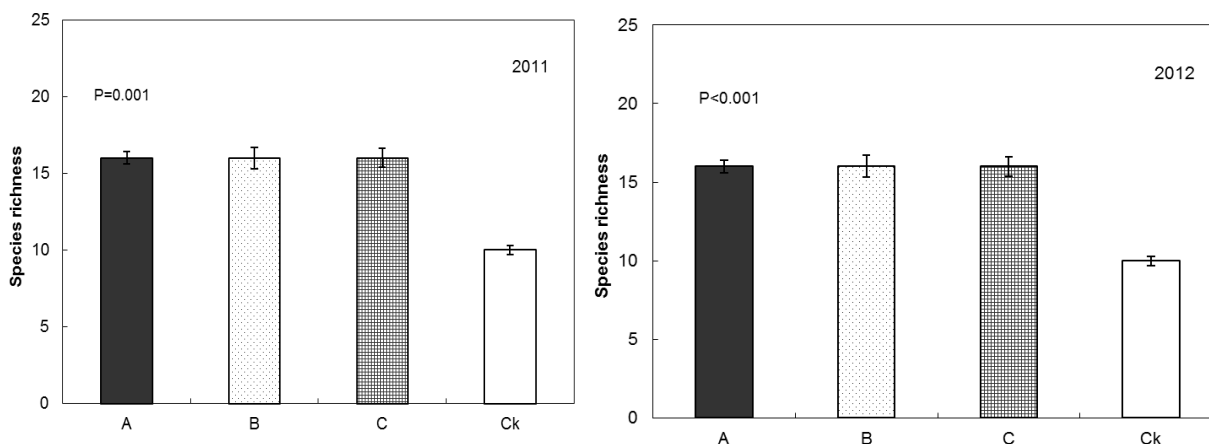
Figure 3. Species richness in predatory arthropod communities in garlic-tobacco systems in Longyan during 2011 and 2012 (Mean±S.E.). Note: Treatment A consisted of two rows of garlic planted on one edge of a ridge; Treatment B consisted of two rows of garlic planted on each edge of a ridge; Treatment C consisted of garlic planted between two individual K326 tobacco plants; and the control treatment (Ck) consisted of all ridges planted with K326 only. P value is significantly different at 5% level of significance (determined by a Fisher's protected least significant different (LSD) test for means separation).

populations in tobacco fields were followed by changes in *Aphidius gifuensis* Ashmead populations (Shi, 2000). Similarly, Cai and You (2007) found that the dynamics of the parasitoid *Diaeretiella rapae* M'Intosh population paralleled the dynamics of aphids in garlic-cabbage fields.

The Shannon-Weaner index is widely used to estimate arthropod diversity (Shannon and Weaner, 1949; Renio et al., 2008; Wu et al., 2011b). The results of this study indicated that the diversity indices for predatory arthropods

did not differ significantly between intercropping tobacco fields and tobacco fields (Figure 2). This result is consistent with the findings of Lin et al. (2011), who have demonstrated the same pattern in paddy fields (Lin et al., 2011). However, the present study found that the abundances of predators or spiders were significantly higher in garlic-tobacco fields (Figures 4 and 5). These results are not consistent with the findings of Chen et al. (2011) and Lin et al. (2011). Their results showed that
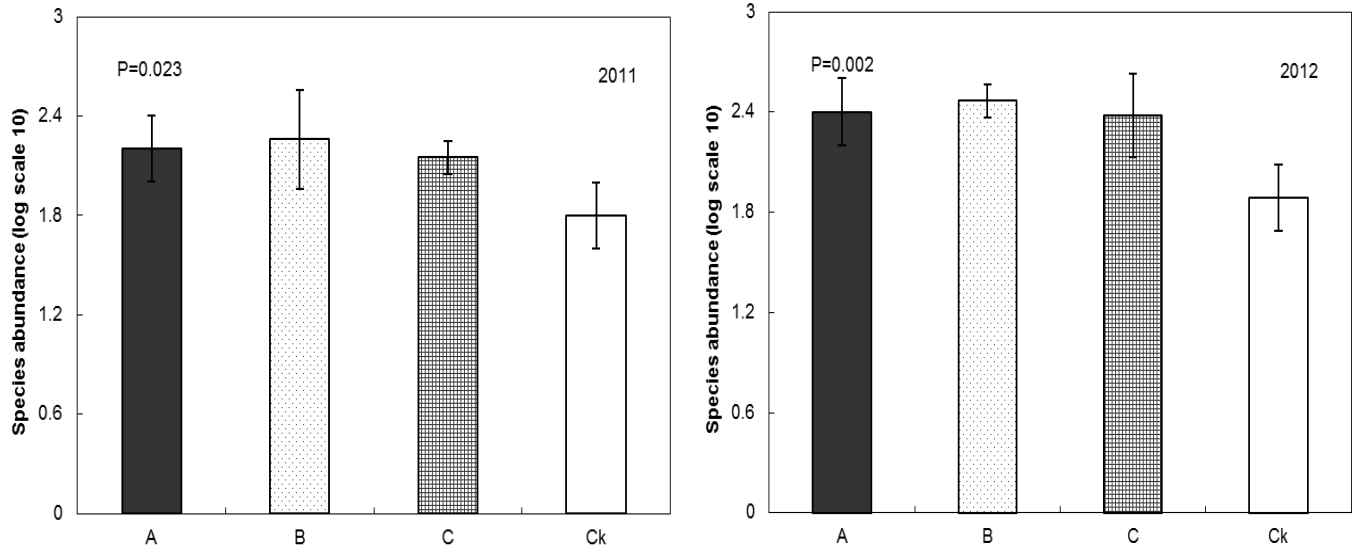
Figure 4. Species abundance in predatory arthropod communities in garlic-tobacco systems in Longyan during 2011 and 2012 (Mean±S.E.). Note: Treatment A consisted of two rows of garlic planted on one edge of a ridge; Treatment B consisted of two rows of garlic planted on each edge of a ridge; Treatment C consisted of garlic planted between two individual K326 tobacco plants; and the control treatment (Ck) consisted of all ridges planted with K326 only. P value is significantly different at 5% level of significance (determined by a Fisher's protected least significant different (LSD) test for means separation).
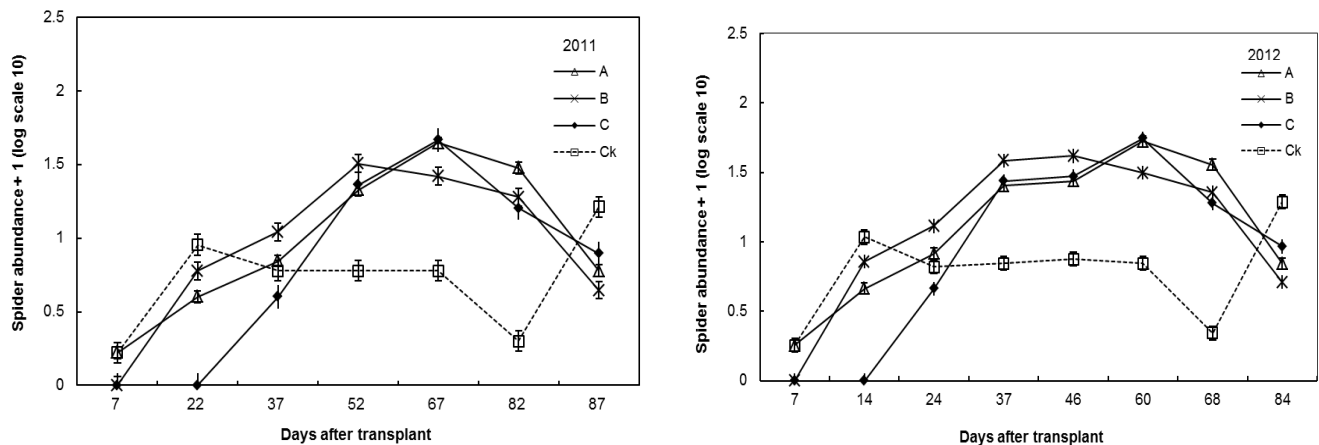

Figure 5. Spider abundance in garlic-tobacco systems in Longyan during 2011 and 2012 (Mean±S.E.). Note: Treatment A consisted of two rows of garlic planted on one edge of a ridge; Treatment B consisted of two rows of garlic planted on each edge of a ridge; Treatment C consisted of garlic planted between two individual K326 tobacco plants; and the control treatment (Ck) consisted of all ridges planted with K326 only.

spider abundances and richess were not significantly affected by a cover crop in tea plantations or by polycultural manipulation in paddy fields (Chen et al., 2011; Lin et al., 2011). The smaller number of individuals in arthropod communities may make the abundance or diversity of predators or spiders more consistent in tobacco fields than in other crop fields. Moreover, further studies are required to quantify the differences among the richness, abundance and diversity of predators or spiders

in tobacco fields, and attention should be focussed on long-term studies that use larger experimental sites.

Conclusion

The results of this study show that the species richness and abundance of predator arthropods and spider abundance can be significantly enhanced by intercropping

garlic in tobacco fields. The natural enemy hypothesis is clearly supported by this work. The higher abundance of predators or spiders in garlic-tobacco fields may be helpful for controlling pests.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Levels of the amino-acid lysine in rations for broilers

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Synthetic amino acid supplementation has provided facilities in the adjustment of feed formula, making it possible to obtain the required levels of essential amino acids. Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. In order to characterize the requirements of lysine, 240 male broilers of the lineage Avian Farms were kept in an environment with average temperature of 25.6°C. The 22 to 42 days old broilers presented initial average weight of 541 ± 3.6 g. The basal ration contained 19.57% of crude protein (CP), 3100 kcal of metabolizable energy (ME)/kg and 0.88% of total lysine, which was supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% de L-lysine hydrochloric acid (HCL), resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine. The experimental design was completely randomized with five treatments of total lysine, six repetitions and eight broilers per repetition. During experiments, the temperature was kept at 25.6 ± 0.24°C, relative moisture ate 68.4 ± 6.30%, black globe temperature at 25.7 ± 0.25°C and the wet-bulb globe temperature (WBGT) at 74 ± 0.6°C. Treatments influenced the absolute weight of carcass, breast with bone, leg, thigh, abdominal fat and relative weight of breast with bone. Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine.

Key words: Chickens for meat production, growth phase, lysine, thermal environment.

INTRODUCTION

Birds, as well as mammals, are homeothermic animals, which indicated that means that even there are fluctuations at the environmental temperature, they can keep the body temperature constant (Borges et al., 2002). Any environmental change out of the thermal comfort of these animals requires behavioral, physical or physiological adjustments as attempts to adapt to the new condition. Oba et al. (2007) reported that the range of thermal neutrality for broilers is between 24 and 28°C. Considering these adjustments, the ration intake stands out, which are reduced as temperature increases, thus causing the decrease of growth rate and worsening of food conversion (Baziz et al., 1996). However, the effect of temperature over metabolism is more complex than how it is frequently reported. According to Mendes et al. (1997), the combination of high temperature and

environment with high levels of protein reduces the growth rate and production of breast meat from broilers of the fast growth commercial lineage.

High temperatures also reduce the weight of organs in broilers, in order to reduce the metabolic rate of animals with consequent reduction of heat production. Studies conducted by Zaboli et al. (2016) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance. Therefore, considering the different responses associated with the effect of high temperatures, it is evident that the broiler requirements vary not only due to the lineage but also in function of the thermal environment to which such animals are submitted in the different periods.

In some tropical areas of Brazil State, such as the North Region, a temperature range of 35 to 45∘C from August to May is very common, and the reduction of growth performance in poultry is the most important issue. The year-on-year amplification in growth rate of modern poultry due to constant genetic improvement, global heating, and the expanding of the poultry industry in hot climates requires correct ways to lighten the consequences of heat stress. Therefore, diverse methods of growth performance in poultry have been corroborated in the world, such as climate-controlled housing, low providing density, nutritional management, lowering marketing weight, among others. Though, in greatest situations many of these practices are overpriced with low efficiency (Zaboli et al. 2016).

Protein remains the most expensive dietary nutrient, though the use of crystalline amino acids offers multiple advantages in that they provide reductions in both dietary crude protein concentrations and the excretion of dietary nitrogen into the environment (Franco et al., 2017). Belloir et al., (2017) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance.

Lysine is considered the second most limiting amino acid in broiler diets. It is used as the reference amino acid in poultry and swine nutrition because it is mainly utilized for protein synthesis and accounts for 7.5% of carcass protein (Viola et al., 2009). The present work was conducted in order to characterize the lysine requirements for male broilers from 22 to 42 days old kept under average temperature of 25.6°C.

MATERIALS AND METHODS

Animals

The experiment was conducted in the climatic chambers of the Laboratory of Animal Bioclimatology of the Zootechny Department, Federal University of Viçosa (UFV), Brazil. Overall, 240 male broilers of the lineage Avian farms with initial average weight of 541 ± 3.6 g, vaccinated against the diseases Marek and Avian pox. Broilers remained in the experiment from the 22nd to 42th day at high temperature (25.6°C). The experimental design was completely randomized with five treatments (levels of lysine), six repetitions and eight broilers per repetition.

Experimental diets (Table 1), isoproteic and isoenergetic, based on maize, soybean meal and corn gluten were formulated to attend the nutritional requirements of broilers regarding protein, energy, calcium, phosphorous and amino acids, except for lysine. Rations were supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% of L-lysine HCl 78.4%, resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine.

Experimental design

During the initial period (1 to 21 days old), broilers were created in a conventional shed under traditional management and feeding. When 22 days were completed, broilers were weighed and transferred to climatic chambers and the experimental period started, where they remained until 42 days old. Broilers were placed in metal batteries composed by 12 compartments with area of 0.72 m^2 per compartment, all of them trough-type, with each compartment representing an experimental unit.

The temperature and moisture monitoring of each room was made by thermometers of maximum and minimum, dry bulb, wet bulb and black globe placed at an intermediate height in relation to the battery central compartment. Temperatures were daily recorded at two moments (8 and 18 h) during all the experiment.

The thermal environment was expressed in terms of the Wet-bulb Globe Temperature (WBGT) proposed by Buffington et al. (1981) and calculated by the following equation: WBGT = Bgt + 0.36 Dpt - 330.08 in which Bgt is the black globe temperature in °K and Dpt the dew point temperature in °K. Rations and water were provided at will and water was changed twice a day to avoid heating.

A continuous light program was adopted along all the experiment 24 h of artificial light by means of two fluorescent lamps of 25 Watts per room. The studied variables were: ration intake, weight gain, food conversion, total lysine intake, carcass yield, protein deposition and absolute and relative weights of prime cuts (breast, thigh and drumstick).

The calculation of ration intake during the experimental period was obtained by the difference between the amount of ration provided and lost and wastes of rations, which were weighed in the beginning and end of experiment. The weight gain of broilers was obtained by the difference between the weight in the end and beginning of experiment. The food conversion was calculated for the period from 22 to 42 days old based on data of ration intake and weight gain.

Analyses of crude protein and body composition

In the end of experiment, broilers were weighed after 12 h of fasting and posteriorly four of each repetition were chosen to be slaughtered considering the average weight of the experimental unit (± 5%). After broilers were bled and plucked, eviscerated carcasses were weighed. Posteriorly the abdominal fat was removed and weighed. The two entire carcasses (including feet and head) of each repetition were ground during 15 min, one by one, in commercial cutter of 30 Horse Power (HP) and 1,775 rpm, and after homogenization one sample was collected. Due to the high fat content of carcasses, samples were pre-dried in forced ventilation stove at ± 60°C for 72 h and pre-degreased by hot method in extractor *Soxhlet* for 4 h. After this step, samples were ground and placed in glasses for posterior evaluations. Analyses of crude protein were made in the Laboratory of Animal

Table 1. Calculated composition of the experimental diets (%).

Ingredients	Total lysine level (%)				
	0.88	0.94	1.00	1.06	1.12
Corn (7.98% PB)	65.500	65.500	65.500	65.500	65.500
Soybean meal (45.61% PB)	21.870	21.870	21.870	21.870	21.870
Corn gluten meal (60.38% PB)	7.122	7.122	7.122	7.122	7.122
Dicalcium phosphate	1.524	1.524	1.524	1.524	1.524
Limestone	1.228	1.228	1.228	1.228	1.228
Soybean oil	1.200	1.200	1.200	1.200	1.200
Salt	0.415	0.415	0.415	0.415	0.415
Mineral mix[1]	0.050	0.050	0.050	0.050	0.050
Vitamin mix[2]	0.100	0.100	0.100	0.100	0.100
Butylated Toluene Hydroxide	0.010	0.010	0.010	0.010	0.010
Cocxistac[*]	0.050	0.050	0.050	0.050	0.050
Choline chloride	0.125	0.125	0.125	0.125	0.125
Virginiamicin	0.055	0.055	0.055	0.055	0.055
Caulin	0.596	0.520	0.443	0.366	0.290
L-Lysine HCL (78.4%)	0.000	0.076	0.153	0.230	0.306
DL-Methionine (99%)	0.153	0.153	0.153	0.153	0.153
Tryptophan (99%)	0.005	0.005	0.005	0.005	0.005
Calculated composition					
Crude protein (%)	19.570	19.570	19.570	19.570	19.570
Metabolizable energy (kcal/kg)	3.100	3.100	3.100	3.100	3.100
Calcium (%)	0.918	0.918	0.918	0.918	0.918
Available phosphorus) (%)	0.389	0.389	0.389	0.389	0.389
Sodium (%)	0.200	0.200	0.200	0.200	0.200
Total lysine	0.880	0.940	1.000	1.060	1.120
Digestible lysine (%)[3]	0.780	0.840	0.900	0.960	1.020
Digestible tryptophan (%)[3]	0.150	0.150	0.150	0.150	0.150
Digestible valine (%)[3]	0.814	0.814	0.814	0.814	0.814
Digestible threonine (%)[3]	0.632	0.632	0.632	0.632	0.632
Digestible meth + cys	0.515	0.515	0.515	0.515	0.515

[1]Content/kg – Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g vehicle q.s.p. 500 g. 2 Content/kg - vit. A - 15,000,000 UI, vit. D3 - 1,500,000 UI, vit. E - 15,000 UI, vit. B1 - 2.0 g, vit. B2 - 4.0 g, vit. B6 - 3.0 g, vit. B12 - 0.015 g, nicotinic acid - 25 g, pantothenic acid - 10 g, vit. K3 - 3.0 g, pholic acid - 1.0 g, zinc bacitracin - 10 g, selenium - 250 mg and vehicle q.s.p. - 1,000 g. 3 Digestible amino acids calculated based on the coefficients of digestibility from Rhodimet-Rhône-Poulenc (1993) tables). *Active principle – salinomicine.

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An additional group of 21 days old broilers was slaughtered to determine the body composition in the beginning of experiment. The protein deposition in the carcass was calculated by the difference between the values of carcass composition between 22 and 42 days old.

The two remaining carcasses of each repetition (total of 12 carcasses per treatment) were used to obtain the prime cuts in which the absolute weight (grams) and yield (%) of entire carcasses (with feet and head), legs, thigh, drumstick, breast, feathers and abdominal fat. The carcass yield was obtained by the ratio between the weight of clean and eviscerated carcass (with feet and head) and the live weight after fasting, while the yield of prime cuts was determined considering the weight of the eviscerated carcass without feathers.

Statistical analysis

Statistical analyses were carried out through the SAEG software (1997). Estimates of total lysine requirements were established by means of linear regression and/or quadratic models and by Linear Response Plateau (LRP) according to the better adjustment.

RESULTS AND DISCUSSION

Table 2 presents the average values of environmental conditions within the climatic chambers obtained during the experimental period. The Avian Farms manual recommends the temperature around 22.5°C and moisture around 70% for the category between 22 and 42

Table 2. Average environmental conditions observed during the experimental period in the climatic chambers with broilers from 22 to 42 days old.

Variable	Values
Average air temperature (°C)	25.6 ± 0.24
Average relative humidity (%)	68.4 ± 6.30
Average black globe temperature (°C)	25.7 ± 0.25
Black globe humidity index (BGHI)	73.7 ± 0.60

Table 3. Performance, total lysine intake and protein deposition rate of male broilers from 22 to 42 days of age fed with rations containing different levels of lysine, under high temperature environment.

Parameter	Lysine levels (%)					CV (%)
	0.88	0.94	1.00	1.06	1.12	
Weight gain (g)[1]	1,219	1,265	1,293	1,315	1,283	4.53
Feed intake (g)	2,371	2,350	2,369	2,383	2,390	4.62
Feed:gain ratio[2]	1.94	1.86	1.83	1.81	1.86	2.76
Total lysine intake (g)[3]	21	22	24	25	27	4.81
Protein deposition rate (g)	164	166	177	180	173	3.46

[1,2,4]Quadratic effect (P<0.09), (P<0.01) and (P<0.03), respectively. [3]Linear effect (P<0.01). CV: Coefficient of variation.

days. The temperature limit of thermal-neutrality is about 25°C, and then it is possible to infer that the experimental conditions of the present work represent a moderately hot environment. A comfortable environment for such category of animals those with Black Globe Humidity Index (BGHI) around 72 and as stressing those with BGHI of 84 (Borges et al., 2002).

Table 3 presents results of performance (weight gain, ration intake and food conversion), total lysine intake and rates of fat and protein deposition in broiler carcasses from 22 to 42 days old receiving rations with different levels of lysine and kept at high temperature (25.6°C). A quadratic effect (P<0.09) was observed in the lysine levels of the ration over the weight gain (WG) of broilers up to the level 1.05%, which was associated to an intake of 25 g of total lysine. Such result was higher than 0.92 and 0.98% of total lysine obtained by Barboza (1998) for Hubbard and Ross male broilers from 22 to 40 days old, as well as to 0.85 and 1.00% of total lysine obtained by Barboza (1998) also for the weight gain of male broilers from 21 to 42 and 22 to 40 days old, respectively. On the other hand, Conhalato (1998) obtained a better result of weight gain for broilers from 22 to 42 days old created in hot periods of the year (average temperature superior to 26°C) with levels of total lysine (1.20%) higher than those found in the present work. Contrarily, Mendes et al. (1997) did not observe any influence of the lysine level over the weight gain in 22 to 42 days old broilers submitted to high environmental temperature (25.5 to 33°C).

The differences of results among the above mentioned studies may be associated to the genetic factors, as well as to the differences of environmental temperature where they were conducted. According to Cahaner et al. (1995),

the nutritional requirements of broilers are influenced by the environmental temperature and genetics. No effects of lysine levels over the ration intake (RI) were observed for the broilers. Mendes et al. (1997) worked with 21 to 42 days old broilers kept under high temperature (25.5 to 33.3°C) and also did not verify such effect. Considering that in general the animals, when exposed to high temperature, reduce the ration intake to avoid an increase of heat production (Baziz et al., 1996). it is possible to deduce that under this condition the capacity to adjust the intake is committed due to the nutrient concentration of the ration.

The lysine levels of ration had a quadratic effect (P<0.01) over the food conversion (FC), which improved up to the level 1.03% of total lysine (Table 4), what corresponded to 0.93% of digestible lysine and an estimate total lysine consumption of 24.5 g. A quadratic effect of the lysine level over the food conversion in 22 to 42 days old broilers was also recorded by Barboza (1998) and Conhalato (1998).

Levels of lysine had a quadratic effect (P<0.01) over the weight gain (WG), which improved up to 1.05% of total lysine (Table 5).

Results obtained by Trindade Neto et al. (2011) in a similar study with broilers from the same lineage and age verified that the weight variation occurred according to the equation $\hat{Y} = -1622.36 + 6602.5422X - 3019.1834X^2$, $r^2 = 86.76$ and 1.09% of lysine was indicated as optimal. As observed for final weight, weight gain ($\hat{Y} = -2489.08 + 6596.5735X - 3028.5724X2$, $r^2 = 83.87$) and feed conversion ($\hat{Y} = 8.55 - 12.4257X + 5.6735X2$, $r^2 = 89.56$) also had quadratic responses (P <0.05) and estimated

Table 4. Dietary lysine level and feed:gain ratio (g/g) of broilers from 22 to 42 days old under high environmental temperature (25.6°C).

Food conversion	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1.96	0.00		
1.93	0.88		
1.90	0.94	1.03	$\hat{Y} = 7.63131 - 11.2593X + 5.45108X^2$
1.87	1.00		$r^2 = 0.98$
1.84	1.06		
1.81	1.12		

r^2 = coefficient of determination.

Table 5. Weight gain (g) and lysine levels of rations for 22 to 42 days old broilers kept under high temperature (25.6°C).

Weight gain	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1215	0.00		
1235	0.88		
1255	0.94	1.05	$\hat{Y} = 21.7717 - 66.5417X + 3179.67X^2$
1275	1.00		$r^2 = 0.96$
1295	1.06		
1315	1.12		

Table 6. Lysine levels of ration and ideal ration intake for the protein deposition of 22 to 42 days old broilers at high temperature (25.6°C).

Total lysine of ration	Absolute value of ration for protein deposition (g)	Linear equation
0.00		
0.88		
0.94	25	$\hat{Y} = -1.23113 + 24.9651lys$
1.00		$r^2 = 0.99$
1.06		
1.12		

the same lysine level (1.09%) as the best model. Considering that the protein deposition is more efficient than fat deposition since it aggregates more water, the increase of PDR in the carcass up to 1.05% of total lysine justifies the improvement observed for the WG and FC of broilers.

The total lysine intake increased linearly (P<0.01) due to the improvement of the lysine levels in the ration according to the equation $\hat{Y} = -1.23113 + 24.9651lys$ ($r^2 = 0.99$). This result is justified by the fact that the ration intake did not vary among treatments. Although there was a linear increase of total lysine intake, the intake of 25 g provided the highest absolute values of gain and deposition of protein in the carcass (Table 6).

Regarding the carcass composition, a quadratic effect (P<0.03) of the total lysine level was observed over the protein deposition rate (PDR), which increased up to the level 1.05% (Table 7). This result corroborates what was found by Summers et al. (1992) and Deschepper and Groote (1995), who also verified alterations in the chemical composition of 42 days old broiler carcasses when low protein rations supplemented with essential amino acids in thermo-neural environments.

According to Trindade Neto et al. (2010), determining the peak of protein deposition allows estimating the maximum efficiency of amino acids used for the synthesis and accumulation of muscle mass in broiler carcasses. The effect of increase on protein deposition is characterized by the increase of water on body composition. Similarly, the reduction of body fat suggests an increase of protein synthesis efficiency and favors the accumulation of muscle mass. The deposition of protein in the carcass may be related not only to the lysine content of the diet, but also to the genetic strain and to the age of the birds utilized, as well as to thermal-environmental factors, immunologic challenge, among

Table 7. Protein deposition rate (g) and levels of lysine in rations for 22 to 42 broilers kept at high temperature (25.6°C).

PDR*	Total lysine of ration (%)	Ideal total lysine for PDR (%)	Quadratic equation
180	0.00		
176	0.88		
172	0.94	1.05	$\hat{Y}= 428.074-1154.76X+550.523X^2$
168	1.00		$r^2 = 0.81$
164	1.06		
160	1.12		

*Protein deposition rate.

Table 8. Absolute and relative weights of carcass, prime cuts and abdominal fat of 42 days old broilers under high environmental temperature.

Parameter	Lysine levels					CV (%)
Absolute weight (g)	0.88	0.94	1.00	1.06	1.12	-
Weight after fasting	1,720	1,765	1,794	1,857	1,789	5.24
Carcass	1,371	1,412	1,439	1,474	1,407	5.07
Breast with bone	349	390	403	398	405	6.70
Legs	369	368	389	397	382	487
Drumstick	183	184	188	194	187	4.93
Thigh	186	183	202	202	193	6.38
Abdominal fat	19	16	14	16	18	16.7

others de Oliveira et al. (2013).

The lysine levels of ratio influenced (P<0.03) the carcass absolute weight and yield, which increased up to the levels 1.01 and 0.96% of lysine, respectively, according to the quadratic equations $\hat{Y}$ = -3340.89 + 9392.72X+4627.2X^2, r^2 = 0.83 and $\hat{Y}$ = 27.5967 + 109.332 – 56.907X^2, r^2 = 0.96 (Table 8). Contrarily, Moran Jr. and Bilgili (1990), Kidd et al. (1997) and Conhalato (1998) did not verify any influence of lysine levels over the carcass yield of 21 to 42 days old broilers created during hot months. Barboza (1998) also did not observe effect of lysine levels over the carcass yield of 22 to 40 days old broilers in thermal-neutral environment.

There was a quadratic effect of lysine levels over the absolute weight of breast with bone (P<0.01), which increased up to the level 1.06% (Table 8), and a linear effect (P<0.01) over the relative weight of breast with bone, which increased according to the equation $\hat{Y}$ = 16.81124 + 10.6134lys (r^2=0.67).

Barboza (1998) observed an increase of 2.8% in the breast yield as the lysine levels increased from 0.95 to 1.15% and 0.8 to 0.98%, respectively. Results obtained in the present work are coherent since according to Baker (1991) the lysine is greatly important for the composition of muscle protein. Furthermore, Moran Jr and Bilgili (1990) state that the lysine supply in adequate levels is fundamental for the production of meat and breast. Moreover, according to Kidd et al. (1997), the requirement of essential amino acids for the maximum yield of breast is higher than what is considered adequate for the maximum growth.

The lysine levels had a quadratic effect (P<0.07) over the absolute weight of legs (Table 8), which increased up to the level 1.06% of total lysine according to the equation $\hat{Y}$ = - 497.899 + 1673.27lys – 788.216 lys^2 (r^2=0.80). Similarly, Conhalato and Barboza (1998) did not observe effect of lysine levels over the yield of legs from 22 to 42 and 22 to 40 days old broilers, respectively. On the other hand, Mendes et al. (1997) verified an influence of increasing levels of lysine over the yield of legs from 21 to 42 days old broilers created at cyclic high temperature (25.5 to 33.3%).

Levels of lysine influenced (P<0.07) the absolute weight of thigh, which had a quadratic increase up to the level 1.02% according to the equation $\hat{Y}$ =- 713.3 – 1773.73lys – 866.164lys² (r^2 = 0.63) (Table 8). Moreover, levels of lysine influenced (P<0.01) the absolute weight of abdominal fat (Table 8), which presented a quadratic reduction up to the level 1.00% according to the equations $\hat{Y}$ = -315.5 – 602.109lys + 301.385lys2 (r^2=0.98) and $\hat{Y}$ = 25.6898 - 49.1016 lys + 24.4444 lys² (r^2=0.99).

Mendes et al. (1997) also verified effects of the lysine levels over the abdominal fat content of 21 to 42 days old broilers created at high temperature, however, Barboza (1998) did not observe effect of levels from 0.80 to 1.10%

over the abdominal fat in 22 to 40 days old broilers when kept in neutral environment.

Conclusion

Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis.

Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine. The total lysine intake increased linearly due to the improvement of lysine content in the ration. An effect of total lysine was observed over the protein deposition rate (PDR), which presented a quadratic increase up to the level 1.05%.

Lysine levels of the ratio influenced the absolute weight and carcass yield, which presented a quadratic increase up to the level 1.01 and 0.96%, respectively. There was a quadratic effect of lysine levels over the absolute weight of breast with bone, which increase up to the level 1.06%, and a linear effect over the relative weight of breast with bone. Lysine levels had a quadratic effect over the absolute weight of legs, which increased up to the level of 1.06%. Lysine levels influenced the absolute weight of thigh, which increased up to the level 1.02%. Lysine levels influenced the absolute weight of abdominal fat, which presented a quadratic decrease up to the level 1.00%.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest

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