



# Variety and environmental effects on crude protein concentration and mineral composition of *Arachis pintoi* (Kaprovickas & Gregory) in Benin (West Africa)

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## ABSTRACT

In tropical regions of West Africa, low crude protein and mineral concentrations of forages are often reported as the most constraints for livestock nutrition. Forage legumes have generally better crude protein and mineral concentrations than grasses. Three year trials were conducted in Benin on four *Arachis pintoi* varieties (CIAT 17434, CIAT 18744, CIAT 22160 and AFT 495) in two ecological regions (humid and subhumid). The objective was to identify their crude protein content and mineral concentrations in order to make recommendations for their use in ruminant production systems. Varieties were established through randomised complete block design arranged in a split-block where regions were main plots and varieties were subplots replicated four times per variety. Plant forages were harvest five months after establishment, dried and analysed for crude protein and mineral nutrient contents. Data were analysed with GLM model. Results showed that year had no significant influence on crude protein nor mineral contents of forages, but region and variety had both significant influence ( $P < 0.05$ ) on crude protein and mineral concentrations (Ca, K, Mg, P, Na, S, Cl, Zn Cu, Fe, Mn, Se, Co, Mo and Cr). Region×variety interaction was also significant. In humid region and for crude protein content, varieties ranked in CIAT 18744 (20.43%) = AFT 495 (19.66%) > CIAT 22160 (18.03%) = CIAT 17434 (17.77%) and in subhumid region the ranking was : CIAT 18744(19.72%) > CIAT 22160 (18.61%) > CIAT 17434(17.89%) = and AFT 495 (17.50%). Variety CIAT 18744 showed generally the highest concentrations in Ca (1.60%) and P(0.21%).

## 1. INTRODUCTION

*Arachis pintoi* Krapov. & W.C. Greg is collected from its native environment (Brazil) in 1954, and was introduced to Central American countries after some preliminary researches. In those countries, *A. pintoi* is used in grazing systems for both beef and dairy cattle and has been recognized as a legume with high intake potential and with a high defoliation and trampling tolerance [1]. *A. pintoi* maintained a large proportion of its areal parts in spite of drought. The plant had a root-deepness characteristic which can confer drought tolerance by allowing the plant access to water in deeper soil profile. The plant grows well in soils low in P although some P fertilizer is advisable for soils extremely low in P. In association, *A. pintoi* can increase up to 137% grasses forage yield [2]. The plant was reported to have a high nutritive value [3]. The nitrogen accumulated by the plant from biological fixation can reach up to 85% [4, 5]. *A. pintoi* plant mineral concentration are

affected by soil composition and [6] had reported that concentrations of K, P, Mg and S in the plants were higher on clay soils than on sandy soils, and both K and Mg were more consistently taken up than either P or S [7] have reported interesting forage yields for *A. pintoi* when compared to other herbaceous legume forage grown in West Africa. But information regarding variety type and environment on the plant crude protein (CP) and mineral composition in West Africa region had never been published. In order to increase the information upon which decisions can be made regarding the widespread attention receiving by the plant as forage crop in Benin, a study was conducted to examine the effects of variety and environment on CP and mineral concentrations. These effects were evaluated using four genotypes (CIAT 17434, CIAT 18744, CIAT 22160 and AFT 495) sown in two contrasted ecological region (humid and subhumid) of Benin.

## 2. MATERIALS AND METHODS

### 2.1. Site and climatic conditions

The experiment was carried out on two sites. The first was

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located in the humid region of Benin (coastal Atlantic Ocean) at 6°05'N, 1°32'E and 0-20 m altitude. The area is covered by savannah vegetation with grasses dominated by *Panicum maximum* and *Brachiaria* sp. Soils are ferrallitic, which are red in colour, characterised by a deep profile (> 2 m) with no gravel (Table 1). The rainfall follows a quadrimodal distribution. The rainy season extends from March until the beginning of November, with a short dry season in July and August. Annual precipitations during the three experimental years (2008, 2009, 2010) were 1211, 1147 and 1232 mm, respectively. Average monthly minimum and maximum temperatures were 22 and 30 °C, respectively. The second experimental site was located in the subhumid region of Benin (12° 45'N, 2° 28'E, altitude 400 m) with a grass savannah dominated by *Andropogon gayanus*, *Rottboellia cochinchinensis* and *Pennisetum polystachion*.

The soil on the second experimental site contained a high proportion of gravel (> 50%) with a lower depth (40 – 80 cm) (Table 1). The climate is characterized by strongly contrasting seasons with a single rainy season from May to mid-October followed by a dry season of 6 to 7 of months. Annual precipitation during the three experimental years (2008, 2009, 2010) were 894, 902 and 932 mm, respectively. Average monthly minimum and maximum temperatures were 21 and 33 °C, respectively. A general trend of the rainfall and temperature in both sites is a slightly decrease of rainfall and the increase of temperature during the last three decades.

## 2.2. Characteristics of plant genotypes

*A. pintoii* genotypes CIAT 17434, CIAT 18744, CIAT 22160 and AFT 495 were used in this trial. They were selected on the basis of their outstanding performance reported by the literature in terms of dry matter (DM) production, soil cover and plant dry season survival (Table 2).

## 2.3. Land preparation, treatments and sowing

Prior to the study (year 2007), both sites were covered by the previously described savannah vegetation. Soil sites were sampled and analysed for physical and chemical properties (Table 1). After that, sites were manually cleared, weed residues were burnt on site, and ash spread over the field in conformity with local practices. This was followed by conventional peasant's practice tillage at a depth of approx. 40 cm. For each site, the design was a randomised complete block arranged in a split-block where sites were main plots and varieties were subplots replicated four times

per variety of four blocks. The experimental scheme was as follows: 2 sites × 4 varieties × 4 blocks. Elementary plot size was 6 m × 5 m (30 m<sup>2</sup>) with 2 m spacing between plots. Seeds were sown on April 01 and April 15 2008 in the humid and the sub humid region respectively with 50-cm spacing between lines [13] and 20 cm between plants in the same line. Four seeds per hole were planted (60-80% germination) at a depth of about 2 cm and latter thinned to one vigorous plantlet per hole after emergence. Varieties were planted without *Rhizobium* inoculation or any fertilizer treatment in conformity with smallholder practices in the region. Plots were weeded manually 8 weeks after establishment, after this only shrub regrowth was controlled.

## 2.4. Sample collection and analysis

Samples were clipped five months after sowing from each quadrat into 5-cm strata, and further separated into leaf, stem, and dead material. All leaves collected per plot were dried at 60°C during 72 hours and ground through a 1-mm screen prior to analyses for crude protein (CP, Kjeldahl-method, N×6.25), crude fibre and ash (550°C for 3 h) according to the methods of [14]. Mineral content (Ca, Mg, K, P, S, Cl, Na, Fe, Zn, Mn, Cu, Se, Mo, Cr and Co) was determined as described in [15] using an atomic absorption spectrophotometer (Perkin Elmer AAS-800, Wellesley, MA). P concentration was determined by the colorimetric method using molybdovanadate reagent [16]. Se was determined by atomic absorption spectrophotometry coupled to a hydride generator (FIAS-MHS) after reduction in KCl solution [17] Cl was analysed by titrimetry after fusion of ash with CaO and dilution in 20% HNO<sub>3</sub> [17] Sulphur was determined using turbidimetry [17].

## 2.5. Statistical analysis

Eight treatments (2 sites × 4 varieties) were considered in the trial. For all measurements, the plot was used as the experimental unit. Statistical analysis showed no influence of year on crude protein content and mineral composition and so, data were pooled for two-way (site and varieties) analysis of variance using GLM (General Linear Model) procedure of SAS 8.02 software (SAS Inc., Cary, NC).

The model was  $Y_{ij} = \mu + S_i + V_j + (S*V)_{ij} + e_{ij}$ . Where  $\mu$ =mean,  $S_i$ =fixed site effect,  $V_j$ =fixed variety effect,  $(S*V)_{ij}$  their interaction,  $e_{ij}$ =experimental error. When significant interaction was observed, data were reanalyzed separately by one-way ANOVA. A significant difference was declared at  $P < 0.05$ . Means were compared by Least Significant Difference method.

**Table 1:** Physical and chemical properties of the soils on the experimental sites.

Soil property	Experimental region		Analysis method
	Humid site	Sub humid site	
Sand (%)	70-80	75-95	Hydrometer method
Silt (%)	15-25	4-10	
Clay (%)	3-7	2-14	
pH	5.8 – 6.0	6.2 – 6.5	[18], H <sub>2</sub> O (2:5) (pH-meter PHM82)
Organic carbon (%)	0.30-40	0.4-0.6	[19]
Total N (%)	0.02-0.06	0.06-0.10	Kjeldahl (method 981.10, [14])
Available P (ppm)	2-4	4-8	[16] (spectrophotometer, CE 373) using the Scheel method
Ca (meq/100g)	2.0-4.0	4.2-5.6	[16]
Mg (meq /100g <sup>1</sup> )	0.9-1.3	1.0-1.8	
K (meq /100g)	0.3-0.5	0.4-0.6	
Na (meq /100g)	0.1	0.1-0.3	
CEC (meq /100g)	4.0-5.2	6.0-8.0	

**Table 2:** Characteristics of *Arachis pintoi* plant varieties used in the trial.

Variety	Characteristics
CIAT 17434	It grows slowly than CIAT 18744 but produces more seeds. The most common variety grown through the word.
CIAT 18744	This variety is reported to utilise phosphorous more efficiently than CIAT 17434; it has a greater growth rate, is more productive, covers the soil faster and has denser stolon than CIAT 17434.
CIAT 22160	CIAT 22160 was reported to have better drought tolerance and ability to produce green sprouts during dry season. It has better forage quality and showed more aggressiveness in association with grasses as well as a better resistance to pest and disease than CIAT 17434.
AFT 495	Variety ATF 495 is more erect and performed better in South-East Asia than CIAT 17434. It produces more flowers and bigger seeds than other CIAT varieties. Its upper leaves are normally paler than those of CIAT 17434.

[8];[9]; [10];[11]; [12].

**Table 3:** Crude fibre, ash and crude protein concentrations (% DM) in the leaves of tested varieties (mean of 3 years) (N = 4).

Characteristics	Humid site				Subhumid site				SEM <sup>(2)</sup>
	CIAT 17434	CIAT 18744	CIAT 22160	AFT 495	CIAT 17434	CIAT 18744	CIAT 22160	AFT 495	
Crude fibre (%)	27.33 a <sup>(1)</sup>	21.43 c	25.00 b	28.71 a	27.41 a	22.18 c	24.55 bc	26.67 ab	0.44
Ash (%)	8.91 a	8.52 a	9.09 a	8.66 a	8.71 a	8.09 a	8.90 a	9.14 a	0.29
CP (%)	17.77 Bb	20.43 Aa	18.03 Bb	19.66 Aa	17.89 Bb	19.72 Aa	18.61 Aa	17.50 Bb	0.18

<sup>(1)</sup>For the same characteristic and for the same site, means followed by different lower letters are significantly different for  $p < 0.001$ .For the same characteristic and for the same variety, means followed by different upper letters are significantly different for  $p < 0.05$ .<sup>(2)</sup>SEM :Standard error of means.**Table 4:** Macro- and micro-mineral concentrations in *A. pintoi* leaves growing under humid and sub humid conditions (mean of 3 years) (N = 3) and ruminants requirements.

Varieties	Macro-minerals (% DM)						Micro-minerals (mg/kg DM)								
	Ca	P	K	Mg	Na	S	Cl	Zn	Cu	Fe	Mn	Se	Co	Mo	Cr
	Humid site														
CIAT 17434	1.41a <sup>(1)</sup>	0.15 b	0.50	1.00	0.01	0.11	0.50	25	10	438	254a	0.06	0.22 a	0.17	1.42
CIAT 18744	1.60a	0.21 a	0.51	1.06	0.01	0.09	0.28	23	10	517	198b	0.03	0.11 b	0.11	1.13
CIAT 22160	1.51a	0.14 b	0.49	1.07	0.01	0.12	0.31	22	9	459	225a	0.03	0.12 b	0.15	1.31
AFT 495	1.01b	0.16 b	0.61	0.92	0.01	0.14	0.43	29	11	410	259a	0.02	0.11 b	0.18	1.10
	*	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS
	Subhumid site														
CIAT 17434	1.62a	0.22 b	0.42	1.51	0.01	0.10	0.52	30	14	559	300a	0.05	0.23	0.20	1.80
CIAT 18744	1.55a	0.31 a	0.67	1.96	0.01	0.08	0.31	27	13	664	231b	0.03	0.19	0.18	1.43
CIAT 22160	1.42a	0.24 b	0.51	1.89	0.01	0.11	0.40	29	11	529	350a	0.04	0.24	0.19	1.60
AFT 495	1.09b	0.23 b	0.57	1.17	0.01	0.12	0.38	34	12	487	267b	0.04	0.18	0.21	1.21
	*	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
SEM <sup>(2)</sup>	0.08	0.02	0.03	0.1	0.0	0.01	0.13	2.4	1.59	18	27	0.00	0.02	0.01	0.09
Ruminant requirements <sup>(2)</sup>	0.24	0.18	0.70	0.10	0.07	0.02	0.07	50	10	50	50	0.1	0.1	-	-

<sup>(1)</sup>For the same mineral nutrient and for the same site, means followed by different letters are significantly different for  $p < 0.05$ <sup>(2)</sup>Requirements for cattle according to [20] for Cu, Fe, Mn, Se and Co; and [21]for Mg.

### 3. RESULTS

#### 3.1. CP concentration

Results showed that year had no significant influence on CP and mineral contents of forages ( $P > 0.05$ ). Site and variety influenced ( $P < 0.05$ ) forage CP content and site×variety interaction was also significant ( $P < 0.05$ ) (Table 3). In southern region of Benin, variety CIAT 17434 and AFT 495 showed the highest (CP) content followed by the two others varieties (Table 3).

In northern region, CIAT 18744 and CIAT 22160 were the most interesting. Under humid conditions, varieties CP contents ranked in the following order: CIAT 18744 = ATF 495 > CIAT 22160 = CIAT 17434 (Table 3). In the sub humid location the ranking was different: CIAT 18744 = CIAT 22160 > CIAT 17434 = ATF 495. Significant interaction site×variety difference was observed and was related to AFT 495 which had showed significant lower (CP) content in subhumid area than humid one. Significant differences were observed between varieties for Ca, P, Mn and Co nutrients content depending to the site (Table 4).

Variety CIAT 18744 had the highest P concentrations in its forage than others. Significant difference ( $P < 0.05$ ) was observed in humid site for Co but in subhumid site forage contents for Co were the same.

### 4. DISCUSSION

The aim of this research was to provide information regarding the impact of variety and location on crude protein and mineral concentration of select *A. pintoi* in Benin. A significant site×variety interaction ( $p < 0.05$ ) was observed for forage crude protein content indicating that CP concentrations depend not only on the genetic characteristics of selected varieties but also on plant environment. Crude protein content of forage depends on N available in the soil or via symbiotic fixation. In this study, available soil N was very low (0.02-0.10%) and therefore it is possible that most N available in the forage would be provided by atmospheric fixation. Ability of plants for N fixation depends not only on species but also on variety [22]. Effectiveness of associated

native strains of rhizobia which can infect plants roots would also play an important role. Crude protein concentrations (17.5 to 20.43 % CP) are consistent with [23]. In Colombia, [24] reported CP content of 15, 18 and 15% for CIAT 17434, CIAT 18744 and CIAT 18748, respectively. For all varieties, CP content of forage was above the critical level for ruminants of 6-8% defined by [25] and confirms the potential role of harvested forage as a protein supplement.

This trial had showed forage mineral contents; Forage mineral determination is important as different incidences of mineral imbalances had been reported particularly in tropical forages as the major causes of lowest ruminant production. On the other hand, many problems related to health had been caused by deficiency or toxicity of minerals [26, 27]. Forage mineral content can vary greatly and depends not only to plant variety but also to soil [7, 28, 29]. Therefore, it is imperative to analyze the forage for mineral profile in order to recommend supplements of mineral to livestock. Macro- and micro-nutrient contents of each variety did not differ greatly between sites except for P, Mg, Fe, Co and Mn. For the same site, significant differences were observed between varieties for Ca, P, Mn and Co. For all varieties, these mineral concentrations were generally higher under subhumid than under humid conditions. Mechanisms controlling soil nutrients assimilation by roots via plant-soil relationships are complex and therefore difficult to discuss with limited data available regarding such aspects of the present study. Nevertheless, higher soil P concentration at plant establishment (6 ppm vs. 3 ppm in subhumid and humid sites, respectively) could play an important role in P concentration in plant forage. On other hand, low soil pH may reduce P and Mg uptake by *A. pintoii* in the humid location. Ca concentrations varied from 1.01 to 1.62% and were lower than the mean of 1.8% reported by [30]. *A. pintoii* K concentrations (0.50-0.67%) are higher than 0.23 % recorded by [31] but are in agreement with results of [32](0.57-0.72%). S concentrations (0.09-0.14%) recorded in this experiment are consistent with 0.11 % reported by [33]. Co and Fe concentrations of *A. pintoii* variety forages recorded in this trial (1.11-1.24 mg/kg DM and 410-664 mg/kg, respectively) were particularly high compared to values of other herbaceous legumes (*Aeschynomene histrix*, *Stylosanthes fruticosa*, *Centrosema pubescens* and *Mucuna pruriens*) grown in the same environment [15]. Finally, regarding ruminant nutrition, *A. pintoii* P content was sufficient to meet animal requirements (Table 4). Deficiencies in Ca, Mg, S, and Cu would not occur but for K, Na, Zn and Co, animals fed with *A. pintoii* would need supplementation in order to fulfill the requirements of grazing cattle.

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