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	Agronomic efficiency of bone meal ur			
	acidification in Brachiaria ruziziensis d			
	matter production in Western Amazo			
E	BSTRACT			
	Aims: The objective of this work was to evaluate the agronomic efficiency of the bo			
	meal under acidification in the production of dry matter of Brachiaria ruziziensis			
	relation to a soluble source.			
	Study Design: The experiment was conducted in a completely randomized desi			
	with seven treatments and three replications: Witness; Single Superphosphate (S Bone Meal (BM); BM treated with 10% Oxalic Acids; 10% Acetic Acid and 1% to 0.5			
	Hydrochloric Acid.			
	Place and Duration of Study: The experiment was carried out from October 2014			
	February 2015, at the Experimental Farm of the Federal University of Rondô			
	(UNIR), located 15 km from the city of Rolim de Moura, Rondônia, Brazil.			
	Methodology: The bone meal used in the experiment was produced manually, whe			
	bovine bones were collected and burned for carbon removal and particle reduction			
	The oxalic, acetic and hydrochloric acids were used to to increase the solubility of the bone meal and applied to the soil for growth of <i>B. ruziziensis</i> . It was evaluated			
	Agronomic Efficiency Index (AEI), Phosphorus Conversion Efficiency (PCE), Shoot I			
	Matter (SDM) and Root Dry Matter (RDM).			
	Results: AEI was obtained for acid treatments above 60% and PCE satisfactory wh			
	compared to the soluble source, except for 0.5% hydrochloric acid and significant			
	above the BM without acid treatments. There was higher production of SDM and RI			
	with the soluble source (SS), however the acid treatments promoted dry ma			
	production above the BM without acid treatments.			
	Conclusions: The application of acids in bone meal promoted satisfactory agronor efficiency gains for plants of <i>Brachiaria ruziziensis</i> .			

- **1. INTRODUCTION** 37
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39 The Brazil is the second largest producer of beef in the world, behind only the United States, and according to the Brazilian Institute of Geography and Statistics [1], the herd surpasses 40 41 218 million head. Approximately 80% of the Brazilian herd is extensively raised under 42 planted / natural pastures, since it is the most economical method of cattle feeding to cattle ranchers [2]. Of the fodder used for this purpose, those of the genus Brachiaria correspond 43 44 to 70% and 80% of the area planted in Brazil. The Brachiaria ruziziensis is one of the most planted because of its high palatability, grazing support and high dry matter production (6 to 45 46 15 t ha⁻¹) [3].

- 47 48 It is estimated, however, that about 70 to 80% of the pastures used for this purpose are 49 degraded or in the process of degradation [4]. According to Dias-Filho [2], the main causes 50 of this degradation are focused on the adopted management, such as fire use, low quality 51 seeds, high stocking rates and absence of fertilization. Among these, the absence and 52 inefficiency of fertilization has been noted as a more aggravating factor of pasture 53 degradation in Brazil [5,6]. As soil fertility decreases, there is a marked productive loss of 54 fodder and, consequently, decrease in animal production [3].
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56 When considering the Amazon region, the problem tends to be more pronounced, 57 considering the most acidic soil characteristics, low cation exchange capacity, high 58 aluminum saturation and low nutrient reserves, mainly in phosphorus (P) (between 1 and 3 g 59 dm⁻³) [2]. It is suggested, therefore, that fertilization with this nutrient is carried out, in order 50 to guarantee its adequate supply.

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62 Phosphorus maintenance for pasture production has been performed with the use of liming 63 and the use of soluble phosphorus sources, such as single and triple superphosphate [7]. 64 Although widely used, they come from the exploration of phosphate rocks, whose reserves 65 are estimated to deplete in up to 100 years [8 9]. In this sense, it is necessary the study of 66 new sources of phosphorus in order to guarantee the supply of this element [10,6].

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68 Recently, studies by several authors have shown that the use of solid animal waste 69 processed, bone meal (BM), have been pointed as potential source of phosphorus and 70 calcium plants [11,11]. Under heat action, bones can also supply magnesium, potassium 71 and iron from the blood [13]. However, due to the chemical nature of the bones 72 (hydroxyapatite), BM has low solubility of phosphorus in water (0.26% of P_2O_5) despite its 73 high concentration, between 30, 6 and 38.8% P₂O₅ [14]. However, it is necessary to study 74 acidifying agents capable of readily providing the available phosphorus in the bones, as tested for 2% citric acid, which provided 23% solubility to bone meal [15]. 75

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Thus, the objective of this study was to evaluate the agronomic efficiency of the bone meal under acidification in the production of dry matter of *Brachiaria ruziziensis* in relation to a soluble source.

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2. MATERIAL AND METHODS

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The experiment was conducted at the Experimental Sector of Agronomy of the Federal University of Rondônia - UNIR, Campus Rolim de Moura, RO - Brazil, located at coordinates 11° 34 '58.60 "S and 61°46'22.30" W with an altitude of 277 m. The climate of the region, according to the classification of Koppen, is Aw type, defined dry season, mean temperature of 28 ° C, mean annual precipitation of 2,250 mm and relative humidity of 85% [16].

The study was conducted in open-air pots of 3.9 kg in a completely randomized design, with seven treatments and three replicates, totaling 21 experimental units (Table 1).

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Table 1. Treatments used in experimentation.

Treatments	Description	Symbol
1	Unmanaged control	Wit.
2	P (Simple Superphosphate)	SS
3	P (Bone Meal)	BM
4	(Bone Meal + 10% Oxalic Acid)	Oxa ^{10%}
5	(Bone Meal + 10% Acetic Acid)	Ace ^{10%}
6	(Bone Meal + 1% hydrochloric acid)	HCI ^{1%}
7	(Bone Meal + 0.5 % hydrochloric acid)	HCI ^{0,5%}

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94 The conduction of the open - air experiment aimed to study the effect of plants under natural 95 conditions. As a source of phosphate fertilization, bone meal (37.5% P_2O_5) and simple 96 superphosphate (18% P_2O_5) were used. For each source, the equivalent of 100 kg ha⁻¹ of 97 P₂O₅ was used, depending on the concentration of each source. For the evaluations under 98 equal conditions, both P sources were subjected to grinding and sieving of 2.00 mm.

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100 As substrate for the plants, the arable layer (0-20 cm) of the experimental area classified as 101 Eutrophic Yellow Red Oxisol [17] was used, with the following chemical and physical characteristics: pH in water = 5.4 ; Organic matter = 3 dag kg⁻¹; P = 3.7 mg dm⁻³; K = 102 mg dm⁻³; S-SO⁻² = 3.6 mg dm⁻³, Ca = 4.1 cmol_c dm⁻³; Mg: 1.4 cmol_c dm⁻³; Fe = 88 mg dm⁻³; Cu = 1.8 mg dm⁻³; Zn = 1.5 mg dm⁻³; Mn = 25 mg dm⁻³; B = 0.14 mg dm⁻³, Potential Acidity= 4.8 cmol_c dm⁻³; Al = 0.12 cmol_c dm⁻³; Sum of Bases = 5.7 cmol_c dm⁻³; Potential cation exchange 102 103 104 105 capacity = 10.5 cmol_c dm⁻³; Basis Saturation= 54%; Saturation by aluminum = 2%; Sand = 106 530 g kg⁻¹; Silt = 83 g kg⁻¹ and Clay = 381 g kg⁻¹. 107

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109 The bone meal was produced under burn for elimination of organic compounds and for maceration in smaller particles. For this, the bovine bones were arranged inside a masonry 110 barbecue and accommodated in coin type screens, using charcoal for the burning for 111 112 approximately eight hours. After cooling the material, about 24 hours later, the bones were 113 ground with the use of gral and pistil and sieved in a 2.00 mm mesh. The material was 114 characterized in laboratory for analysis of the contents of P and Ca, presenting 37.5% P_2O_5 115 and 43.76% CaO.

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As acid extractors of phosphorus, the oxalic, acetic and hydrochloric acids (PAs) were used. 117 118 For Oxalic and Acetic acids, 10 ml dilution in 100 ml of distilled water was used. For 119 hydrochloric acid, dilution of 1 and 0.5 ml in 100 ml of distilled water was used. The solutions 120 were applied to 100 g of bone meal. Subsequently, the material was forced to air drying at 121 65 ° C for approximately 72 hours. The bone meal was homogenized to the soil and 122 arranged in the experimental units.

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124 The Brachiaria ruziziensis sprouts collected in the experimental area and standardized (12 125 cm in height and mean mass of 4.52 g) were used as indicator of the treatments. Three 126 shoots of B. ruziziensis were arranged in each experimental unit. In addition to the 127 treatments, complementary fertilization with macro and micronutrients was applied as 128 recommended for the forage described by Costa et al. [3]. The water supply was performed 129 by daily manual irrigation, applying 300 ml / pot / day. Cultural treatments were carried out 130 regarding the manual removal of invasive plants from the soil seed bank. 131

132 The plants were cultivated for 120 days, with Shoot Dry Matter (SDM) at 60, 90 and 120 days. The forage was cut at 20 cm from the soil level and the SDM determined from drying 133 134 in a forced circulation oven at 65 °C for approximately 72 hours. At the end of the periods the 135 sum of the SDM produced in the three periods was calculated for the calculation of the 136 Agronomic Efficiency Index (AEI) and Phosphorus Conversion Efficiency (PCE).

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138 The AEI was calculated from equation 1:

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 $AEI(\%) = [(Y2-Y1)/(Y3-Y1)] \times 100$ (Equation 1)

142 Where: AEI = Agronomic Efficiency Index, in%; Y1 = SDM obtained in the control, in g; Y2 = 143 SDM obtained with the treatments, in g: $Y_3 = SDM$ obtained from the reference source (SS), in a.

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The PCE was calculated from equation 2:

PCE $(mg mg^{-1}) = (SDM Treatments - SDM Witness) / APA (Equation 2)$

150 Where: PCE = Phosphorus Conversion Efficiency, mg mg-1; SDM Treatments = Dry Matter of the Aerial Part Total obtained in each treatment, in mg; SDM Witness = Dry matter of the 151 152 Total Aerial Part obtained in the control; APA = Amount of Phosphorus Added, in mg / pot.

153 The dry matter of the roots (RDM) was obtained after 120 days of treatments. For this, the 154 155 roots were removed from the experimental units, washed and subjected to drying in forced 156 circulation oven at 65 °C for approximately 72 hours. SDM and RDM were determined using 157 a precision digital scale. The obtained data were submitted to the analysis of variance and 158 the contrast of the means realized by the test of Tukey to 5% of probability. For the 159 analyzes, the statistical program ASSISTAT 7.7 was used.

3. RESULTS AND DISCUSSION 161

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Significant results were observed in all studied variables (P <0.01). When analyzing table 1, 163 164 it is noticed that the Total Shoot Dry Matter (SDM) was significantly higher in the commercial 165 soluble source (SS) application. However, acidification of bone meal (BM) with 10% acetic 166 acid, 10% Oxalic acid and 1% Chloride promoted an increase of 224%, 231% and 249% in 167 relation to treatment that did not receive acidification (BM). In relation to the control, the 168 application of BM without acidification promoted increments of only 56.1%.

169 With the exception of the treatment with 0.5% hydrochloric acid, the other acid treatments 170 promoted a significantly similar effect on the Agronomic Efficiency Index (AEI), with the 171 lowest AEI observed, confirming its natural phosphate characteristics. Oxa 10%, Ace 10% and HCI 1% acidifying treatments promoted I AEI on average 31% lower, taxing much lower 172 173 by observing the AEI without acidification of FO. Owing to the efficiency scale, it can be 174 noted that the AEI decreased in the following order: SS> HCI1%> Ace10%> Oxa10%> HCI 175 0.5%> BM (Table 2).

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177 Phosphorus Conversion Efficiency (PCE) was significantly higher in the standard treatment 178 (SS), however high PCE values were obtained with acid treatments, when compared to 179 those that did not receive acidification (BM). Among the acid treatments, HCl0.5% was the 180 one with the lowest efficiency. The PCE obeyed the following order: SS> HCl1%> Ac10%> 181 Ox10%> HCl0.5%> BM (Table 2).

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183 Table 2. Values of Shoot Dry Matter (SDM) of Brachiaria ruziziensis, Agronomic 184 Efficiency Index (AEI) and Phosphorus Conversion Efficiency (PCE) of bone meal 185 (BM) under acidification.

Treatments	SDM	AEI	PCE
moutinonito	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		$mg mg^{-1}$
	y	%	nig nig
Witness	4,1 d		
SS	23,1 a	100 a	223,13 a
BM	7,3 cd	16,84 d	37,58 c
BM+ Oxa ^{10%}	16,4 abc	64,73 b	144,45 abc
BM+ Ace ^{10%}	16,9 ab	67,36 b	150,32 abc

BM+ HCl 1%	18,1 ab	73,68 b	164,41 ab
BM+ HCI ^{0,5%}	10,3 bcd	32,63 c	72,81 bc
C.V	8,28	7,03	23,09
F	182,7	166, 8	7,50
<i>p</i>	0,001	0,001	0,002

C.V: Coefficient of Variation; BM: Bone Meal; SS: Simple Superphosphate; Oxa10%: Bone Meal + 10% Oxalic Acid; Ace10%: Bone Meal + 10% Acetic Acid; HCl1%: Bone Meal + 1% hydrochloric acid; HCl 0,5%: Bone Meal + 0.5 % hydrochloric acid

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Due to the high solubility of simple superphosphate (SS) in water [18], shoot dry matter production (SDM) was substantially higher, given the high availability of phosphorus (P) in solution. The same behavior was observed by Simões et al. [19] when using SS compared to bone meal (BM) in Tifton grass. However, these same authors observed satisfactory results with BM.

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In a study using bone meal and meat, Oliveira et al. [20] observed results of SDM of Tifton grass varying between 65 and 93% in relation to fertilization with SS, a fact not observed in this work, where the application of the alternative source of P resulted in about 30% and the extraction of the acids between 70.9 and 78%. In general terms, the production of dry matter with natural phosphate sources depends on the forage species and the BM production method.

The low natural solubility of BM may compromise the short-term response of annual crops, such as B. ruziziensis, and is not fully indicated for efficient production of SDM, especially in soils with pH above 5.0, where the reactions with phosphate naturally occur slowly and progressively [15].

Agronomic Efficiency Index (AEI) data differ from results obtained in other studies by Balbino et al. [15] and Farias et al. [21], which verified high BM efficiencies when compared to the control (soluble phosphate) treatment. Compared with Arad phosphate and triple superphosphate, [15] verified an increase in the availability of P in the soil and in the production of sugarcane in the use of BM. However, in the present study low AEI was observed using this source of P.

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The low efficiency can be related to the pH considered high in the application of bone meal on the substrate, preventing the transformation of the bound Ca + P compounds from BM to available soil phosphorus [14]. Also, according to Damaceno et al. [6], raising the pH can reduce BM efficiency and increase SS efficiency.

Like the soluble sources that undergo acidic attacks in reaction with water, like 2% citric acid on triple superphosphate, the action of the acidic extractors is pronounced in this work. With the exception of 0.5% Hydrochloric Acid, the other extractors showed AEI above 60%, considered high by Silva et al. [22] when compared to the standard source, evidencing its potential use in increasing the solubility of natural phosphate. These same authors, when studying the matter production of pigeon pea (*Cajanus cajan*), obtained similar responses between the use of natural phosphate and triple superphosphate.

Due to the high availability of phosphorus from the soluble source (SS), high phosphorus conversion efficiency (PCE) was obtained, a result also observed by Zambrosi et al. [23], which obtained a superiority of 31% of the same source in relation to the use of a natural phosphate (thermophosphate) in millet. However, the application of the acids resulted in satisfactory phosphorus efficiencies in dry matter, when observed the behavior without its 228 presence. The PCE is directly correlated with the capacity of the phosphate source to 229 release the nutrient in the soil solution [23, 6]. Therefore, the low capacity observed by the 230 bone meal compromises the use of the nutrient by the forage.

231 Another fact intrinsic to PCE is the extent and efficiency of root system absorption, which 232 responds to the available P in the soil. The distinction in the morphology and root physiology 233 modifies the uptake of P and other nutrients, compromising PCE [23]. The difference 234 mentioned can be observed in the distribution of roots of B. ruziziensis according to the 235 phosphate source and the acid treatment used (Figure 1).

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- 1 2
- 1 Witness 2 - Simples Superphosphate
- 3 Bone Meal
- Bone Meal + 10% Oxalic Acid
- Bone Meal + 10% Acetic Acid
- 6 Bone Meal + 1% Hydrochloric acid 7 - Bone Meal + 0.5% Hydrochloric acid

237 238 Figure 1. Root system of B. ruziziensis according to the treatments applied.

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240 According to Singh et al. [24], the increase in P availability in the soil allows root growth in 241 depth and the emergence of lateral roots in grasses, such as B. ruziziensis. Therefore, when 242 the opposite scenario is found, the result responds in the same way, as verified for Dry 243 Matter of Roots (RDM). The observed similarity between the control and the BM evidences 244 the low supply of available phosphorus, resulting in poor root architecture (Figure 1). All 245 acidic treatments provided an increase in weight and volume and in the presence of B. 246 ruziziensis root hairs, both related to the increased availability of phosphorus in plants.

247 Observing the behavior of the root: aerial part, the acidification of BM provided a balance 248 between what was produced in the aerial part and in the roots. This fact is reported by Taiz 249 and Zeiger [25], arguing that values close to one represent balance between the 250 photosynthetic rate benefited by adequate nutrition and root expansion promoted by the 251 presence of nutrients in the soil.

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4. CONCLUSION 253

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255 The Shoot Dry Matter and Root Dry Matter from B. ruziziensis were positively influenced by bone meal treatment with acids. Other than acidification with HCI 0.5%, the other treatments 256

provided AEI's above 60%. Acid treatments provided higher PCE's in the absence of acidification. It is recommended to use acid extractors to increase the availability of phosphorus from bone meal, which is an alternative to the shortage of fortified mineral sources in the world

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CONFLICT OF INTERESTS

265 Authors have declared that no competing interests exist.

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