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Nutritive Value and Digestibility of Aruana Guinea Grass under N Fertilization of NBPT- Treated Urea

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Abstract **– Forage plants are the most important food source for ruminants. The nutritive value and digestibility of forage grass depends on N supply. This study aimed to evaluate the effects of N fertilization, rates and nitrogen sources on chemical composition, in vitro digestibility, in Aruana Guinea grass (*Panicum maximum* Jacq.) cultivated in good soil fertility, under greenhouse conditions. The experiment was performed at the Institute of Animal Science, at the municipality of Nova Odessa, Sao Paulo State (Brazil), from April to June (Fall season) using a randomized blocks experimental design with four replication, in a factorial scheme 4 x 2 (rates and nitrogen sources). The grass was grown in clay Oxisol red in which were applied 0; 150; 300 and 450 N kg/ha and two sources of urea (urea untreated and coated urea with the urease inhibitor N-(n-butyl) thiophosphate triamide - NBPT).Two harvests were performed and each of them the grass was harvested about 30 days of growth, the vegetal material was separated into: a) blade leaf and b) stems. The nitrogen application significantly ($P< 0.01$) increased the dry matter accumulation and crude protein (CP) on leaves and stems in both cuts. Nitrogen fertilization increased linearly IVDMD and decreased NDF and ADF. Moreover, there was improved in the values of cell wall constituents and therefore the forage quality. The use of UR + urease inhibitor (NBPT) in regrowth with interval of 28 days resulted in higher CP contents and forage digestibility. Forage quality was improved with N rates greather than 150 kg/ ha.**

Keywords **– Digestibility, *Panicum maximum*, Pasture, Urease Inhibitor.**

I. INTRODUCTION

Grasslands occupy about 174 million hectares of Brazilian territory, cultivated mainly in poor fertility soils. Although the genus *Urochloa* (before classified as *Brachiaria*) is the principal grass for the animal diet, its has a narrow genetic variability, which makes its vulnerable pasture to pasture spittlebug (*Mahanarva* spp.) and a syndrome called “sudden death”, in large areas of Marandu grass in the Northern Brazilian territory, usually related to temporarily flooded areas [1]. Guinea grass (*Panicum maximum* Jacq.) is widely distributed in the tropical and sub-tropical areas of the world [2] and it has been used in the for more intensive cattle production systems as an alternative to *Urochloa*. Aruana guinea grass (*Panicum maximum* cv. Aruana) is the most cultivated Guinea grass in Brazil as pasture for sheep; this grass has small size, high capacity to issue leaves and tillers, rapid regrowth after harvesting, due to the high number of basal buds, besides the excellent acceptability by the animals [3]. Aruana grass has stood out for presenting best annual distribution production [4]; [5] associated with reasonable nutritional value during the critical dry period in the year [5] and it requires medium and high soil fertility [6].

High dry matter production of grasses Guinea with values between 18 and 21 ton/ha/year were obtained with fertilization of 150-200 kg N/ha [7]. Nutritive value of forage is dependent on management adopted, climatic conditions (light, water, temperature) and soil fertilization. Regrowth interval, stubble height and nitrogen (N) fertilization are important management pratices that affect herbage accumulation and nutritive value. For Aruana Guinea grass, the pre-grazing capony height recommended is 30 cm, when the canopy intercepts 95% of the incident light, with post-grazing height at 15 cm [8]. Tropical grasses cultivated in high temperatures have higher dry matter yields, but can exhibit low digestibility [9].The poor quality of tropical grasses, due the high temperature can be improved through if N provided is adequate. The N application from low and moderate rates increases the digestibility by 3-5 percentage units in a tropical grass (*Panicum maximum* Jacq. trichoglume), according to [10].

Urea (UR) is the N source most widely used in Brazilian pastures. UR contains high N concentration (460 g /kg of N) and lower price than ammonium sulfate. UR is hydrolyzed by urease enzyme, abundant in soils, and it is subject to substantial losses by volatilization of ammonia (NH_3) when applied to the surface of both acidic and alkaline soils [11]. Several studies reported average losses of 20-60% of the applied N [12], [13] and [14]. Urease inhibitors, such as N-(n-butyl) thiophosphoric acid triamide (NBPT), have the potential to retard the hydrolysis of urea, is an alternative to reduce the losses by volatilization [15] and to improve N-use efficiency of applied urea minimizing N losses via gaseous emissions of ammonia [16].

The study of protein contents (CP), neutral detergent fiber (NDF) and acid detergent fiber (FDA) is fundamental in qualitative analysis of grasses because these parameters can influence directly or indirectly the consumption of dry

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matter by animals [17].

There is a growing interest in the formulations of coating chemical fertilizers with urease inhibitor. However, there is little information on herbage accumulation and nutritive value of Aruana Guinea grass in tropical regions, under N fertilization with urea treated with urease inhibitor NBPT. We hypothesized that UR treated with NPBT could increase the nutritive value and intermediate rates of N could improve grass quality and production.

Our objective was to evaluate the chemical composition and *in vitro* digestibility of Aruana Guinea grass subjected to N rates, using UR or Urea treated with urease inhibitor (NBPT).

II. MATERIAL AND METHODS

Study site description

This study was carried out from April to July at the Institute of Animal Science and Pastures (IZ) at Nova Odessa, São Paulo State, Brazil (22°42'S, 47°18'W and 528 m altitude). According to the Köppen system, the climate of this region is considered, esothermal humid with an ubtropical dry winter and is classified as Cwa.

The soil for this study was collected in Sertãozinho County, from the 0-20 cm surface layer of a cultivated soil, classified as Typic Hapludox or Red Latosol. After collection, the sample was air-dried, passed through a 2-mm sieve and analyzed to determine its chemical and physical properties (Table 1). Soil analyses were conducted according to the methods escribed by [18] and [19].

Treatment and experimental design

This experiment was conducted in a greenhouse condition, using the forage Aruana Guinea grass (*Panicum maximum* cv. Aruana). Ceramic pots with capacity of 3.5 L were filled with 4.5 kg of dry and sifted soil (mesh sieve = 4 mm). The fertilizer formulations tested were prilled urea (UR, 1-2 mm in diameter, 456 g/kg N). The experiment was arranged in a complete randomized block design with four replicates, in factorial arrangement 4 x 2, four N rates (0; 75; 150 and 225 mg/dm^3, applied 15 days after sowing – 0; 25; 50 and 75 mg/dm^3, after the first harvest 0; 50; 100 and 150 mg/dm^3, totaling the equivalent of 0; 150; 300 and 450 kg/ha) and combined with urea treated or untreated with the urease inhibitor N-(n-butyl) thiophosphate triamide (NBPT). This resulted in a total 32 experimental units, arranged randomly.

NBPT (produced by AGROTAIN International, LLC, and St. Louis, MO, USA) was used as a solution containing 200 g/kg of the active ingredient. The NBPT solution was applied to 1 kg of prilled urea at a rate of 1060 mg NBPT per kg of urea one day prior to application of the fertilizer to the soil. The rates of NBPT used in the present study are comparable to the ones used in the works of [20], [21] and [22]. Recommendations of NBPT vary from 400 to 1040 mg/kg urea [23] and [24].

Twenty five Aruana Guinea grass were sown in each pot and after successive thinnings, remained five plants per pot and a basic fertilizer was performed at that time. Pots were fertilized with phosphorus = 35 mg/dm^3, potassium = 30 mg/dm^3 and sulphur = 15 mg/dm^3, provided through KH_2PO_4 and Na_2SO_4.

Two harvests of the plants were performed at the following times: 30 days after germination and 28 days after the first harvest. Each plant was harvest at 6 cm height from the soil surface, and the plant material was separated into leaf blades and stem (stems and sheaths), dried in an oven with forced ventilation at 65°C until a constant mass was reached. During the experiment, soil moisture was maintained at 80% of field capacity by an automatic subsurface irrigation system, which replaced the water according to the evapotranspiration of the soil-plant system.

The chemical analyzes were performed at the Bromatological Laboratory of Animal Science Institute. The samples were ground in a Willey mill with a 1mm sieve. For chemical analysis sub-samples were dried at 105ºC and determined the contents of crude protein (CP) by the semi-micro Kjeldahl method [25], neutral detergent fiber (NDF) and acid detergent fibre (ADF) by the method described by [26]. The contents of lignin (sa) according to [27] and in vitro dry matter digestibility (IVDMD) by [28].

Statistical analyses

The statistical model used was the following: $Yij = \mu + Ti + Nj + TNij + Bl + Eij$, with Yij = observation; μ= overall mean; Ti = nitrogen rate effect (i = 1- 4); Nj = nitrogen source (1-2); TNij = interaction N rate and sources; Bl = block effect (l = 1-4) and Eij random residual error associate with Yij and Eij ~$N(o\sigma^2)$

Data were subjected to variance analysis using SAS® (Statistical Analysis System, version 9.3) statistical package and a 5% significance level. For the N rates, regressions were performed using the General Linear Model (GLM) procedure. A level of significance ($P < 0.05$ or $P < 0.01$) was used for all statistical tests. Regression analysis (linear and quadratic effects) was determined.

Table 1: Chemical and physical properties* of soil sample used.

pH	OM	P	K	Ca	Mg	H+ Al	CEC	V	Clay	Silt	Sand
	g/dm^3	Mg/dm^3	------------------mmol$_c$ /dm^3---------------					(%)	--------- g/kg ---------		
6.0	34	10.0	3,4	54	25	22	104.6	79	701	180	119

* pH $CaCl_2$: $CaCl_2$ 0.0125 mol/ L; OM: organic matter: oxi-reduction; P, K, Ca, Mg: extracted with ion-exchange resin; H + Al: buffer solution at pH 7.0; CEC: cation-exchange capacity; V: base saturation; soil texture: densimeter method.

III. RESULTS AND DISCUSSION

Forage accumulation and Chemical composition

The forage accumulation and chemical composition of leaf blades and stem of Aruana Guinea grass are shown in Table 1, Table 2 and Table 3.

Dry matter accumulation of leaf and stem of Aruana Guinea grass increased significantly as the level of applied N increased. The regressions analysis showed linear responses for dry matter accumulation for leaf and stem (both use of urea or coated urea NPBT). In first and second harvest, dry matter accumulation of leaf represents an average of 76% of total biomass and the 24 % of stem. All nutrients in leaf blades were affected by N fertilization, except dry matter digestibility of leaf blades. Leaf and stem crude protein content increased linearly with N rates and were similars for both N sources - UR or UR +NBPT, with values for leaf blades: $CP_{UR} = 16.61 + 0.033N$ and $CPU_{R+NPBT} =16.58 + 0.029 N$. Stem CP values ($CP_{UR} = 9.20 + 0.02N$ and $CP_{UR+NPBT} = 9.34 + 0.015N$) were lower ($P < 0.05$) in comparison to leaf blade contents. Applying 450 kg N/ha increased CP from 15.8% to 24.6% in leaf blades and from 9.0 to 14.0% in pseudocolms, an increase of 56% in first harvest was observed - corresponding to the initial growth period (30 days).

NDF and ADF varied according to N rates. NDF leaf blades was quadratically decreased ($NDF_{UR} = 61.5 - 0.073N + 0.0002N^2$ and $NDF_{UR+NBPT} = 61.7 - 0.062N + 0.0002N^2$) with N fertilization, resulting in higher NDF contents with application rates of 181.0 kg/ha for UR and 154.0 kg/ha for UR +NBPT, respectively. Probability N source as UR + NPBT promotes the acceleration of plant growth and stimulates incorporation of cell wall constituents. This results in N stocking amounts to be applied to the system, which reduces environmental impact due to application of excess N. NDF stem and both ADF leaf blades and stem were significantly reduced ($P < 0.0001$) by N fertilization. NDF and ADF in stem were higher for all N rates than leaf blades. Unfertilized Aruana Guinea grass had NDF contents in blades and stems that averaged 61.9% and 71.5%, respectively. Such fact is justified once the pseudostems show higher non-digestible fiber proportions in comparison to leaf blades, needed to maintain the architecture of each stem in the pasture field. In first harvestting, no effect of N rate and sources on digestibilities of DM on leaf blades (average 82.5%) and stem (60.3%) were observed. These values of digestibility were considered high.

Table 2: Forage accumulation (g/pot), chemical composition and *in vitro* dry matter digestibility (IVDMD) in leaf blades (LB) and stem (ST) of Aruana Guinea Grass subjected to nitrogen fertilization, first harvest cycle.

Chemical component	**Nitrogen sources**	**Nitrogen application rates (kg/ha)**					**Probability**^{a}	
		0	**150**	**300**	**450**	**SEM**	**L**	**Q**
Forage accumulation - LB	UR	2.72	9.58	12.28	13.31	1.14	<0.01	0.18
	UR+NBPT	2.72	8.32	12.85	13.50	1.05	<0.01	0.14
Forage accumulation - ST	UR	2.71	8.75	11.98	12.09	1.32	<0.01	0.38
	UR+NBPT	2.71	8.11	10.73	11.82	1.17	<0.01	0.43
Crude protein (%) - LB	UR	15.8	20.6	23.9	25.6	1.22	<0.01	0.15
	UR+NBPT	15.8	20.3	23.1	24.1	1.19	<0.01	0.16
Crude protein (%) - ST	UR	9.00	11.4	13.5	14.9	1.01	<0.01	0.17
	UR+NBPT	9.00	11.0	13.9	13.2	1.02	<0.01	0.15
Neutral detergent fiber (%) LB	UR	61.9	54.9	55.7	56.3	1.72	<0.01	<0.01
	UR+NBPT	61.9	56.7	57.4	59.4	1.73	<0.01	<0.01
Neutral detergent fiber (%) ST	UR	71.5	64.9	63.5	57.6	1.54	<0.01	0.14
	UR+NBPT	71.5	65.0	63.8	62.9	1.41	<0.01	0.13
Acid detergent fiber (%) - LB	UR	27.1	24.0	22.7	22.8	1.08	<0.01	0.42
	U+NBPT	27.1	24.4	24.3	22.5	1.10	<0.01	0.54
Acid detergent fiber (%) - ST	UR	34.7	32.0	31.2	29.8	0.98	<0.01	0.16
	UR+NBPT	34.7	33.3	32.1	29.4	0.89	<0.01	0.18
IVDMD (%) - LB	UR	81.8	83.0	82.1	82.8	0.40	0.43	0.58
	UR+NBPT	81.8	83.6	82.2	82.9	0.39	0.31	0.54
IVDMD (%) - ST	UR	67.3	60.0	54.4	55.9	0.70	0.21	0.51
	UR+NBPT	67.3	66.7	53.7	57.4	0.68	0.19	0.52

[a] L: linear effect; Q: quadratic effect.

Crude protein content (CP) in regrowth both leaf blades and stem increased linearly with N fertilization with values varying, for leaf blades in the range 7.9-16.1% ($CP_{UR} = 0.018N + 7.79$) and 7.9-19.9% ($_{CPUR+NBPT} = 0.028N + 7.18$) and to stem from 4.6 to 7.4% (CPUUR =0.006N + 4.97) and 4.6 to 7.3 ($CP_{UR+NBPT} = 0.007N + 4.31$). Each kilogram of N resulted in a 0.18 and 0.28 percentage unit increasing leaf blades CP content with urea and coated urea with NBPT. Applying 300 kg N/ha as UR+NBPT increased CP leaf blades from 13.2% to 16.3% related to the use of UR uncoated, an increase of 23.5% was shown. These values were lower than those found in the first harvest, indicating lower residual effects of nitrogen contained in the soil, due to the losses and the lower share it in soil (Table 3). In general, leaf blades show higher N values than pseudostems, due to the

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metabolic process, which is more intense in the leaves. N fertilization led to a linear decreased of NDF leaf blades: $NDF_{UR} = -0.0181N + 66.7$ and $NDF_{UR+NBPT} = - 0.0175N + 66.9$ and ADF stem $ADF_{UR} = -0.011N + 35.65$ and $ADF_{UR+NBPT} =- 0.010N + 35.1$. Unfertilized Aruana guinea grass had NDF and ADF content in leaf blades and stem that averaged 67.2%, 69.1% and 27.7% and 35.6%, respectively. Digestibility of DM in leaf blades and stem were increased linearly with N fertilization ($IVDMDLB = 0.035N + 61.85$ and $IVDMDST = 0.016 N + 60.88$).

Table 3: Chemical composition and in vitro dry matter digestibility (IVDMD) in leaf blades (LB) and stem (ST) of Aruana Guinea grass subjected to nitrogen fertilisation, second harvest cycle.

Chemical component	Nitrogen sources	Nitrogen application rates (kg/ha)				SEM	Probability[a]	
		0	150	300	450		L	Q
Forage accumulation - LB	UR	0.49	1.93	4.33	5.13	1.02	<0.01	0.21
	UR+NBPT	0.49	2.06	4.03	4.82	1.04	<0.01	0.15
Forage accumulation - ST	UR	0.52	2.08	3.82	4.32	1.12	<0.01	0.18
	UR+NBPT	0.52	2.17	3.90	3.49	1.09	<0.01	0.22
Crude protein (%) - LB	UR	7.9	10.4	13.2	16.1	1.22	<0.01	0.14
	U+NBPT	7.9	10.0	16.3	19.9	1.18	<0.01	0.23
Crude protein (%) - ST	UR	4.6	6.2	7.5	7.4	0.80	<0.01	0.14
	U+NBPT	4.6	4.6	7.1	7.3	0.73	<0.01	0.15
Neutral detergent fiber (%)- LB	UR	67.2	63.0	61.5	58.7	1.02	<0.01	0.16
	U+NBPT	67.2	64.3	61.0	59.6	0.98	<0.01	0.14
Neutral detergent fiber (%)- ST	UR	69.1	66.7	67.7	68.5	1.88	0.12	0.15
	U+NBPT	69.1	69.6	68.9	69.2	1.59	0.13	0.17
Acid detergent fiber (%)- LB	UR	27.7	28.9	27.9	29.1	1.40	0.14	0.56
	UR+NBPT	27.7	29.8	28.9	29.1	3.42	0.15	0.48
Acid detergent fiber (%)- ST	UR	35.6	33.2	32.3	30.6	1.10	<0.01	0.25
	UR+NBPT	35.6	34.0	32.1	30.4	1.21	<0.01	0.28
IVDMD (%)- LB	UR	63.1	62.2	73.0	76.4	0.70	<0.01	0,16
	UR+NBPT	63.1	65.8	73.6	79.3	0.84	<0.01	0.35
IVDMD (%)- ST	UR	61.5	62.5	66,0	68.5	0.73	<0.01	0.17
	UR+NBPT	61.5	62.5	65.0	68.7	0.79	<0.01	0.16

[a] L: linear effect; Q: quadratic effect.

In general, the N fertilization increased the dry biomass production of leaf blades in comparison to the increase of pseudostem biomass, presenting lower participation in the capony. Such characteristic of the Aruana Guinea Grass is desirable in the management of pasture, once the blades play important role in the pasture system ecology producing essential factors for plant maintenance and growth.Positive responses to N fertilization on the dry matter of forage grasses were reported by several authors [29] and [4]. On the other hand, according to [30] pseudostem development can increase the dry matter production but causes a direct influence on the sward structure, leadind to negative effects on the forage quality and its animal grazing performance reducing the leaf blade production. The relation from leaf blades and stems is a tool for the management of forage, considering critical limits of 1.0, indicating decrease on the amount and quality of produced forage [31]. In the present work, such relation is between 2.59 and 5.55, above the critical level.

The nutritive values, determined by the chemical composition of the crude protein (CP) unsoluble acid fiber (ADF) and digestibility were affected by N fertilization. The consistent increase in CP content in response to N fertilization was observed in both the UR and UR + NBPT application. [32] observed in *Panicum maximum*, the N media concentration in budding and Green leaves were around 15 and 25 g /kg equivalent to 9.38 and 15.63% of CP. [29] had values from 18.8 and 21.6 g/kg (equivalent to 11.8% and 13.5% CP) as N critical concentration in leaf blades of Aruana grass respectively during first and second cuts. In second harvest CP contents in Aruana Guinea grass leaves with the addition of NBPT, were higher in comparison to untreated UR. Several studies conducted with Brazilian soils have shown that surface-apllied UR presents high losses of NH3 ranged from 18 to 64% of the N applied [12], [13] and [14]. The UR with urease inhibitor treatment reduces NH_3 losses by volatilization and to improve N-use efficiency that results in increase of CP contents. In pasture of *Urochloa* (syn. *Brachiaria*) *brizantha* Marandu the application of urease inhibitor NBPT reduced NH_3 volatilization 50% in comparison to untreated urea. This reduction provides an increase of total N in the forage, major recovery of N applied and increased in the dry biomass yield [14]. With addition of NBPT delayed the peak of NH_3 losses due to urease inhibition and reduced NH_3 volatilization between 54 and 78% in comparison to untreated UR [16]. Although the nutritional requirements of Aruana Guinea grass are higher when compared with other forage plants, such as those the genus *Urochloa* (syn. *Brachiaria*). The CP leaf blade in first harvest was 15.8% for the treatment unfertilized. The soil of this experiment classified as Typic Hapludox, is considered of good fertility, with organic matter (34 g/dm^3), which mineralization can supply N requirements of Aruana grass, resulting in high contents of CP in first harvest for the treatment unfertilized, and added the effects of N foliar concentration due the lower growth of grass. CP or N contents in leaf blades (which represent 76% of the biomass aerial) are considered adequate (between 15 and 25 g N /kg) and not limiting for nutrition Aruana Guinea grass (first harvest) and second harvestting subjected to rates from 150 kg N per hectare. Concerning crude protein concentration in Aruana Guinea grass leaves, the recorded values ranged from 15.8 to 25.6 % (first harvest) and from 7.9 to 19.9 % (second harvest) of crude protein and were higher than the 7.0%, which is considered by [17] to be the minimum amount necessary to maintain a microorganism population in the rumen of cattle.

A percentage of NDF and ADF were lower with the application of N rates. As expected, leaf blades were smaller than stems. Similar results were obtained from the different N doses and did not differ statistically (P>0.05). The FDN found are according to the ones reported by [30], [33] around 58 and 72% of NDF, once values above 65% are common in young tissues [30]. Values of ADF were around 22.5 and 35.6%, when above 40%, it indicates low consumption levels [34]. N fertilization had a positive impact on Aruana Guinea Grass forage quality increasing CP and reducing NDF and ADF, once N fertilization could be a result of better plant growth. To [17], addition of N for forage can increase protein concentration in the cell and it has effect in the cell wall reducing FDN concentration. Under conditions of rapid growth, the stem tracheids mature rapidly, even with thin walls, because the very active meristematic regions drain most of the available assimilate [35]. Thus, less carbohydrate is available for secondary thickening and lignifications of the cell walls before the tracheids mature and lose their cytoplasm [36]. Nitrogen fertilization stimulated the formation of neutral monossaccharides (glucose and arabinose) in cell walls of Milenio grass (*Panicum maximum*), resulting in better-quality fiber and higher forage digestibility [36]. This process possibly occurred in this trial, as suggested by chemical composition and digestibilities of DM and fiber components.

DM digestibility is primarly a function of NDF and ADF concentration. In this way the re growth leads to higher ADF content and a lower NDF was observed for the high N application rates. Leaf blades digestilibilities of DM with UR and UR+NBPT was improved by 13.4 and 16.2%, respectively, with higher N dosages. The harvest period, in this work was adjusted to 28 days maintaining the high regrowth proportion for leaf blades (76%) and 24% for stem with nule senescent material, keeping nutritive value of Aruana Guinea grass. Digestibility values for Aruana Guinea Grass were observed by [37] during the first 40 days of growth, causing the decrease of nutritive values due to the higher number of stems and higher cell wall proportion (NDF and ADF), limitating the disponibility of this food in the pasture. Mulato Grass and Aruana present lower FDA levels in comparison to Massai Grass, as well as the in vitro digestibility of organic matter (DIVMO) [38]. Besides that, the increase in CP may favor forage digestion [39].

As general, the efficiency of NBPT addition in the urea was low for the conditions in the warm tropical soil with natural high fertility, high moisture through irrigation in comparison to exclusive urea application. Another alternative is a combination of nitrification (for example – dicyandiamide) and urease inhibitors added to UR, but may not always produce the desired purpose of both inhibitors to reduced N losses [40] and [16].

IV. CONCLUSION

Nitrogen fertilization increased CP and decreased NDF and ADF. The use of UR + urease inhibitor (NBPT) in regrowth with interval of 28 days resulted in higher CP contents and forage digestibility. Forage quality was improved with N rates above 150 kg/ha.

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