BUKTI KORESPONDENSI ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel	: Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia
Jurnal	: Tropical Grasslands-Forrajes Tropicales (2019) Vol. 7(5):524–530
Penulis	: Asep Indra Munawar Ali, Sofia Sandi, Riswandi & Muhakka

No	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit	26/01/2016
2	Bukti konfirmasi review dan hasil review	-
3	Bukti konfirmasi submit artikel dan artikel yang disubmit	-
4	Bukti konfirmasi resubmit artikel dan artikel yang diresubmit	-
5	Bukti konfirmasi review dan hasil reviewers	25/11/2019
6	Bukti konfirmasi submit artikel dan artikel yang disubmit	27/11/2019
7	Bukti konfirmasi artikel accepted	-
8	Terpublish	30/11/2019

Tropical Grasslands-Forrajes Tropicales



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1 Seasonal influence on mineral concentration of forages on flooded pastures in South

2 Sumatra of Indonesia

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6 Abstract

7 This study was conducted to evaluate macro and micro mineral concentration of forages on 8 flooded pastures in non tidal swamp of South Sumatra of Indonesia. Five species of Poaceae, 9 four species of Mimoceae, three species of Cyperaceae and one species of Onagraceae were 10 collected during flooded and dry seasons. The results showed that mineral concentrations of 11 forages greatly varied between seasons. Higher concentration of Ca and Fe in flooded season and 12 higher concentration of Mg in dry season were found in most forages. All forages had deficient P 13 but most forages had sufficient Mg concentration and just Mimoceae and L. peploides had 14 sufficient Ca for grazing buffalo requirement. Sulphur sufficiency occured in some of Mimoceae 15 and Cyperaceae species. Most forages had Cu, Fe, Zn, and Mn concentrations which more than 16 critical level for buffalo grazing even though all deficiencies were found in flooded season. 17 Copper deficiency occurred in Brachiaria muticum, Mimosa gigantica, Scirpus grossus and 18 Scleria pterora; Zn deficiency occured in Oryza Rupifogon; Mn deficiency occured in 19 *Eleocharis dulcis* in flooded season. Copper toxicity occured in *Neptunia oleraceae* in dry 20 season, Aeschynomene sensitive and Ludwigia peploides during all seasons. Iron toxicity occured 21 in Leersia hexandra in flooded season, Eleocharis dulcis and Ludwigia peploides in all seasons 22 and Mn toxicity occured in Neptunia oleraceae in dry season. 23 Keywords: buffalo, deficiency, toxicity 24

25 Introduction

26 Natural pasture species in non-tidal swamp have been the main source of feed for swamp buffalo 27 in South Sumatra. Forage nutritive values fluctuate with seasons and the dry season is the most 28 limiting in terms of nutrient supply to grazing buffaloes. Most grazing livestock in tropical 29 countries including Indonesia fulfill their mineral requirements usually only from the forages. 30 Forages represent important source of minerals for grazing ruminants, and play essential roles in 31 preventing diseases and inhibiting or stimulating ruminal microbial activity (Spears 1994). 32 The concentration of individual minerals in forages varies greatly depending on interaction of 33 number of factors including soil, plant species and stage of maturity, yield, management factor, 34 and climate. Mineral profiles of tropical forages in flooded pasture have been received little 35 attention, and in some situation they are deficient in one or more elements, thus supplementation 36 is required for optimal animal performance and health (Camarao et al. 2002; Camarao et al. 37 2004).

Data on variation of the mineral concentrations in forage species and their variability between
seasons are essential for proper or cost effective feed formulation and swamp buffalo nutrition.
Thus, objectives of this study were to estimate and compare seasonal dynamics of Ca, P, Mg, S,
Cu, Fe Zn, and Mn of native flooded pasture species in non-tidal swamp of South Sumatra,
Indonesia.

43 Materials and methods

44 Site description

45 Research was conducted on February until September 2014 located in non-tidal swamp

46 administrated in Rambutan subdistrict, Banyuasin district and Pampangan subdistrict, OKI

47	(Ogan Komering Ilir) district of South Sumatra Province and geographycally covered on
48	3°05'27.2" – 3°11'24.9" South Latitude and 104 °55'23.9" – 104 °58'57.9" East Longitude.
49	The dry season begins from April to September while the rainy season relatively occurs from
50	October to March with the average of rainfall being 2,100 - 3,264 mm per year. The research
51	location has lowest temperatures ranged from 22 to 25°C (at 05.00 - 08.00 a.m.) and highest
52	temperatures ranged from 30 to 34°C (11.00 a.m 02.00 p.m.).
53	Forage sampling
54	Native vegetation species consumed by buffaloes include Poaceae (Brachiaria muticum, Leersia
55	hexandra, Hymenachne acutigluma, Ischaemum rugosum, Oryza rupifogon), Mimoceae
56	(Mimosa gigantica, Sesbania exasperata, Neptunia oleracea, Aeschynomene sensitive),
57	Cyperaceae (Scirpus grossus L, Scleria pterora Presl, Eleocharis dulcis), and Onagraceae
58	(Ludwigia peploides) were collected from different part of flooded pasture.
59	Observation of forage was conducted by observing the species of vegetation which consumed by
60	grazing buffaloes. This observation was played on 08.00-11.00 a.m. and 02.00-05.00 p.m (Hirata
61	et al. 2008; Setianah et al. 2004) in our previous study (Ali et al. 2013). The forages were
62	collected by hand picking during flood in wet season (March 2014) and dry season (August and
63	September 2014) from at least nine different sites. Samples were chopped, dried at 50 °C, pooled,
64	and then coarsely milled to pass 1 mm screen for mineral analysis.
65	Mineral analysis
66	For each season and each forage species, representative samples (duplicate) were digested
67	stepwise with nitric acid (HNO ₃). Concentrations of minerals of the forage samples were

68 analyzed by using inductively coupled plasma emission spectrometer (SPS7700, Seiko

Instruments Inc., Chiba, Japan) in Laboratory of Feed Technology and Dairy Nutrition, Faculty
of Animal Husbandry, Bogor Agricultural University.

71 Statistical analysis

72 Data were analyzed using StatView SAS (1999). The differences of means between seasons were

73 determined using unpaired t-test. The difference was assumed statistically significant (P<0.05).

74 Result

75 There was difference (P<0.05) in Ca. P, Mg, and S concentration within seasons (Table 1).

76 Calcium concentration in most forages was higher in flooded than in dry season except in *B*.

77 *muticum*, *N. oleracea*, *S. pterora Presl*, *E. dulcis*, and *L. peploides*. With an exception of *B*.

78 *muticum*, Ca concentrations in Poaceae and Cyperaceae lower than Ca concentrations in

79 Mimoceae. Similar to Ca, the concentration of P in forages varied (P<0.05) between seasons on

80 most forages except in O. rupifogon, A. sensitive, S. pterora Presl and E. dulcis. On the contrary

81 to Ca concentration, Mg concentration in most forages was higher in dry than in flooded season

82 except in S. grossus L, I. rugosum, N. oleracea, A. sensitive, and H. acutigluma which was

83 higher in flooded than in dry season.

84 There was difference (P<0.05) in micro minerals concentration within seasons (Table 1). In most

85 Poaceae species, Cu concentrations not significantly different between seasons except in *B*.

86 *muticum* while Fe, Zn and Mn concentrations fluctuated between seasons. Higher Fe

87 concentrations in flooded season were found in most forages while other micro minerals

88 fluctuated between seasons in most forage.

89

Species	Sagar	Macı	ro mine	ral (%]	DM)	Mi	Micro mineral (ppm DM)			
Species	Season	Ca	Р	Mg	S	Cu	Fe	Zn	Mn	
Poaceae										
B. muticum	dry	0.62^{b}	0.10^{b}	1.59 ^b	0.50 ^b	11.6 ^b	105.0 ^a	41.8 a	366.2 ^b	
	flooded	0.11 ^a	0.01 ^a	0.86 ^a	0.04 ^a	9.6 ^a	107.0 ^a	60.2 ^a	128.0 ^a	
L. hexandra	dry	0.08^{a}	0.16^{b}	1.01^{b}	0.13 ^a	15.3 ^a	193.2 ^a	128.0 ^b	274.0 ^a	
	flooded	0.12^{b}	0.09 ^a	0.84^{a}	0.14 ^a	12.3 ^a	806.0 ^b	97.8 ^a	283.0 ª	
H. acutigluma	dry	0.07^{a}	0.11 ^b	1.02^{a}	0.17 ^a	15.5 ^a	139.0 ^a	40.9 ^a	225.0 ^a	
	flooded	0.19 ^b	0.08 ^a	1.09 ^b	0.09^{a}	17.5 ^a	208.0 ^b	56.8 ^b	252.0 a	
I. rugosum	dry	0.08^{a}	0.04^{a}	1.03 ^a	0.06^{a}	13.2 a	184.0 ^b	57.0 ^a	125.0 a	
	flooded	0.14 ^b	0.07 ^b	1.11 ^b	0.38 ^b	12.2 ª	121.0 ^a	78.1 ^a	297.0 ^b	
O. rupifogon	dry	0.12^{a}	0.05 ^a	1.19^{b}	0.03 ^a	16.8 ^a	499.0 ^b	45.8 ^b	906.0 a	
	flooded	0.16 ^b	0.08^{a}	0.95 ^a	0.03 ^a	13.6 ^a	188.0 ^a	26.1 ^a	1044.0 ^b	
Mimoceae										
M. gigantica	dry	0.32 ^a	0.14^{b}	1.68^{b}	0.30 ^b	22.1 ^b	206.0 ^a	76.9 ^b	252.2 ª	
	flooded	0.39^{b}	0.10^{a}	1.54 ^a	0.14 ^a	8.6 ^a	317.0 ^a	33.7 ^a	342.1 ^a	
S. exasperata	dry	0.42^{a}	0.17^{b}	1.41^{b}	0.30 ^b	23.4 ª	100.0 ^a	76.4 ^a	153.0 ^a	
	flooded	0.64^{b}	0.15 ^a	1.17^{a}	0.23 ^a	21.3 ^a	181.0 ^b	64.3 ^a	128.0 ^a	
N. oleracea	dry	0.66 ^b	0.07^{a}	1.80^{a}	0.05 ^a	43.3 ^b	202.0 a	176.0 ^b	1076.0 ^b	
	flooded	0.43 ^a	0.14^{b}	2.01 ^b	0.18 ^b	21.7 ^a	358.0 ^b	43.2 ^a	402.0 a	
A. sensitive	dry	0.46 ^a	0.12 ^a	1.98 ^a	0.43 ^a	29.2 ª	129.0 ^a	86.7 ^a	407.0 ^b	
	flooded	0.48^{b}	0.13 ^a	2.35 ^b	0.40 ^a	42.1 ^b	145.0 ^a	94.4 ^a	286.0 a	
Cyperaceae		0110	0.120	2100	0110					
S. grossus L	dry	0.08^{a}	0.21 ^b	0.65 ^a	0.31 ^a	14.3 ^b	89.4 a	49.6 ^b	291.0 a	
-	flooded	0.20 ^b	0.11 ^a		0.33 ^a	9.9 a	318.0 ^b	43.3 a	568.0 ^b	
S. pterora Pres	<i>l</i> dry	0.25 ^b	0.06^{a}		0.05 ^a	14.7 ^b	69.0 ^a	48.1 ^a	487.0 ^b	
1	flooded	0.20^{a}	0.08^{a}		0.03 ^b	9.6 ^a	151.0 ^b	62.3 ^a	210.0 a	
E. dulcis	dry	0.08 ^b	0.08^{a}		0.08 ^a	20.1 ^a	936.0 ^a	55.1 ^b	231.0 ^b	
	flooded	0.03^{a}	0.14^{a}		0.29 0.49 ^b		1629.0 ^b	28.0 ª	34.4 ^a	
Onagraceae	1100000	0.05	0.20	0.17	0.49	17.1	102910	20.0	5111	
L. peploides	dry	0.56 ^b	0.16 ^a	2.62^{b}	0.16 ^a	31.2 ^a	728.0 ^a	198.0 ^a	969.0 ^b	
	flooded	0.33 ^a	0.21 ^b		0.15 ^a	244.0 ^b	926.0 ^b	207.0 ^a	691.0 ^a	
Critical level*		0.30	0.21	0.20	0.15	11	50	33	40	
Toxic level *						25	500	750	1000	

Table 1. Concentrations and seasonal variation of macro and micro minerals in forages

^{a,b}Means with different superscripts within a species between season are significantly different (P<0.05).

* in accordance with McDowell et al. (1997).

91 **Discussion**

92 *Macro minerals*

93 In most forages, the concentration of minerals declined with advancing maturity and could 94 probabably be explained by their genetic variations, climate or mineral level in soils (Minson 95 1990; Underwood and Suttle 1999). It was observed that Ca concentrations of Mimoceae 96 (legume) and most Poaceae (grass) were higher in flooded season than in dry season. This was 97 agreed with a study in dry land of South Sumatra, Indonesia (Evitayani et al. 2006) which 98 showed that Ca concentration in two species of grasses and four species of legumes in rainy 99 season was relatively higher than in dry season and varied from 0.57 (*Pennisetum purpuphoides*) 100 to 2.61% (Leucaena leucocephala) in rainy and from 0.51 (P. purpuphoides) to 2.44% (L. 101 *leucocephala*) in dry season. Oppositely, Warly et al. (2006a) in dry land of West Sumatra 102 concluded that Ca concentration of seven species of grasses and 13 species of legumes in rainy 103 season were lower than in dry season and ranged from 0.39 (Euchlaena mexicana) to 1.69% 104 (Gliricidia maculata) while Nasrullah et al. (2004) in dry land of South Sulawesi showed that the 105 concentration ranged from 0.3 (Cenchrus ciliaris) to 4.0% (Desmanthus virgatus). Results of the 106 current study showed that range of Ca concentration was lower than previous studies (Nasrullah 107 et al. 2004; Evitayani et al. 2006; Mtui et al. 2006; Warly et al. 2006a). Calcium concentration in 108 Mimoceae and Onagraceae was more than 0.30% which meet the ruminant's requirement (Mc 109 Dowell et al. 1997), in agreement with the previous studies that Ca concentration was normally 110 higher in legumes than in grasses. This indicated that Mimoceae is good Calcium source for 111 grazing buffalo in all seasons on flooded pasture.

Phosphor concentration in all forages in present result was lower than ruminant's requirement
(0.25%) and fluctuated in both seasons. Nasrullah et al. (2004) reported that P concentration

114 ranged from 0.20 (E. mexicana) to 1.80% (Centrocema plumieri) and was not significantly 115 affected by seasons. Warly et al. (2006a) concluded that P concentration was higher in dry 116 season than in rainy season and range from 0.21 (*E.mexicana*) to 0.47% (Setaria sphacelata). 117 Study by Evitayani et al. (2006), which sampled same species but different location, showed that 118 P concentration ranged from 0.29 (*Centrocema pubescens*) to 0.68% (*P. purpuphoides*) and was 119 higher in rainy season than in dry season and all the forages had P concentration more than 120 0.25% in rainy season. The low concentration of Ca and P in current results related to acid soil 121 condition in study location with low availability of Ca and P for plants (Ali et al. 2013). Study of 122 Jumba et al. (1995) showed the significant correlation between soil P and herbage P while Kano 123 et al. (2006) showed that soil pH not only related to grass growth and P uptake but also 124 mycorrhizal dependency. 125 Underwood and Suttle (1999) recomended that diets of livestock should have Ca: P ratio about 126 1:1 to 2:1 and more important than the absolute concentration for utilization by ruminants. This 127 present results showed that the Ca:P ratio range from 0.16 (E. dulcis) to 10.0 (B. muticum) and 128 implicated that high Ca concentration increase the demand of P in buffalo diets. Study by Jones 129 (1990) and Miller et al. (1990), which described some low cost strategies in dry land, was

130 recommended for overcoming P deficiency in the flooded pasture.

131 With an exception of *E. dulcis* in flooded season, all forages had sufficient Mg concentration for

132 ruminant's requirement (Mc Dowell et al. 1997) which indicated that buffalo consuming

133 varieties of forages in the area are unlikely to had Mg sufficiency. This agreed with Minson

134 (1990) that grass tetany, which caused by Mg deficiency, in the ruminant grazing on tropical

135 forage have not been reported. The sufficiency of Mg concentration in most forages also reported

136 by previous studies (Nasrullah et al. 2004; Evitayani et al. 2006; Warly et al. 2006a). Unlike Ca 137 and P concentrations, range of Mg concentrations was higher than previous studies in dry land. 138 Sulphur provides microbial protein synthesis in rumen and its concentration depends on the 139 availability of soil sulphur, nitrogen and phosphor, and the maturity of the forage. The 140 concentration of S varied (P < 0.05) between seasons in seven of thirteen forage species. Sulphur 141 deficiency occured in most Poaceae species except on B. Muticum, and I. Rugosum and most 142 Mimoceae and Cyperaceae species had sufficient S concentrations for ruminant's requirement. 143 Sulphur deficiency in dry season was reported by Evitayani et al. (2006). Warly et al. (2006a) 144 reported that percentage of forage species with S deficiency was higher in dry season than in 145 rainy season. The present results indicated that high level of Mimoceae and Cyperaceae in 146 buffalo diet lead sufficient consumption of Ca, Mg, and S in the flooded pasture, which 147 dominated by Poaceae and Cyperaceae spesies (Ali et al. 2013). 148 Micro minerals 149 Based on mineral requirement (Mc Dowell et al. 1997) most forages in flooded pasture had Cu, 150 Fe, Zn, and Mn concentration more than critical level of requirement for grazing ruminants in 151 tropical region and had range of concentrations higher than in dry land. Copper deficiency was 152 found in B. muticum, M. gigantica, S. grossus, and S. pterora in flooded season while Cu toxicity 153 was found in N. oleraceae in dry season, in A. sensitive and L. peploides in all seasons. The 154 range of Cu concentration in Poaceae and Mimoceae in present results was higher than range

reported by Fariani (2008) from 5.6 to 10.1 ppm and 3.8 to 16.6 ppm in three species of grasses

and four species of legumes in dry land of South Sumatra. Warly et al. (2006b) reported that Cu

157 concentration of thirteen species of grasses varied from 2.3 to 14.4 ppm.

158 In present results, Cu concentration of most Poaceae species not significantly varied between 159 seasons except *B. muticum*. Warly et al. (2006b) also reported similar result but Cu deficiency 160 was higher in dry season than in rainy season while Fariani (2008) reported that Cu 161 concentration of Axonopus compressus and Panicum maximum was higher in dry season than in 162 rainy season. Copper concentrations of Mimoceae in current results varied between seasons. 163 Other studies also reported similar result (Mtui et al. 2006), or higher in rainy than dry season 164 (Khan et al. 2006 and Evitayani and Warly 2010). Khan et al. (2006) reported range from 10.91 to 25.69 ppm while Evitayani and Warly (2010) reported range from 3.8 to 16.6 ppm in West 165 166 Sumatra.

167 All forages had Fe concentration more than critical level for ruminant's requirement while 168 toxicity was found in *L. hexandra* in flooded season, *E. dulcis*, and *L. peploides* in all seasons. 169 Present results showed that Fe concentration in most Mimoceae, Cyperaceae, and Onagraceae 170 species was higher in flooded season than in dry season and could be explained probably due to 171 natural dilution process and translocation of minerals to the root. Similar result was reported by 172 Mtui et al. (2006) with range from 66.76 to 618.9 ppm, and Warly et al (2006b) with range from 173 96.3 to 569.2 ppm. Khan et al. (2006) also reported that all forages had Fe sufficient and higher 174 concentration in winter than in summer and ranged from 92.53 to 201.73 ppm. Differently 175 Fariani (2008) reported that the higher Fe concentration was just found in grasses with range 176 116.40 to 511.40 ppm. The excessive Fe concentration was also reported by Rubanza et al. 177 (2006) and Warly et al. (2006b).

178 It was observed that Zn concentration in all forages was less than toxic level while Zn deficiency

179 was just found in O. rupifogon and E. dulcis in flooded season. Mn deficiency was found in E.

180 *dulcis* in flooded season while toxicity in *N. oleraceae* in dry season. The low Zn and Mn

concentration was also reported by Fariani (2008), Mtui et al. (2006), Rubanza et al. (2006), and
Warly et al. (2006b). Khan et al. (2006) found that Zn and Mn concentration in all forages was
above critical level and Zn concentration was higher in winter than in summer. Similar to Cu and
Fe concentration, range of Zn and Mn concentration in current results was higher than studies in
dry land (Fariani 2008, Khan et al. 2006, Kakengi et al. 2007, Mtui et al. 2006, Rubanza et al.
2006, and Warly et al. 2006b).

187 This finding could probably be explained by mineral level in soils (Minson 1990; Underwood 188 and Suttle 1999). Soil condition in study location characterized with low pH, low availability of 189 Ca, P, and excessive micro mineral solubility for plants (Ali et al. 2013 and Waluyo et al. 2012). 190 Beside that high micro mineral concentration in aquatic plants could be associated with the 191 ability to accumulate the minerals in their tissue (Abdul-Wahab et al. 2014a, 2014b, Asikin and 192 Thamrin 2012, and Veschasit 2012) and excessive micro minerals in water, which probably 193 caused by poor management of wastewater in study area and intensive agricultural practices as 194 significant sources of metal pollution in soils and water. Research by Jan et al. (2011) showed 195 that there was higher concentration of Cu, Zn, Cr, Ni, Pb, and Mn in meat and milk in 196 contaminated area than in control area and concluded that consumption of the foods had 197 significantly increased the minerals concentration in human blood. Similar results was also 198 reported by Miranda et al. (2009) Skalická et al. (2005) and Blanco-Penedo et al. (2006) or 199 reviewed by Rajaganapathy et al. (2011). Therefore, high concentration of Cu, Fe, and Mn in N. 200 oleraceae, A. sensitiva, E. dulcis, and O. rupifogon in current study might not only raise toxicity 201 for buffaloes, when especially consumed in large amounts, but also contaminate to meat and 202 milk of buffalo as food for human consumption. Hence, there is a need to further investigate the 203 status of trace and toxic minerals in blood, liver, kidney, meat, and milk of buffalo in the region.

204 Conclusion

- 205 It could be concluded that mineral concentration of forages greatly varied between seasons on
- 206 flooded pastures in South Sumatra. All of forages had deficient P but most forages had sufficient
- 207 Mg concentration for grazing buffalo except E. Dulcis during flooded season and just Mimoceae
- and L. peploides had sufficient Ca for grazing buffalo in all seasons. Sulphur sufficiency was just
- 209 found in some of Mimoceae and Cyperaceae species.
- 210 Most forages had Cu, Fe, Zn, and Mn concentration which more than critical level for buffalo
- 211 grazing even though all deficiencies were found in flooded season. Copper deficiency was found
- in B. muticum, M. gigantica, S. grossus and S. pterora. Zinc deficiency found in O. Rupifogon
- and Mn deficiency in E. dulcis in flooded season. Copper toxicity was found in N. oleraceae in
- 214 dry season, A. sensitive and L. peploides during all seasons. Iron toxicity was found in L.
- 215 hexandra in flooded season, E. dulcis and L. peploides in all seasons and Mn toxicity was found
- 216 in *N. oleraceae* in dry season.
- 217

218 Acknowledgment

- 219 The authors would like to thank a financial support from the Indonesian Ministry of Research
- and Technology at Research Centre for Sub-optimal Lands (PUR-PLSO), University of
- 221 Sriwijaya, Indonesia.

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asep ali <asepali76@gmail.com>

VERY URGENT: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Schultze-Kraft, Rainer (CIAT) <R.SchultzeKraft@cgiar.org> To: "Ali, Asep (ILRI)" <asepali76@gmail.com> Mon, Nov 25, 2019 at 7:35 PM

Dear Dr. Ali

I need your immediate reply.

Regards

Rainer Schultze-Kraft

From: Schultze-Kraft, Rainer (CIAT) Sent: Wednesday, November 20, 2019 10:46 AM To: asep ali <indranutrisi@yahoo.co.id> Subject: VERY URGENT: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales Importance: High

Dear Dr. Ali,

It is now very urgent that you reply to my email of 14 November and provide the citation substitutes.

Thank you for your cooperation.

Regards

Rainer Schultze-Kraft

From: Schultze-Kraft, Rainer (CIAT) Sent: Thursday, November 14, 2019 8:03 PM

To: 'asep ali' <indranutrisi@yahoo.co.id>

Subject: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Dear Dr. Ali,

During the process of checking and verifying the references cited in your manuscript, we found that the following five correspond to articles published in journals that are considered to be potentially predatory:

Evitayani; Warly L; Fariani A; Ichinohe T; Hayashida M; Abdul Razak SA; Fujihara T. 2006. Macro mineral distribution of forages in South Sumatra during rainy and dry seasons. Journal of Food, Agriculture and Environment 4(2):155–160. http://bit.ly/370gPTe

Mtui DJ; Lekule FP; Shem MN; Rubanza CDK; Ichinohe T; Hayashida M; Fujihara T. 2006. Seasonal influence on mineral content of forages used by smallholder dairy

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Rajaganapathy V; Xavier F; Sreekumar D; Mandal PK. 2011. Heavy metal contamination in soil, water and fodder and their presence in livestock and products. A review. Journal of Environmental Science and Technology 4:234–249. doi: http://dx.doi.org/10.3923/jest.2011.234.249

Rubanza CDK; Shem MN; Ichinohe T; Fujihara T. 2006. Polyphenolics and minerals composition of selected browse tree species leaves native to north-western Tanzania traditional fodder banks. Journal of Food, Agriculture and Environment 4(2):328–332. http://bit.ly/2KisFOL

Warly L; Fariani A; Evitayani; Hayashida M; Fujihara T. 2006a. Mineral status of forages and grazing goats in West Sumatra, Indonesia: 1. Macro minerals. Journal of Food, Agriculture and Environment 4(2):234–236. http://bit.ly/2rx0Hbt

Since Tropical Grasslands-Forrajes Tropicales does not encourage citing articles from such journals, I would appreciate it if you could identify substitutes for these citations. Just let me know any new substitute citations – we will adjust the text accordingly.

In order to send you the proofs of your paper soon, kindly reply at your earliest convenience.

Best regards

Rainer Schultze-Kraft

From: asep ali <indranutrisi@yahoo.co.id>

Sent: Thursday, October 31, 2019 11:49 PM

https://mail.google.com/mail/u/0/?ik=34918bf17b&view=pt&search=all&permmsgid=msg-f:1651177261849018104&simpl=msg-f:16511772618490... 2/4

To: asep_ali@fp.uncri.ac.id; Schultze-Kraft, Rainer (CIAT) <R.SchultzeKraft@CGIAR.ORG>

Subject: Re: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Dear Dr. Schultze-Kraft

All your comments and questions have been followed and so I just deleted your comments in the document. Kindly I also attached a file about your comments and my further explanations and the line number which might you need.

Thank you so much

Asep Ali

Pada Rabu, 30 Oktober 2019 23.08.48 WIB, Schultze-Kraft, Rainer (CIAT) <mailto:r.schultzekraft@cgiar.org> menulis:

Dear Dr. Ali,

I would greatly appreciate hearing from you whether I can expect an amended version of manuscript 301 from you for publication in the next issue of Tropical Grasslands-Forrajes Tropicales.

There is one more issue that I would like to ask you to clarify, for the benefit of better understanding of the reader: What does 'flooded' resp. 'dry' season mean regarding water in the pasture? Is there standing water only in the wet (= 'flooded') season and the soil surface is dry in the other season.

Looking forward to hearing from you.

Kind regards

Rainer Schultze-Kraft

From: Schultze-Kraft, Rainer (CIAT)

Sent: Monday, October 28, 2019 1:53 PM

To: mailto:indranutrisi@yahoo.co.id; mailto:asep_ali@fp.uncri.ac.id

Subject: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Dear Dr. Ali:

A few weeks ago I received from my editor colleague, Lyle Winks, a version of your manuscript, Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia, modified and edited on the basis of the Journal's peer review. I read the manuscript thoroughly and after consultation with my colleague we are prepared to publish it as a Short Communication in the next issue of Tropical Grasslands-Forrajes Tropicales. However, there are still a few minor queries that we have. Please refer to the attached document where the queries are expressed in the form of comments. Kindly address them and return the manuscript to me. It is imperative that any modification of yours (deletion, correction, addition) to the manuscript is done using the "track changes" mode. I would furthermore appreciate it if in each "Comment" you could very briefly annotate how you reacted to the respective query, e.g. by using the "Reply to Comment" mode.

For your information, I have replaced 'Mimosaceae' with 'Leguminosae' in the attached text because Sesbania and Aeschynomene are not mimosoids. In the Journal we prefer that the GRIN plant taxonomy be used; therefore I have modified several of the species names and their authorities.

Kindly acknowledge receipt of this email and let me know if you are in the position to send me the amended manuscript within the next few days. This would enable us to include your paper in the next Journal issue which is scheduled for 30 November.

Looking forward to hearing from you.

Kind regards,

Rainer Schultze-Kraft

Professor Rainer Schultze-Kraft Co-Editor, Tropical Grasslands-Forrajes Tropicales http://tropicalgrasslands.info International Center for Tropical Agriculture (CIAT) Apartado Aéreo 6713 Cali, Colombia Email: http://cn.mc151.mail.yahoo.com/mc/compose?to=R.Schultzekraft@cgiar.org



asep ali <asepali76@gmail.com>

VERY URGENT: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

asep ali <asepali76@gmail.com> To: "Schultze-Kraft, Rainer (CIAT)" <R.SchultzeKraft@cgiar.org>

Mon, Nov 25, 2019 at 8:20 PM

I searching other citatations now. Thank you so much for your effort to find my current email.

Asep Ali [Quoted text hidden]



asep ali <asepali76@gmail.com>

VERY URGENT: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Schultze-Kraft, Rainer (CIAT) <R.SchultzeKraft@cgiar.org> To: "Ali, Asep (ILRI)" <asepali76@gmail.com> Mon, Nov 25, 2019 at 9:02 PM

Dear Dr. Ali,

Good to know that you received my communication.

I consider that the citations Rubanza et al. (2006) could be deleted, also Mtui et al. (2006) and Rajaganapathy et al. (2011). For the other three, hopefully you find a substitute.

Regards

[Quoted text hidden]

Short Communication

Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia

Concentración de minerales en forrajes nativos en pastizales estacionalmente inundados en South Sumatra, Indonesia

ASEP INDRA MUNAWAR ALI, SOFIA SANDI, RISWANDI AND MUHAKKA

Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang, South Sumatra, Indonesia. <u>unsri.ac.id</u>

Abstract

This study was conducted to evaluate macro- and micro-mineral concentrations in forages growing on seasonally flooded native pastures in non-tidal swamps of South Sumatra, Indonesia and grazed by buffalo. The upper part of native forage plants from 5 species of Poaceae, 4 species of Leguminosae, 3 species of Cyperaceae and 1 species of Onagraceae were sampled by hand-plucking during flooded and dry seasons. The results showed that mineral concentrations of forages varied greatly between seasons. In general concentrations of most minerals were adequate to supply the dietary needs of grazing ruminants in both wet and dry seasons. Phosphorus (P) concentrations were low in all species in both wet and dry seasons but growing animals should select a diet adequate in P, while lactating females could benefit from P supplementation. In cut-and-carry situations, animals would probably respond to additional P in the diet. These hypotheses need testing in the field.

Keywords: Buffalo, mineral deficiency, mineral toxicity.

Resumen

En South Sumatra, Indonesia se realizó un estudio para evaluar las concentraciones de macro- y micro-minerales en plantas forrajeras nativas comunes en pastizales estacionalmente inundados y utilizadas con búfalos. Para el estudio see muestrearon por el método 'hand-plucking' plantas de 5 especies de Poaceae, 4 especies de Leguminosae, 3 especies de Cyperaceae y 1 especie de Onagraceae durante las estaciones inundada y seca. Los resultados mostraron que las concentraciones minerales variaron considerablemente entre estaciones. No obstante, las concentraciones de la mayoría de los minerales fueron adecuadas para satisfacer los requerimientos nutricionales de rumiantes en pastoreo en ambas estaciones. Las concentraciones de fósforo (P) fueron bajas en todas las especies en ambas estaciones, pero se considera que bajo condiciones de pastoreo los animales en crecimiento seleccionan una dieta adecuada en P, mientras que hembras lactantes podrían beneficiarse de P suplementado. En situaciones de corte y acarreo, los animales probablemente responderían a P adicional en la dieta. Estas hipótesis necesitan ser probadas en el campo.

Palabras clave: Búfalos, deficiencia mineral, toxicidad mineral.

Introduction

The concentrations of individual minerals in forages vary greatly depending on the interactions of a range of factors including soil, plant species, stage of maturity, yield, management factors and climate. Forages represent an important source of minerals for grazing ruminants and play essential roles in preventing diseases and inhibiting or stimulating ruminal microbial activity (<u>Spears 1994</u>). Natural pasture species in non-tidal swamps have been traditionally the main source of feed for swamp buffalo in e.g. Brazil (<u>Camarão et al. 2004</u>) and South Sumatra (<u>Ali</u>

Correspondence: A.I.M. Ali, Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang 30662, South Sumatra, Indonesia. Email: <u>asep_ali@fp.uncri.ac.id</u> et al. 2014). Forage nutritive values fluctuate with seasons and the dry season is the most limiting in terms of nutrient supply to grazing buffalo. As most grazing livestock in tropical countries including Indonesia are usually unsupplemented, they must fulfill their mineral requirements from forage. Camarão et al. (2004) failed to find evidence of mineral deficiencies in grazing buffalo. Data on mineral concentrations in forage species and their variability between seasons are essential for correct and cost-effective ration formulation and swamp buffalo nutrition.

However, mineral profiles of tropical forages in flooded pasture have received little attention and limited studies showed that forages can be deficient in one or more elements. In this study we aimed to estimate and compare the concentrations of mineral elements including calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) in native flooded pasture species in non-tidal swamps of South Sumatra, Indonesia during the flooded and dry seasons.

Materials and Methods

Site description

Research was conducted during February–September 2014 on communal pastures in non-tidal swamps in Rambutan subdistrict, Banyuasin district and Pampangan subdistrict, OKI (Ogan Komering Ilir) district of South Sumatra Province (between $3^{\circ}05'$ and $3^{\circ}11'$ S, and $104^{\circ}55'$ and $104^{\circ}58'$ E). The study area was selected as representative of non-tidal swamps used for grazing buffalo where the highest population of swamp buffalo were found. The study area (\pm 567 ha) is part of the Batang hari river watershed and is surrounded by settlements and plantations of oil palm and rubber. The soils are poorly drained, acid fluvisols with low availability of Ca, P and Mg and high solubility of Fe and Zn (Ali et al. 2014).

The dry season extends from April to September while the rainy season normally occurs from October to March with annual rainfall of 2,100–3,264 mm. The study area is regularly inundated for 3–8 months during rainy seasons. Minimum temperatures range from 22 to 25 °C (at 05.00–08.00 h) and maximum temperatures range from 30 to 34 °C (at 11.00–14.00 h). Farmers also use the communal pastures for paddy fields and vegetable production (\pm 304 ha) when the water level is low.

Forage sampling

In the representative areas forage plants were never fertilized, nor had any management intervention. Observations of forage consumed by grazing buffalo during 08.00–11.00 h and 14.00–17.00 h (Setianah et al. 2004; Hirata et al. 2008) and in our previous study (Ali et al. 2014) revealed that native vegetation species consumed by buffalo included: Poaceae [Urochloa mutica (Forssk.) T.Q. Nguyen (syn. Brachiaria mutica (Forssk.) Stapf), Leersia hexandra Sw., Hymenachne amplexicaulis (Rudge) Nees, Ischaemum rugosum Salisb., Oryza rufipogon Griff.]; Leguminosae [Mimosa pigra L., Sesbania exasperata Kunth, Neptunia oleracea Lour, Aeschynomene sensitiva Sw.]; Cyperaceae [Actinoscirpus grossus (L.f.) Goetgh. & D.A. Simpson, Scleria gaertneri Raddi, Eleocharis dulcis (Burm.f.) Trin. ex Hensch.]; and Onagraceae [Ludwigia peploides (Kunth) P.H. Raven].

The study area on both left and right sides of the river was divided into 2 subareas based on land use. Samples of the forages eaten by buffalo were randomly collected by hand-plucking during flooding in the wet season (March 2014) and in the dry season (August and September 2014) from at least 2 different sites within each subarea. A mix of leaf and stem (\pm 250 g) was handplucked from the upper parts of herbaceous plants in the pre-flowering stage and from younger twigs of the shrubs, *M. pigra* and *S. exasperata*. Samples for each species were washed with distilled water, chopped and dried at 50 °C. The oven-dried samples were pooled within the subarea and then coarsely milled to pass 1 mm screen for mineral analyses.

Mineral analyses

Representative samples (in duplicate) were digested stepwise with nitric acid (HNO₃). Concentrations of minerals in the forage samples were analyzed by using inductively coupled plasma emission spectrophotometer (SPS7700, Seiko Instruments Inc., Chiba, Japan) in the Laboratory of Feed Technology and Dairy Nutrition, Faculty of Animal Husbandry, Bogor Agricultural University.

Statistical analyses

Data were analyzed using StatView SAS (<u>1999</u>). The differences in means between seasons were determined using an unpaired t-test and were considered significant if P<0.05.

Results

While season had a significant effect (P<0.05) on concentrations of Ca, P, Mg and S, the effects were

Species	Season	М	lacro-mine	eral (% DI	M)	Mi	Micro-mineral (ppm DM)			
		Ca	Р	Mg	S	Cu	Fe	Zn	Mn	
Poaceae										
Urochloa mutica	dry	$0.62b^{1}$	0.10b	1.59b	0.50b	11.6b	105a	41.8a	366b	
	flooded	0.11a	0.01a	0.86a	0.04a	9.6a	107a	60.2a	128a	
Leersia hexandra	dry	0.08a	0.16b	1.01b	0.13a	15.3a	193a	128.0b	274a	
	flooded	0.12b	0.09a	0.84a	0.14a	12.3a	806b	97.8a	283a	
Hymenachne amplexicaulis	dry	0.07a	0.11b	1.02a	0.17a	15.5a	139a	40.9a	225a	
· ·	flooded	0.19b	0.08a	1.09b	0.09a	17.5a	208b	56.8b	252a	
Ischaemum rugosum	dry	0.08a	0.04a	1.03a	0.06a	13.2a	184b	57.0a	125a	
č	flooded	0.14b	0.07b	1.11b	0.38b	12.2a	121a	78.1a	297b	
Oryza rufipogon	dry	0.12a	0.05a	1.19b	0.03a	16.8a	499b	45.8b	906a	
	flooded	0.16b	0.08a	0.95a	0.03a	13.6a	188a	26.1a	1,044b	
Leguminosae										
Mimosa pigra	dry	0.32a	0.14b	1.68b	0.30b	22.1b	206a	76.9b	252a	
	flooded	0.39b	0.10a	1.54a	0.14a	8.6a	317a	33.7a	342a	
Sesbania exasperata	dry	0.42a	0.17b	1.41b	0.30b	23.4a	100a	76.4a	153a	
-	flooded	0.64b	0.15a	1.17a	0.23a	21.3a	181b	64.3a	128a	
Neptunia oleracea	dry	0.66b	0.07a	1.80a	0.05a	43.3b	202a	176.0b	1,076b	
-	flooded	0.43a	0.14b	2.01b	0.18b	21.7a	358b	43.2a	402a	
Aeschynomene sensitiva	dry	0.46a	0.12a	1.98a	0.43a	29.2a	129a	86.7a	407b	
·	flooded	0.48b	0.13a	2.35b	0.40a	42.1b	145a	94.4a	286a	
Cyperaceae										
Actinoscirpus grossus	dry	0.08a	0.21b	0.65a	0.31a	14.3b	89a	49.6b	291a	
	flooded	0.20b	0.11a	1.32b	0.33a	9.9a	318b	43.3a	568b	
Scleria gaertneri	dry	0.25b	0.06a	0.89b	0.05a	14.7b	69a	48.1a	487b	
-	flooded	0.10a	0.08a	0.74a	0.08b	9.6a	151b	62.3a	210a	
Eleocharis dulcis	dry	0.08b	0.14a	1.01b	0.29a	20.1a	936a	55.1b	231b	
	flooded	0.03a	0.20a	0.17a	0.49b	19.1a	1,629b	28.0a	34a	
Onagraceae										
Ludwigia peploides	dry	0.56b	0.16a	2.62b	0.16a	31.2a	728a	198.0a	969b	
	flooded	0.33a	0.21b	2.06a	0.15a	244.0b	926b	207.0a	691a	
Critical level ²		0.30	0.25	0.20	0.26	11	50	33	40	
Toxic level ³						25	500	750	1,000	

Table 1.	Concentrations	of macro- and	micro-minerals	in forages	in swamps in	South Suma	atra during flood	ed and dry seasons.

¹Means followed by different letters within a species between seasons are significantly different (P < 0.05).

²Levels for growth and production of ruminant animals (McDowell et al. 1997).

³Levels suggested to produce toxic symptoms in ruminant animals (McDowell et al. 1997).

inconsistent across species (Table 1). Calcium concentrations in most forages were higher in the flooded season than in the dry season except for *U. mutica*, *N. oleracea*, *S. gaertneri*, *E. dulcis* and *L. peploides*. With the exception of *U. mutica*, Ca concentrations in Poaceae and Cyperaceae were lower than those in Leguminosae. Concentrations of P in most forages also varied (P<0.05) between seasons with the exception of *O. rufipogon*, *A. sensitiva*, *S. gaertneri* and *E. dulcis*. Some species had higher P concentrations in the dry while others had higher concentrations, Mg concentrations in most forages were higher during the dry than in the flooded season.

Concentrations of micro-minerals also varied (P<0.05) between seasons and among species (Table 1). In most

Poaceae species, Cu concentrations did not differ significantly between seasons, while Fe concentrations were usually higher during flooding periods and Zn and Mn concentrations were usually higher in the dry season.

Discussion

Macro-minerals

The variation between species in occurrence of peak concentrations of nutrients could be due to a combination of main growth periods, time of maturity and genetic variation (Minson 1990; Underwood and Suttle 1999).

The higher Ca concentrations in Leguminosae and most Poaceae during the flooded season than in the dry

season agree with a study in dry land of South Sumatra, Indonesia by Evitayani et al. (2006) who showed that Ca concentration in 2 species of grasses and 4 species of legumes was higher in the rainy season than in the dry season and varied from 0.57 in Napier grass [Cenchrus purpureus (Schumach.) Morrone syn. Pennisetum purpureum Schumach.] to 2.61% in leucaena [Leucaena leucocephala (Lam.) de Wit] in rainy and from 0.51 (Napier) to 2.44% (leucaena) in dry seasons. On the contrary, Warly et al. (2006a) in dry land of West Sumatra concluded that Ca concentrations of 7 species of grasses and 13 species of legumes were lower in the rainy season than in the dry season. The higher concentrations in legumes than in grasses agree with the findings of Warly et al. (2006a) of 0.39 [Zea mays L. subsp. mexicana (Schrad.) H.H. Iltis, syn. Euchlaena mexicana (Schrad.) Kuntze)] to 1.69% [Gliricidia maculata (Kunth) Kunth.] and Nasrullah et al. (2004) in dry land of South Sulawesi of 0.3 (Cenchrus ciliaris L.) to 4.0% [Desmanthus virgatus (L.) Willd.]. Results of the current study showed that the range of Ca concentrations was lower than in previous studies (Nasrullah et al. 2004; Evitayani et al. 2006; Mtui et al. 2006; Warly et al. 2006a). Calcium concentration in the Leguminosae and Onagraceae species exceeded the 0.30% which meets the requirements of ruminants (McDowell et al. 1997).

Phosphorus concentrations in all forages in this study were lower than the critical level for ruminants (0.25%); McDowell et al. 1997) and fluctuated in both seasons. At the early heading stage, Nasrullah et al. (2004) reported that P concentration ranged from 0.20 (Z. mays subsp. mexicana, syn. E. mexicana) to 1.80% [Centrosema plumieri (Turpin ex Pers.) Benth.] and was not significantly affected by season, while Warly et al. (2006a) found that P concentration was higher in the dry season than in the rainy season and ranged from 0.21 (Z. mays subsp. mexicana, syn. E. mexicana) to 0.47% [Setaria sphacelata (Schumach.) Stapf & C.E. Hubb.]. In a further study, which sampled the same species but at a different location, Evitayani et al. (2006) showed that P concentration ranged from 0.29 (Centrosema molle Mart. ex Benth., syn. C. pubescens auct.pl.) to 0.68% (Cenchrus purpureus syn. Pennisetum purpureum) being higher in the rainy season than in the dry season and that all forages had P concentration >0.25% in the rainy season.

The low concentrations of Ca and P in forage in our study would be a function of acid soil conditions at this location with low availability of Ca and P for plants (<u>Ali</u> et al. 2014). Jumba et al. (<u>1995</u>) showed a significant positive correlation between soil P and herbage P, while Kanno et al. (<u>2006</u>) showed that soil pH related to not only grass growth and P uptake but also mycorrhizal

dependency. Underwood and Suttle (1999) recommended that diets of livestock should have a Ca:P ratio in the range 1:1 to 2:1, and claimed this was more important than the absolute concentration for utilization by ruminants. In our study the Ca:P ratio for individual species ranged from 0.16 (E. dulcis) to 10.0 (U. mutica); thus grazing livestock would have a wide range from which to select an acceptable diet. Depending on the ability to select from available pasture, grazing buffalo, especially lactating females, could suffer from a P deficiency in the diet and would benefit from P supplementation. Studies by Jones (1990) and Miller et al. (1990) described some low-cost strategies on dryland pastures for overcoming P deficiency in grazing livestock. Direct P supplementation through the use of non-protein supplementation in ureamineral blocks may serve as a more valuable alternative.

With the exception of *E. dulcis* in the flooded season, all forages had sufficient Mg concentration for requirements of ruminants (McDowell et al. 1997); thus buffalo consuming forages in the area are unlikely to suffer Mg deficiency. The adequacy of Mg concentration in most forages has been reported in previous studies (Nasrullah et al. 2004; Evitayani et al. 2006; Warly et al. 2006a).

Sulphur is essential for microbial protein synthesis in the rumen and its concentration in ruminant diets is important. There was wide variation between seasons and species in S concentrations in the forages examined and some species contained sufficient S to satisfy ruminant requirements effective for rumen functioning. Availability of the various species and the opportunity to select by animals would determine the need for supplementation with sulphur. Sulphur deficiency in the drv season has been reported by Evitayani et al. (2006). Warly et al. (2006a) found that the percentage of forage species with S deficiency was higher in the dry season than in the rainy season.

Micro-minerals

Based on mineral requirements (McDowell et al. 1997) concentrations of Cu, Fe, Zn and Mn in most forages in flooded pasture exceeded the critical levels for grazing ruminants in tropical regions. Very high levels of Cu were found in *N. oleracea* in the dry season and in *A. sensitiva* and *L. peploides* throughout the year. If these species formed a significant portion of the diet, Cu toxicity could possibly occur in grazing animals. The range in Cu concentration in Poaceae and Leguminosae was higher than that reported by Fariani (2008) of 5.6–10.1 ppm and 3.8–16.6 ppm in 3 species of grasses and 4 species of legumes, respectively, in dry land of South

Sumatra. Warly et al. (2006b) reported that Cu concentration in 13 species of grasses varied from 2.3 to 14.4 ppm, while Khan et al. (2006) reported a range from 10.9 to 25.7 ppm in Pakistan and Evitayani and Warly (2010) a range from 3.8 to 16.6 ppm in West Sumatra.

The generally higher Fe concentration in most Leguminosae, Cyperaceae and Onagraceae species during the flooded season than in the dry season was probably a function of a natural dilution process and translocation of minerals to the roots as plants mature. Similar results were reported by Mtui et al. (2006) with a range from 619 to 678 ppm, and Warly et al. (2006b) with a range from 96 to 569 ppm. Khan et al. (2006) also reported that all forages had higher Fe concentrations in winter than in summer with a range from 93 to 202 ppm. As in our study, Khan et al. (2006) found that Zn and Mn concentrations in all forages were above critical levels and overall concentrations were higher in winter than in summer. Similar to Cu and Fe concentrations, ranges of Zn and Mn concentrations in our study were higher than studies in dry land (Khan et al. 2006; Mtui et al. 2006; Rubanza et al. 2006; Warly et al. 2006b; Fariani 2008).

Our findings could probably be explained by mineral level in soils (Minson 1990; Underwood and Suttle 1999). Soil condition in the study location was characterized by low pH, low availability of Ca and P, and excessive micro-mineral solubility for plants (Waluyo et al. 2012; Ali et al. 2014). High micromineral concentrations in aquatic plants could be associated with the ability to accumulate these minerals in their tissues (Asikin and Thamrin 2012; Veschasit et excessive and micro-mineral al. 2012) with concentrations in soils and water, possibly caused by intensive agricultural practices. Research by Jan et al. (2011) showed that there were higher concentrations of Cu, Zn, Cr, Ni, Pb and Mn in meat and milk in contaminated areas than in control areas and concluded that consumption of the foods had significantly increased the mineral concentrations in human blood. Similar results have been reported by Skalická et al. (2005), Blanco-Penedo et al. (2006) and Miranda et al. (2009) or reviewed by Rajaganapathy et al. (2011). Therefore, high concentrations of Cu, Fe and Mn in N. oleracea, A. sensitiva, E. dulcis and O. rufipogon might not only raise toxicity risks for grazing buffalo, when consumed in large amounts, but also contaminate meat and milk of buffalo when milked or slaughtered for human consumption. Hence, there is a need to further investigate the status of trace and toxic minerals in blood, liver, kidney, meat and milk of buffalo in the region.

Conclusion

Mineral concentrations of forages varied greatly between seasons on flooded pastures in South Sumatra. Concentrations of most minerals appeared adequate for the dietary needs of grazing buffalo given that the samples we collected were not whole plant samples but attempted to be similar to forage that buffalo would select. However, all forages had low P concentrations and grazing animals, especially lactating females, might suffer P deficiency in the diet, while stalled animals fed on a cut-and-carry basis would certainly suffer a deficiency. Feeding studies would be warranted to test benefits from supplementing animals with P.

Acknowledgment

The authors thank the Indonesian Ministry of Research and Technology at Research Centre for Sub-optimal Lands (PUR-PLSO), University of Sriwijaya, Indonesia for financial support for the project.

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(Note of the editors: All hyperlinks were verified 12 November 2019.)

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(*Received for publication XX; accepted 20 September 2019; published 30 November 2019*)

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asep ali <asepali76@gmail.com>

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asep ali <asepali76@gmail.com> To: "Schultze-Kraft, Rainer (CIAT)" <R.SchultzeKraft@cgiar.org> Tue, Nov 26, 2019 at 4:36 PM

Dear Dr. Schultze-Kraft

The attached file is the revised manuscript after deleting/substitution the predatory citations/references. I couldn't find a study with the identic result as Evitayani et al. (2006) and Warly et al. (2006a, 2006b) so the change in sentences was accordingly made as inserted new references (Espinoza et al. 1991; Pastrana et al. 1991; Lundu et al. 2012; Uemura et al. 2014) in the revised manuscript. Thank you so much Asep

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Schultze-Kraft, Rainer (CIAT) <R.SchultzeKraft@cgiar.org> To: "Ali, Asep (ILRI)" <asepali76@gmail.com> Tue, Nov 26, 2019 at 5:08 AM

Dear Dr. Ali,

Attached please find the latest version of your ms 301, in Word format.

Please perform the requested literature citation changes (and corresponding text adjustments) <u>using the track</u> <u>changes mode</u> and return the ms to me. It is important that I receive your feedback tomorrow, 26 November.

Kind regards

Rainer Schultze-Kraft

From: Schultze-Kraft, Rainer (CIAT)
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Subject: RE: VERY URGENT: RE: Your manuscript 301 for publication in Tropical Grasslands-Forrajes Tropicales

Dear Dr. Ali,

Good to know that you received my communication.

I consider that the citations Rubanza et al. (2006) could be deleted, also Mtui et al. (2006) and Rajaganapathy et al. (2011). For the other three, hopefully you find a substitute.

Regards

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301 Ali et al_TypesettingR1-RSK-2-JLU-AR-RSK-2.docx 113K *Tropical Grasslands-Forrajes Tropicales (2019) Vol. 7(5):524–530* DOI: <u>10.17138/TGFT(7)524-530</u>

Short Communication

Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia

Concentración de minerales en forrajes nativos en pastizales estacionalmente inundados en South Sumatra, Indonesia

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Abstract

This study was conducted to evaluate macro- and micro-mineral concentrations in forages growing on seasonally flooded native pastures in non-tidal swamps of South Sumatra, Indonesia and grazed by buffalo. The upper part of native forage plants from 5 species of Poaceae, 4 species of Leguminosae, 3 species of Cyperaceae and 1 species of Onagraceae were sampled by hand-plucking during flooded and dry seasons. The results showed that mineral concentrations of forages varied greatly between seasons. In general concentrations of most minerals were adequate to supply the dietary needs of grazing ruminants in both wet and dry seasons. Phosphorus (P) concentrations were low in all species in both wet and dry seasons but growing animals should select a diet adequate in P, while lactating females could benefit from P supplementation. In cut-and-carry situations, animals would probably respond to additional P in the diet. These hypotheses need testing in the field.

Keywords: Buffalo, mineral deficiency, mineral toxicity.

Resumen

En South Sumatra, Indonesia se realizó un estudio para evaluar las concentraciones de macro- y micro-minerales en plantas forrajeras nativas comunes en pastizales estacionalmente inundados y utilizadas con búfalos. Para el estudio see muestrearon por el método 'hand-plucking' plantas de 5 especies de Poaceae, 4 especies de Leguminosae, 3 especies de Cyperaceae y 1 especie de Onagraceae durante las estaciones inundada y seca. Los resultados mostraron que las concentraciones minerales variaron considerablemente entre estaciones. No obstante, las concentraciones de la mayoría de los minerales fueron adecuadas para satisfacer los requerimientos nutricionales de rumiantes en pastoreo en ambas estaciones. Las concentraciones de fósforo (P) fueron bajas en todas las especies en ambas estaciones, pero se considera que bajo condiciones de pastoreo los animales en crecimiento seleccionan una dieta adecuada en P, mientras que hembras lactantes podrían beneficiarse de P suplementado. En situaciones de corte y acarreo, los animales probablemente responderían a P adicional en la dieta. Estas hipótesis necesitan ser probadas en el campo.

Palabras clave: Búfalos, deficiencia mineral, toxicidad mineral.

Introduction

The concentrations of individual minerals in forages vary greatly depending on the interactions of a range of factors including soil, plant species, stage of maturity, yield, management factors and climate. Forages represent an important source of minerals for grazing ruminants and play essential roles in preventing diseases and inhibiting or stimulating ruminal microbial activity (<u>Spears 1994</u>). Natural pasture species in non-tidal swamps have been traditionally the main source of feed for swamp buffalo in e.g. Brazil (<u>Camarão et al. 2004</u>) and <u>South Sumatra (Ali</u>

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Tropical Grasslands-Forrajes Tropicales (ISSN: 2346-3775)

et al. 2014). Forage nutritive values fluctuate with seasons and the dry season is the most limiting in terms of nutrient supply to grazing buffalo. As most grazing livestock in tropical countries including Indonesia are usually unsupplemented, they must fulfill their mineral requirements from forage. Camarão et al. (2004) failed to find evidence of mineral deficiencies in grazing buffalo. Data on mineral concentrations in forage species and their variability between seasons are essential for correct and cost-effective ration formulation and swamp buffalo nutrition.

However, mineral profiles of tropical forages in flooded pasture have received little attention and limited studies showed that forages can be deficient in one or more elements. In this study we aimed to estimate and compare the concentrations of mineral elements including calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) in native flooded pasture species in non-tidal swamps of South Sumatra, Indonesia during the flooded and dry seasons.

Materials and Methods

Site description

Research was conducted during February–September 2014 on communal pastures in non-tidal swamps in Rambutan subdistrict, Banyuasin district and Pampangan subdistrict, OKI (Ogan Komering Ilir) district of South Sumatra Province (between $3^{\circ}05'$ and $3^{\circ}11'$ S, and $104^{\circ}55'$ and $104^{\circ}58'$ E). The study area was selected as representative of non-tidal swamps used for grazing buffalo where the highest population of swamp buffalo were found. The study area (\pm 567 ha) is part of the Batang hari river watershed and is surrounded by settlements and plantations of oil palm and rubber. The soils are poorly drained, acid fluvisols with low availability of Ca, P and Mg and high solubility of Fe and Zn (Ali et al. 2014).

The dry season extends from April to September while the rainy season normally occurs from October to March with annual rainfall of 2,100–3,264 mm. The study area is regularly inundated for 3–8 months during rainy seasons. Minimum temperatures range from 22 to 25 °C (at 05.00–08.00 h) and maximum temperatures range from 30 to 34 °C (at 11.00–14.00 h). Farmers also use the communal pastures for paddy fields and vegetable production (\pm 304 ha) when the water level is low.

Forage sampling

In the representative areas forage plants were never fertilized, nor had any management intervention. Observations of forage consumed by grazing buffalo during 08.00–11.00 h and 14.00–17.00 h (Setianah et al. 2004; Hirata et al. 2008) and in our previous study (Ali et al. 2014) revealed that native vegetation species consumed by buffalo included: Poaceae [Urochloa mutica (Forssk.) T.Q. Nguyen (syn. Brachiaria mutica (Forssk.) Stapf), Leersia hexandra Sw., Hymenachne amplexicaulis (Rudge) Nees, Ischaemum rugosum Salisb., Oryza rufipogon Griff.]; Leguminosae [Mimosa pigra L., Sesbania exasperata Kunth, Neptunia oleracea Lour., Aeschynomene sensitiva Sw.]; Cyperaceae [Actinoscirpus grossus (L.f.) Goetgh. & D.A. Simpson, Scleria gaerineri Raddi, Eleocharis dulcis (Burm.f.) Trin. ex Hensch.]; and Onagraceae [Ludwigia peploides (Kunth) P.H. Raven].

The study area on both left and right sides of the river was divided into 2 subareas based on land use. Samples of the forages eaten by buffalo were randomly collected by hand-plucking during flooding in the wet season (March 2014) and in the dry season (August and September 2014) from at least 2 different sites within each subarea. A mix of leaf and stem (\pm 250 g) was handplucked from the upper parts of herbaceous plants in the pre-flowering stage and from younger twigs of the shrubs, *M. pigra* and *S. exasperata*. Samples for each species were washed with distilled water, chopped and dried at 50 °C. The oven-dried samples were pooled within the subarea and then coarsely milled to pass 1 mm screen for mineral analyses.

Mineral analyses

Representative samples (in duplicate) were digested stepwise with nitric acid (HNO₃). Concentrations of minerals in the forage samples were analyzed by using inductively coupled plasma emission spectrophotometer (SPS7700, Seiko Instruments Inc., Chiba, Japan) in the Laboratory of Feed Technology and Dairy Nutrition, Faculty of Animal Husbandry, Bogor Agricultural University.

Statistical analyses

Data were analyzed using StatView SAS (1999). The differences in means between seasons were determined using an unpaired t-test and were considered significant if P < 0.05.

Results

While season had a significant effect (P<0.05) on concentrations of Ca, P, Mg and S, the effects were

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Species	Season	М	lacro-mine	eral (% DN	(M	Mi	cro-miner	al (ppm D	M)
		Ca	Р	Mg	S	Cu	Fe	Zn	Mn
Poaceae									
Urochloa mutica	dry	$0.62b^{1}$	0.10b	1.59b	0.50b	11.6b	105a	41.8a	366b
	flooded	0.11a	0.01a	0.86a	0.04a	9.6a	107a	60.2a	128a
Leersia hexandra	dry	0.08a	0.16b	1.01b	0.13a	15.3a	193a	128.0b	274a
	flooded	0.12b	0.09a	0.84a	0.14a	12.3a	806b	97.8a	283a
Hymenachne amplexicaulis	dry	0.07a	0.11b	1.02a	0.17a	15.5a	139a	40.9a	225a
	flooded	0.19b	0.08a	1.09b	0.09a	17.5a	208b	56.8b	252a
Ischaemum rugosum	dry	0.08a	0.04a	1.03a	0.06a	13.2a	184b	57.0a	125a
	flooded	0.14b	0.07b	1.11b	0.38b	12.2a	121a	78.1a	297b
Oryza rufipogon	dry	0.12a	0.05a	1.19b	0.03a	16.8a	499b	45.8b	906a
	flooded	0.16b	0.08a	0.95a	0.03a	13.6a	188a	26.1a	1,044b
Leguminosae									
Mimosa pigra	dry	0.32a	0.14b	1.68b	0.30b	22.1b	206a	76.9b	252a
	flooded	0.39b	0.10a	1.54a	0.14a	8.6a	317a	33.7a	342a
Sesbania exasperata	dry	0.42a	0.17b	1.41b	0.30b	23.4a	100a	76.4a	153a
	flooded	0.64b	0.15a	1.17a	0.23a	21.3a	181b	64.3a	128a
Neptunia oleracea	dry	0.66b	0.07a	1.80a	0.05a	43.3b	202a	176.0b	1,076b
	flooded	0.43a	0.14b	2.01b	0.18b	21.7a	358b	43.2a	402a
Aeschynomene sensitiva	dry	0.46a	0.12a	1.98a	0.43a	29.2a	129a	86.7a	407b
	flooded	0.48b	0.13a	2.35b	0.40a	42.1b	145a	94.4a	286a
Cyperaceae									
Actinoscirpus grossus	dry	0.08a	0.21b	0.65a	0.31a	14.3b	89a	49.6b	291a
	flooded	0.20b	0.11a	1.32b	0.33a	9.9a	318b	43.3a	568b
Scleria gaertneri	dry	0.25b	0.06a	0.89b	0.05a	14.7b	69a	48.1a	487b
	flooded	0.10a	0.08a	0.74a	0.08b	9.6a	151b	62.3a	210a
Eleocharis dulcis	dry	0.08b	0.14a	1.01b	0.29a	20.1a	936a	55.1b	231b
	flooded	0.03a	0.20a	0.17a	0.49b	19.1a	1,629b	28.0a	34a
Onagraceae									
Ludwigia peploides	dry	0.56b	0.16a	2.62b	0.16a	31.2a	728a	198.0a	969b
	flooded	0.33a	0.21b	2.06a	0.15a	244.0b	926b	207.0a	691a
Critical level ²		0.30	0.25	0.20	0.26	11	50	33	40
Toxic level ³						25	500	750	1,000

Table 1. Concentrations of macro- and micro-minerals in forages in swamps in South Sumatra during flooded and dry seasons.

¹Means followed by different letters within a species between seasons are significantly different (P<0.05).

²Levels for growth and production of ruminant animals (McDowell et al. 1997).

³Levels suggested to produce toxic symptoms in ruminant animals (McDowell et al. 1997).

inconsistent across species (Table 1). Calcium concentrations in most forages were higher in the flooded season than in the dry season except for *U. mutica*, *N. oleracea*, *S. gaertneri*, *E. dulcis* and *L. peploides*. With the exception of *U. mutica*, Ca concentrations in Poaceae and Cyperaceae were lower than those in Leguminosae. Concentrations of P in most forages also varied (P<0.05) between seasons with the exception of *O. rufipogon*, *A. sensitiva*, *S. gaertneri* and *E. dulcis*. Some species had higher P concentrations in the dry while others had higher concentrations, Mg concentrations in most forages were higher during the dry than in the flooded season.

Concentrations of micro-minerals also varied (P<0.05) between seasons and among species (Table 1). In most

Poaceae species, Cu concentrations did not differ significantly between seasons, while Fe concentrations were usually higher during flooding periods and Zn and Mn concentrations were usually higher in the dry season.

Discussion

Macro-minerals

The variation between species in occurrence of peak concentrations of nutrients could be due to a combination of main growth periods, time of maturity and genetic variation (Minson 1990; Underwood and Suttle 1999).

The higher Ca concentrations in Leguminosae and most Poaceae during the flooded season than in the dry

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season differ from results of previous studies in tropical regions (Espinoza et al. 1991; Pastrana et al. 1991; Lundu et al. 2012). The higher concentrations in legumes than in grasses agree with the findings of Nasrullah et al. (2004) in dry land of South Sulawesi of 0.3 (*Cenchrus ciliaris* L.) to 4.0% [*Desmanthus virgatus* (L.) Willd.]. Results of the current study showed that the range of Ca concentrations was similar to the range in the study of Pastrana et al. (1991) but lower than in previous studies (Espinoza et al. 1991; Nasrullah et al. 2004; Lundu et al. 2012). Calcium concentration in the Leguminosae and Onagraceae species exceeded the 0.30% which meets the requirements of ruminants (McDowell et al. 1997).

Phosphorus concentrations in all forages in this study were lower than the critical level for ruminants (0.25%; <u>McDowell et al. 1997</u>) and fluctuated in both seasons. At the early heading stage, Nasrullah et al. (2004) reported that P concentration ranged from 0.20 (Z. mays subsp. mexicana, syn. E. mexicana) to 1.80% [Centrosema plumieri (Turpin ex Pers.) Benth.] and was not significantly affected by season. <u>A higher P content in</u> rainy season than in dry season was found in acid soil of Paramo region Colombia (Pastrana et al. 1991) while Lundu et al. (2012) found that P concentration was higher in the hot dry season than in the wet season and ranged from 0.02 to 1.57%.

The low concentrations of Ca and P in forage in our study would be a function of acid soil conditions at this location with low availability of Ca and P for plants (Ali et al. 2014). Jumba et al. (1995) showed a significant positive correlation between soil P and herbage P, while Kanno et al. (2006) showed that soil pH related to not only grass growth and P uptake but also mycorrhizal dependency. Underwood and Suttle (1999) recommended that diets of livestock should have a CaP ratio in the range 1:1 to 2:1, and claimed this was more important than the absolute concentration for utilization by ruminants. In our study the Ca:P ratio for individual species ranged from 0.16 (E. dulcis) to 10.0 (U. mutica); thus grazing livestock would have a wide range from which to select an acceptable diet. Depending on the ability to select from available pasture, grazing buffalo, especially lactating females, could suffer from a P deficiency in the diet and would benefit from P supplementation. Studies by Jones (1990) and Miller et al. (1990) described some low-cost strategies on dryland pastures for overcoming P deficiency in grazing livestock. Direct P supplementation through the use of non-protein supplementation in ureamineral blocks may serve as a more valuable alternative.

With the exception of *E. dulcis* in the flooded season, all forages had sufficient Mg concentration for requirements of ruminants (McDowell et al. 1997); thus buffalo consuming forages in the area are unlikely to suffer Mg deficiency. The adequacy of Mg concentration in most forages has been reported in previous studies (Nasrullah et al. 2004; Lundu et al. 2012).

Sulphur is essential for microbial protein synthesis in the rumen and its concentration in ruminant diets is important. There was wide variation between seasons and species in S concentrations in the forages examined and some species contained sufficient S to satisfy ruminart requirements for effective rumen functioning. Availability of the various species and the opportunity to select by animals would determine the need for supplementation with sulphur. <u>Similarly, S, sufficiency</u> was found in two species of legumes consumed by goats in low land of Philippines (Uemura et al. 2014).

Micro-minerals

Based on mineral requirements (McDowell et al. 1997) concentrations of Cu, Fe, Zn and Mn in most forages in flooded pasture exceeded the critical levels for grazing ruminants in tropical regions. Very high levels of Cu were found in N. oleracea in the dry season and in A. sensitiva and L. peploides throughout the year. If these species formed a significant portion of the diet, Cu toxicity could possibly occur in grazing animals. The range in Cu concentration in Poaceae and Leguminosae was higher than that reported by Fariani (2008) of 5.6-10.1 ppm and 3.8-16.6 ppm in 3 species of grasses and 4 species of legumes, respectively, in dry land of South Sumatra, Khan et al. (2006) reported a range from 10.9 to 25.7 ppm in Pakistan while Evitayani and Warly (2010) reported a range from 3.8 to 16.6 ppm in West Sumatra.

The generally higher Fe concentration in most Leguminosae, Cyperaceae and Onagraceae species during the flooded season than in the dry season was probably a function of a natural dilution process and translocation of minerals to the roots as plants mature. Khan et al. (2004) also reported that all forages had higher Fe concentrations in winter than in summer with a range from 93 to 202 ppm. As in our study, Khan et al. (2006) found that Zn and Mn concentrations were higher in winter than in summer. Similar to Cu and Fe concentrations, ranges of Zn and Mn concentrations in our study were higher than studies in dry land (Khan et al. 2006; Fariarji 2008).

Our findings could probably be explained by mineral level in soils (<u>Minson 1990</u>; <u>Underwood and Suttle 1999</u>). Soil condition in the study location was characterized by low pH, low availability of Ca and P,

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from 96 to 569 ppm.

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and excessive micro-mineral solubility for plants (Waluyo et al. 2012; Ali et al. 2014). High micromineral concentrations in aquatic plants could be associated with the ability to accumulate these minerals in their tissues (Asikin and Thamrin 2012; Veschasit et al. 2012) and with excessive micro-mineral concentrations in soils and water, possibly caused by intensive agricultural practices. Research by Jan et al. (2011) showed that there were higher concentrations of Cu, Zn, Cr, Ni, Pb and Mn in meat and milk in contaminated areas than in control areas and concluded that consumption of the foods had significantly increased the mineral concentrations in human blood. Similar results have been reported by Skalická et al. (2005), Blanco-Penedo et al. (2006) and Miranda et al. (2009), Therefore, high concentrations of Cu, Fe and Mn in N. oleracea, A. sensitiva, E. dulcis and O. rufipogon might not only raise toxicity risks for grazing buffalo, when consumed in large amounts, but also contaminate meat and milk of buffalo when milked or slaughtered for human consumption. Hence, there is a need to further investigate the status of trace and toxic minerals in blood, liver, kidney, meat and milk of buffalo in the region.

Conclusion

Mineral concentrations of forages varied greatly between seasons on flooded pastures in South Sumatra. Concentrations of most minerals appeared adequate for the dietary needs of grazing buffalo given that the samples we collected were not whole plant samples but attempted to be similar to forage that buffalo would select. However, all forages had low P concentrations and grazing animals, especially lactating females, might suffer P deficiency in the diet, while stalled animals fed on a cut-and-carry basis would certainly suffer a deficiency. Feeding studies would be warranted to test benefits from supplementing animals with P.

Acknowledgment

The authors thank the Indonesian Ministry of Research and Technology at Research Centre for Sub-optimal Lands (PUR-PLSO), University of Sriwijaya, Indonesia for financial support for the project.

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(Note of the editors: All hyperlinks were verified 12 November 2019.)

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(Received for publication XX; accepted 20 September 2019; published 30 November 2019)

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