Effect of boron and zinc fertilization on white oats grown in soil with average content of these nutrients

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ABSTRACT - The objective of this study was to evaluate the effect of fertilization with zinc or boron on the growth and dry matter production, nutritional value and accumulation of nutrients in white oats. The study comprised two experiments conducted in glasshouses, the first consisting of the application of four doses of zinc (0, 0.2, 0.4 and 0.6 mg/dm³) in the form of zinc sulphate (20% Zn), and the second consisting of the application of four doses of boron (0, 0.2, 0.4 and 0.6 mg/dm³) in the form of Borax (11% B). The experimental design in each case was a randomized block design, with five replicates. Fertilization with zinc and boron increased the growth of white oats, but had no significant effect on the nutritional value of the forage. Higher levels of absorption and accumulation of nutrients in plant tissues were observed following the application of boron and zinc at rates of up to 0.60 mg/dm³ of soil.

Key Words: Avena sativa, crude protein, dry matter

Introduction

Stability in the production of fodder throughout the year facilitates feed management and minimizes variations in the quality of products such as milk and meat and the income of ranchers. In south-central Brazil, the growth of annual grasses in the winter, including the white oat (*Avena sativa* L.) (Primavesi et al., 2004), is crucial to meet the deficit of fodder during this period (Roso et al., 1999).

In many cases, oats are sown without the addition of fertilizers and plant nutrition is dependent on the residual fertilizer from previous crops (Kluthcouski et al., 2003). This may lead to a reduction in the production of fodder and its nutritional value, especially in soils of low fertility, and may even cause nutritional deficiencies in animals fed exclusively with such forage.

In addition to the lack of fertilizer, the inadequate availability of zinc (Zn) and boron (B) in the soil may limit the development of crops (Galrão, 2002) and also affect forage plants. As oats are usually deployed in rotation or succession at agricultural or commercial crops, this micronutrient is subject to unavailability in the soil, because of removal by crops and excessive application of phosphatic fertilizers and/or correctives to the acidity.

These minerals are required for the basic processes of plant life. Zinc participates in the synthesis of tryptophan,

which is a precursor of indol acetic acid (IAA) and is required for plant growth, nitrogen metabolism, starch and chlorophyll synthesis and ATPase activity, as well as the transport of assimilates (Malta et al., 2002). Boron is responsible for the integrity of the cell membrane, cell wall synthesis and lignification (Goldbach et al., 2001). Thus, the lack or excess of these minerals can cause nutritional imbalances, with subsequent effects on dry matter production (Prado et al., 2008).

Little is known about the effect of applying these micronutrients on the production and nutritional value of white forage oats. Thus, the objective of the present study was to evaluate the effect of fertilization with B or Zn on dry matter production, nutritional value and nutrient accumulation in white oats.

Material and Methods

The study comprised two experiments conducted in glasshouse, at Universidade Estadual do Oeste do Paraná, in Marechal Cândido Rondon – PR, Brazil (latitude: 24° 31' S, longitude: 54° 01' W and elevation: 420 m). The climate of the region, according to Koppen, Cfa, is humid subtropical mesothermic, with hot summers, mild winters with infrequent frosts and no set dry season, and an average precipitation and annual temperature of 1,500 mm and 21.4°C, respectively.

The soil used in the experiments was a typical Oxisol of clayey texture, retrieved from the top 0-20 cm of an agricultural area, with the following chemical characteristics: pH in water - 5.29; O.M. - 32.81 g/dm³; P (Mehlich 1) - 25.64 mg/dm³; (H) Al - 4.14 cmol $_c$ /dm³; Ca²⁺ - 6.59 cmol $_c$ /dm³; Mg²⁺ - 1.45 cmol $_c$ /dm³; K - 1.07 cmol $_c$ /dm³; CEC - 13.25 cmol $_c$ /dm³; V% - 68.75; Cu (Mehlich 1) - 12.81 mg/dm³; Zn (Mehlich 1) - 2.4 mg/dm³; Mn (Mehlich 1) - 146.0 mg/dm³; Fe (Mehlich 1) - 30.2 mg/dm³; and B - 0.29 mg/dm³ (Pavan et al., 1992). The soil was relatively fertile, with average levels of B and Zn (Novaes et al., 2007). Field observations showed the potential for positive response of winter cereals in soils under the same conditions.

Each experiment was composed of a randomized block design with five replicates. Each experimental unit comprised a vase made of polyethylene, containing 3.0 dm³ of soil. The first experiment involved the application of four doses of zinc (0, 0.2, 0.4 and 0.6 mg/dm³ Zn) in the form of zinc sulphate (20% Zn). The second experiment involved the application of four doses of boron (0, 0.2, 0.4 and 0.6 mg/dm³ B) in the form of Borax (11% B). The doses were adjusted on the basis of responses observed in the field on private properties with similar soil conditions.

The sources of boron and zinc were diluted in water, with subsequent application of the solution to the vessels. On the same day, 15 oat seeds (*Avena sativa* L., cultivar IPR 126) were sown per pot, and at 20 days after sowing, the seedlings were thinned to leave three plants per pot, simulating a population of 200 plants/m², as recommended for field sowing (CBPA, 2006). The water content of the soil was monitored on a daily basis by weighing 20 vessels, and was corrected whenever necessary to ensure 80% capacity of the soil to water retention. No correctives or fertilizers other than the doses of B or Zn were applied before and during the experiments.

Sixty days after seedling emergence, the height of each plant (from the surface of the soil to the point of flag leaf insertion), plus the number of tiller and leaves per plant were recorded. The plants were then divided into shoots and roots, and dried in a forced ventilation oven at 55 °C±2 °C for 72 hours. Fresh and dry weights were recorded to determine the levels of dry matter (DM) and quantify the production of DM. Dried samples were ground in a Willey mill with blades and a stainless steel chamber, passed through a 0.5 mm sieve and subjected to laboratory procedures.

The concentrations of N, P, K, Ca, Mg, Cu, Zn, Fe, Mn and B in shoot tissue were determined according to Embrapa (2009). The levels of organic matter (OM), mineral matter

(MM) and crude protein (CP) were determined according to the AOAC (1990), and those of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose and cellulose according to Silva & Queiroz (2002).

The results of the two experiments were subjected to analysis of variance (P<0.05). To doses of Zn and B, linear and quadratic regression equations were adjusted, choosing the significant model of greatest coefficient of determination (\mathbb{R}^2).

Results and Discussion

The application of boron significantly affected the development of the shoot and root system of white oat plants (Figure 1). The number of tiller bows and leaves per plant and the production of DM fitted a quadratic regression model (Figure 1a, c, d), while the height of plants and the production of DM of roots and total DM (Figure 1b, e, f) increased linearly with increasing boron. The maximum number of tillers (3.2 tillers/plant) and leaves per plant (9.0 leaves/plant) was obtained at 0.33 and 0.32 mg/dm³ boron, respectively. Maximum aboveground dry matter production (4.26 g/plant) was obtained at 0.47 mg/dm³ boron. Fageria et al. (2000), working with grassland species in soil of savanna, reported an optimal dose for wheat (Triticum aestivum L.) and rice (Oryza sativa L.) of 0.4 mg/dm³ boron. Pavinato et al. (2009) observed that the application of up to 8 mg/L boron in rice, via the nutrient solution, led to reduction in the number of tiller bows per plant.

These results have implications for production, indicating that the dose of boron applied can be adjusted according to whether the purpose of cultivation is to produce forage or grain. In areas intended for the production of fodder, the maximum recommended dose lies between 0.32 and 0.47 mg/dm³ of B, because these systems aim at the maximum production of sheets and DM and high tillering. The large contribution of leaves in total DM is desirable because it results in improved digestibility and increased DM intake (Grise et al., 2001). With regard to tillers, boron deficiency interferes with the levels of indole acetic acid (IAA) in apical tissues, which is followed by the loss of apical dominance caused by the imbalance between IAA and cytokinins. This unbalance allow greater activity of hormones that stimulate the tillering, such as cytokinins, which are responsible for the process of cell division (Awad & Castro, 1983). Among the effects of B deficiency are reduction in the elongation and expansion of new leaves (Pavinato et al., 2009), agreeing with the results of this study, with increments in height and in production of DM.

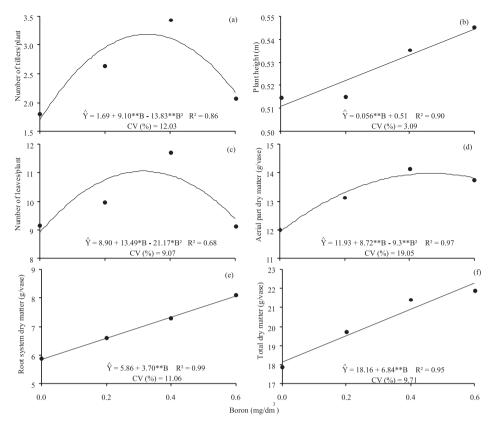


Figure 1 - Number of tillers per plant (a), plant height (b), number of leaves per plant (c), aerial part dry matter (d), root system dry matter (e) and total dry matter (f) of white oat plants following the application of boron.

The low accumulation of DM occurs due to the loss of cell plasticity under boron deficiency, leading to a reduction in leaf area and leaf chlorophyll concentration, while also reducing photosynthesis (Dell & Huang, 1997).

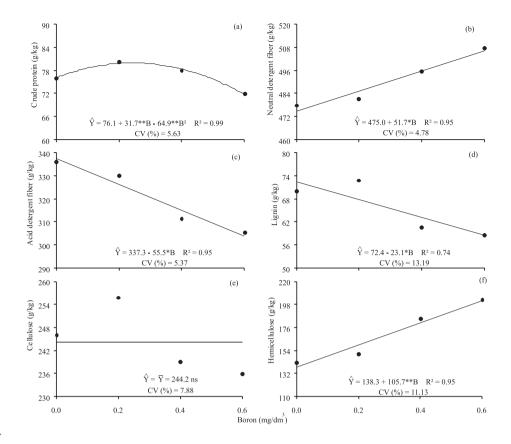
Increments in the root system are of value in cultivated areas where white oats are grown for grazing, since roots contribute to the absorption of nutrients and water and show a high correlation with the production of DM (Fageria et al., 2000), leading to greater plant persistence and ability for regrowth. Castro et al. (2003) observed reduction in primary and side roots of sunflower (*Helianthus annuus* L.) in nutrient solutions containing low concentrations of B, while Fageria et al. (2000) reported optimal doses for the growth of roots of wheat and maize (*Zeamays* L.) of 0.4 mg/dm³ of B, when working with soil from the Brazilian cerrado.

The nutritional value of oats was significantly affected by the application of B (Figure 2), with the exception of the cellulose content (Figure 2f). The concentration of crude protein (CP) fitted a quadratic model regression (Figure 2a), and the highest concentration (80 g/kg) was obtained with the application of $0.24 \, \text{mg/dm}^3 \, \text{B}$. Floss et al. (2003) found CP concentrations of 83.5 g/kg for white oats.

Increase in the CP levels with application of B were expected, since this is calculated as a function of the

content of N in DM (Silva & Queiroz, 2006). Beneficial effects of fertilization with B on levels of N (as NO³⁻) have been observed in other studies (Camacho-Cristóbal & González-Fontes, 1999; 2007). These effects are due to minor changes that B produces in plants, which affect the metabolism of N (Pavinato et al., 2009). Camacho-Cristóbal González-Fontes (2007) studied the relationship between B deficiency and the assimilation of NO³- in tobacco plants (*Nicotiana* tabacum 1.) and found a lower concentration of NO³in plants deficient in B due to the lower net absorption rate as a result of the decline in the activity of protein carriers of NO³-. There is currently no evidence of direct toxic effects of B on the synthesis of proteins (Reid et al., 2004); however, B is directly related to the growth of the aerial parts of plants, requiring the formation of chlorophyll and protein synthesis, of which N is a basic component. Thus, high levels of B affect these processes with consequences for the concentration of N (Epstein & Bloom, 2006).

In terms of fiber components, an increase in the concentrations of NDF (Figure 2b) and hemicellulose (Figure 2) was observed along with a reduction in the concentrations of ADF (Figure 2c) and lignin (Figure 2). The results suggest that except for the non-provision of B, the doses studied were suitable for the cultivation of white



ns - not significant.

Figure 2 - Crude protein (a), neutral detergent fiber, (b) acid detergent fiber (c), lignin (d), cellulose (e) and hemicellulose (f) in white oat plants following application of boron.

oats, because B deficiency causes physiological, anatomical and biochemical changes, including changes in the cell wall structure (Goldbach et al., 2001). Similar concentrations of NDF (408 and 492 g/kg) were reported by Cecato et al. (2001) in black oat cv. IAPAR 61. According to Grise et al. (2001), the increase in NDF concentration can be attributed to the greater participation of stem in detriment of forage leaves. This hypothesis was supported in this study by the increments in the height of plants. The gains in height were due to stretching, a consequence of the elongation of the stem between nodes, and were not accompanied by an increase in the number of leaves, which reached a maximum at 0.32 B mg/dm³. The decrease in hemicellulose is consistent with the reduction in NDF, because hemicellulose is a heterogeneous collection of amorphous polysaccharides (Van Soest, 1994).

For the ADF, Floss et al. (2003), also working with white oats, obtained concentrations in excess (420 g/kg), justified by the older age of plant growth (103 days). Moreira et al. (2005) observed an average concentration of lignin of 52 g/kg in black oats following 64 days of growth, while for cellulose the values found were consistent with those described in the literature, varying from 200 to 400 g/kg in fodder plants according to Van Soest (1994).

Reductions in the concentration of lignin, which is a constituent of the plant cell wall (Jung & Engels, 2002), were observed (Figure 1d). The irregular lignification of cell walls and a significant reduction in total lignin concentration are symptoms of boron deficiency (Epstein & Bloom, 2006). However, a reduction in lignin concentration is also associated with boron toxicity in plants (Reid et al., 2004). The role of boron in the synthesis of lignin may be related to the formation of borate complexes with phenols, thus regulating the availability of free phenol, a precursor of lignin synthesis (Lewis, 1980). Boron also has a structural role in the formation of polysaccharides that compose pectin (O'Neill et al., 1996), thus controlling cell growth (Fleischer et al., 1999) and the mechanical properties of the primary cell walls of plants (Ishii et al., 2001), of which cellulose is a structural component (Arruda et al., 2002).

The application of boron significantly influenced the concentrations of Cu, Mn, B, Fe, Zn, Mg, P, K and Ca in white oats (Figure 3). For Cu, B and Zn, there was quadratic effect of B doses, with the minimum concentrations of Cu (7.35), B (30.42) and Zn (23.46 mg/kg) obtained at the doses of 0.33, 0.32 and 0.35 mg/dm 3 of B respectively (Figure 3a, 3c and 3e). The concentrations of Mn, Mg and Ca increased linearly with B (Figure 3b, 3f and 3i), while the concentrations

of Fe, P and K decreased linearly with B (Figure 3d, 3g and 3 h). There are few reports in the literature about the effects of the application of B on the absorption of other nutrients, especially for white oats, and the information available does not allow the formulation of concrete hypotheses, hence the need for further study. When cultivating tobacco plants in nutrient solutions in the presence and absence of B, Camacho-Cristóbal & González-Fontes (1999) observed reductions in the concentration of Ca, Mg and K in plants under conditions of deficiency. In wheat, the mechanisms of responses to fertilization depend on the cultivar and are different (Mahboodi et al., 2002). The results for Cu and Zn were consistent with those observed by Primavesi et al. (1999). The similarities in the behavior of B and Zn were consistent with Yamada (2000), who reported that these nutrients are required for the optimal functioning of ATPase and that in the absence of B there can be reduction in the efficiency of Zn in plant, and vice versa.

The availability of B in the soil significantly affects its concentration in plant tissues (Epstein & Bloom, 2006). When studying tobacco plants with and without boron deficiency, Camacho-Cristóbal et al. (2005) observed a lower concentration of B in the leaves and root system of the plants under the condition of deficiency.

This nutrient usually moves through the xylem, under the control of the transpiration stream, with a tendency to bind to the pectin on the cell wall of leaves (Furlani et al., 2004); however, it presents low remobilization in plants that do not produce polyols - mannitol, sorbitol and dulcitol (Bastos & Carvalho, 2004) depending on the supply of this nutrient through root absorption and transport via xylem (Dordas & Brown, 2001)

Plant species differ in their requirements for B for growth. The critical range of deficiency varies from 5 to 10 mg/kg of B in DM in grasses and from 80 to 100 mg/kg of B in most species in need (Epstein Bloom, 2006). Engler et al. (2006) found concentrations of B in dry matter ranging from 8.6 to 14.7 mg/kg in rice, while Fageria et al. (1997) found concentrations of 6 to 10 mg/kg B in the early growth stage of the culture.

Meanwhile, the concentration of B in DM can vary, because each group of cultures presents different genetic ability, which can be more or less responsive to fertilization, as a result of active and passive absorption mechanisms of B (Dordas Brown, 2001) and the ability to form complex sugar-alcohol-B through the phloem (Bellaloui et al., 2003). In wheat, the concentration of B in the aboveground parts of the plant increases as the dose applied increases, and may reach up to 318 mg/kg. However, the critical threshold

of toxicity varies from 44 to 318 mg/kg, depending on the cultivar (Furlani et al., 2004).

Primavesi et al. (1999) reported similar concentrations of K (34.2 to 36.0 g/kg) and Mg (3.7 to 4.8 g/kg) and lower concentrations of Fe (169 to 199 mg/kg) and Mn (138 to 158 mg/kg) than those obtained in this study. The concentrations of P obtained were consistent with those observed by Vilela et al. (1978), who reported 6.0 P g/kg for forage yellow oat (*Avena byzantine* L.), and regrowth at 60 days of age. Mondardo et al. (2011) reported similar concentrations of Ca and K in black oats.

The application of Zn had a significant effect on the development of the shoot and root system of white oats (Figure 4). The number of tillers presented linear increase with the application of Zn doses (Figure 4a). The results for plant height and number of leaves per plant fitted the quadratic regression model (Figure 4b and 4c). The tallest plants (0.57 m) and the highest number of leaves per plant (9.74 leaves/plant) were obtained following the application of 0.50 and 0.44 mg/dm³ of Zn, respectively. Increase in plant growth characteristics were expected, since the Zn acts on plant growth (Malta et al., 2002), and according to Römheld (2001), plants deficient in Zn have reduced growth and tillering, shorter internodes and interveinal chlorosis in new leaves and sheaths. However, the results suggest that doses higher than those previously reported to maximum response could be toxic to plants, because at toxic levels, Zn reduces plant growth (Soares et al., 2001).

Zinc also had a significant effect on the production of DM. The DM production of the root system increased linearly in response to Zn doses (Figure 1d), while the DM production of total and of aerial parts fitted the quadratic regression model (Figures 1d and 1f). The maximum production of aboveground (14.3 g/vase) and total (17.8 g/vase) DM occurred at doses of 0.30 and 0.32 mg/dm³ Zn. Similar results were obtained by Rozane et al. (2008), who, studying the application of Zn in the development of rice plants, obtained fit to the quadratic regression model for the production of dry matter of aerial part, and a linear increase in the production of DM of whole plant with increasing zinc. Zinc is required for the basic processes of plant life, including photosynthesis, nitrogen assimilation, respiration and other biochemical and physiological processes, and thus plays a key role in crop growth (Cakmak, 2008). Plants with Zn deficiency in the early stages of development find it difficult to express their maximum genetic potential. The damage both in the maintenance of enzyme activity and the enzyme synthetase of tryptophan leads to a decrease in cell volume and apical growth, due to a reduction in the synthesis or degradation of auxins (Epstein & Bloom, 2006).

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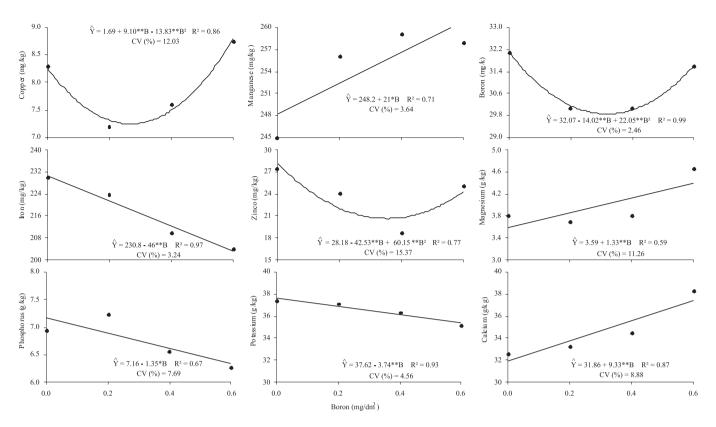


Figure 3 - Concentrations of macro and micronutrients in dry white oatmeal following the application of boron.

Zinc had a significant effect on the nutritional value of white oats, with a quadratic regression model fitting all characteristics studied (Figure 5). The highest concentrations of CP (89 g/kg), cellulose (262 g/kg) and hemicellulose (215 g/kg) were obtained at 0.26, 0.20 and 0.30 mg/dm³ of Zn, respectively. The influence of Zn on the concentration of CP is related to the metabolism of N, because the Zn acts in absorption of N and protein quality (Potarzycki & Grzebisz, 2009). Zinc controls the activity of RNAse, the enzyme that hydrolyzes RNA, leading to a decrease in protein synthesis (Epstein & Bloom, 2006). There was an inverse correlation between the supply of Zn and RNAse activity and also between RNAse activity and the protein content and plant growth. The concentration of cellulose obtained was within the range reported by Van Soest (1994) (200-400 g/kg). Similar concentrations of hemicellulose were obtained by Moreira et al. (2007) in black oats.

Cellulose is a major cell wall constituent (Van Soest, 1994), while hemicellulose is a heteropolysaccharide composed mainly of xylose, arabinose and galacturonic acid. The quantities of these monomers in the molecule determine digestibility. Although both polysaccharides show similar total digestibility, the rumen digestibility of cellulose is greater than that of hemicellulose (Ladeira et al., 2002).

The lowest values of NDF (499 g/kg), ADF (270 g/kg) and lignin (48 g/kg) were obtained at doses of 0.22, 0.21 and 0.29 mg/dm³ of Zn respectively. Similar concentrations of NDF (520 g/kg), ADF (255 g/kg) and lignin (40 g/kg) were obtained by Moreira et al. (2007). The ADF fraction includes cellulose and lignin as primary components, plus varying amounts of ash and nitrogen compounds (Bianchini et al., 2007). Lignin is one of the three compounds that bind to form the fraction of fibrous forage which is considered the main limiting factor in digestibility (Van Soest, 1994). It is a constituent of the cell walls of plants (Jung & Engles, 2002) and its study is crucial to the characterization of forages, because it influences the degradability of stem tissues stems (Jung & Engels, 2002), and its excess can make the dietary protein unavailable, causing reduction in forage for animals (Rogerio et al., 2007).

The concentration of forage fiber has been used as a negative index of dietary quality, since it represents the least digestible fraction of forage. However, fiber plays an important role in the control of voluntary consumption and consequently in nutrient intake (Allen, 2000), and is required for the operation and normal metabolism of the rumen (Matos, 1989), encouraging an environment that promotes the development of rumen microorganisms that are responsible for the digestion of fibrous carbohydrates (Nussio et al., 2006).

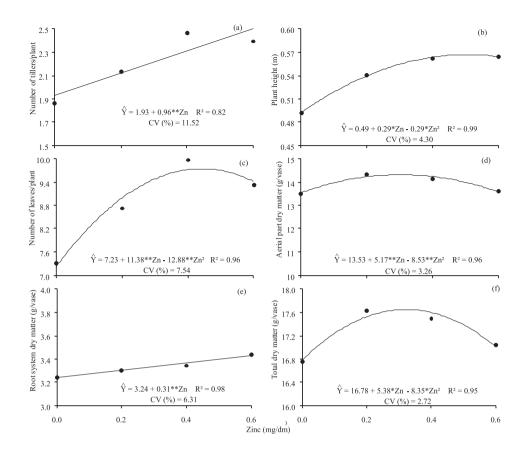


Figure 4 - Number of tillers per plant (a), plant height (b), number of leaves per plant (c), aerial part dry matter (d), root system dry matter (e) and total dry matter (f) of white oat plants following the application of zinc.

Zinc application had a significant effect on the concentration of macro and micronutrients in the shoots of white oats (Figure 6). The lowest concentrations of Cu (0.63) and B (17.55 mg/kg) were obtained with the application of 0.32 and 0.43 mg/dm³ of Zn, respectively. In white oat plants, McDonald & Wilson (1980) observed concentration of 4 mg/kg of Cu in the phase preceding the emergence of the panicle, and Floss et al. (2003) observed concentrations of Cu and B of 7.5 and 23.1 mg/kg, respectively, for 105 days of growth. Low concentrations of Cu in DM white oats may be associated with high organic matter content (32.81 g/dm³) in the soil, because in addition to its low mobility in fine-texture soils with a high organic matter content, Cu can be adsorbed by soil organic matter (Novaes et al., 2007).

The highest concentrations of Mn (278.3), Fe (282.6) and Zn (39.1 mg/kg) were obtained at the doses of 0.24, 0.33 and 0.33 mg/dm 3 of Zn, respectively. As for boron, the effects of zinc supply on the concentration of other nutrients have not yet been established. However, the concentrations of Mn obtained were higher than those observed in other

studies conducted on oats (Nyborg, 1970; Floss et al., 2003) due to the high content of Mn in the soil used in the experiment (146 mg/dm³). Concentrations of Fe were consistent with those observed by McDonald & Wilson (1980), while Primavesi et al. (1999) and Floss et al. (2003) verified lower concentrations of Zn.

There is concern about the concentration of Zn in forage grown in areas intended for direct seeding, due to critical levels of Zn in soils kept under this cultivation system. In the direct seeding system, Zn can potentially become deficient (Motta et al., 2007), and the elevation of its critical level in the soil is due to higher levels of pH, organic matter and P available on the soil surface layer, which act antagonistically on Zn, limiting its absorption by plants (Carneiro et al., 2008).

The highest concentrations of Mg (15.40), P (7.44), K (39.12) and Ca (63.03 g/kg) were obtained at doses of 0.37, 0.30, 0.30 and 0.34 mg/dm³ Zn, respectively, suggesting that doses ranging from 0.30 to 0.37 mg/dm³ Zn provide oats with higher concentrations of macronutrients. The results are relevant, because with the adoption of farming livestock

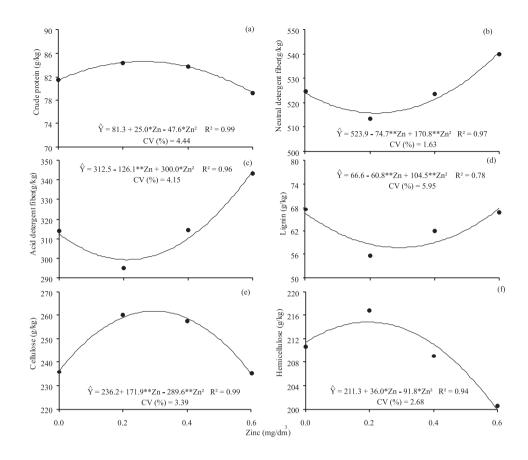


Figure 5 - Crude protein (a), neutral detergent fiber, (b), acid detergent fiber (c), lignin (d), cellulose (e) and hemicellulose (f) in white oat plants following application of zinc.

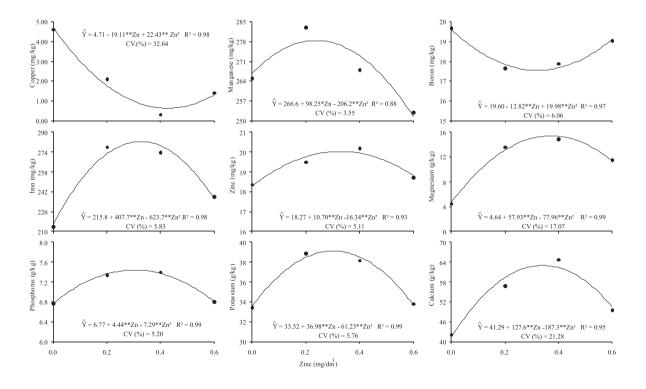


Figure 6 - Concentrations of macro and micronutrients in the dry matter of shoots of white oat plants following the application of zinc.

integration system, nutritionally balanced fodder is crucial, since the objective is the maintenance of cattle exclusively grazing in at least one of the stages of production. Even using mineral supplementation, due to the difficulties in controlling individual intake, providing good quality forage can significantly reduce the occurrence of nutritional problems related to mineral deficiencies.

Conclusions

The application of boron and zinc lead to an increase in the growth of white oats, without, however, changing the nutritional value of the forage. Higher rates of absorption and accumulation of nutrients in plant tissues can be achieved with the application of up to $0.60~\text{mg/dm}^3$ boron and up to $0.43~\text{mg/dm}^3$ zinc.

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