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ORIGINAL PAPER

Zearalenone content in animal fodder samples in relation to weather conditions in Colonia Benítez, northeastern Argentina

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Abstract The northeast of Argentina is a livestock production area. Generally cattle in this area feed on grasslands and improved pastures, but their food is often supplemented with grains, protein sources and reserves in the form of hay and/or silage. However, these food sources may be colonised by fungi including *Fusarium*, which produce mycotoxins, one of which is zearalenone (ZEA). The aim of this study was to relate the natural occurrence of ZEA in Colonia Benítez (Chaco Province) to the temperature and moisture of the autumn preceding the winter collection of fodder samples in 2012 and 2013. The forage resources of 2012 had high ZEA contents (310 to 6279 μg/kg) in 47 % of the samples, whereas those from 2013 exhibited intermediate values of ZEA contents (131 to 296 μg/kg) in 41.6 % of the samples and low contents (< 2 to 91 μg/kg) in 50 % of the samples. The incidence of *F. semitectum* was higher in 2012 compared to 2013. The high concentration of ZEA found in many of the forage samples collected in July 2012 was preceded by autumn weather conditions that were exceptionally conducive to plant stress. In contrast, when meteorological factors were within normal values (as in the autumn of 2013), the pasture and feed collected in winter showed intermediate to low concentrations of ZEA. The temperature and moisture conditions prevailing during the autumn of 2012 accelerated canopy senescence of the forage plants available for livestock at the beginning of the winter. Consequently, the weather conditions during the autumn 2012 likely favoured the sharp increase in ZEA content in these pastures when compared to the other two seasons.

Keywords Weather variables · Zearalenone · Zeranol · *Fusarium semitectum* · Northeastern Argentina

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Introduction

Beef production is an important industry in northeastern Argentina (Canosa et al. 2013; Chiossone 2006) a region that includes the provinces of Formosa, Chaco, Misiones and Corrientes (Fig. 1). Livestock production has been influenced by the progress made in the development of alternative animal feeds, including the management of natural pastures and the use of new forage species, winter supplements with grains and reserves in the form of hay and silage. However, these food resources can be substrates for the development of toxigenic fungi including *Fusarium*, *Aspergillus*, and *Penicillium*. Mycotoxins are a group of highly toxic metabolites (Smith and Moss 1985), which cause mycotoxicosis in both humans and animals (Quiroga 2002). Zearalenone (ZEA) is a mycotoxin produced by several species of the genus *Fusarium*, including *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. semitectum* and *F. sporotrichioides* (Leslie and Summerell 2006). ZEA has important oestrogen-like activity, which leads to deficiencies in conception, ovulation, and embryo implantation in animals. Susceptibility to ZEA differs among animal species, with young and prepubertal sows being particularly sensitive (EFSA 2011; 2004). The production of ZEA in vitro, like that of other mycotoxins, is closely related to the fungal species and type of substrate used. Under field conditions, the production of ZEA is affected by other factors such as environmental, geographic and seasonal factors. In addition, the natural synthesis of ZEA can be influenced by planting species susceptible to *Fusarium* infection (Magan et al., 2011; Reed and Moore 2009; EFSA 2004).

The ZEA content varies according to the plant species and stage of maturity. In some cases, this variation may indicate the presence of a saprophytic habitat favourable for the production of ZEA during late phenological stages of the crop (Di Menna et al., 1987; Towers 1996). Studies in New Zealand have shown that autumn is the season most favourable for production of ZEA, which was found in 80 % of the pastures analysed (Reed et al., 2004). In Europe, Engels and Krämer (1996) found ZEA in annual and perennial ryegrass, whereas in the Czech Republic, Skladanka et al. (2011, 2013) found a range of ZEA contents in different plant species destined for grazing and silage, which was related to the prevailing weather conditions.

In Argentina, there are few records of intoxication in ruminants by the consumption of pastures containing ZEA. However, G.A Lori (personal communication) detected *F. graminearum* and ZEA in species of the family Poaceae in Bragado (Buenos Aires Province), which was related to cases of abortions in sows. Also, Louge Uriarte et al. (2006) found ZEA in a pasture of southeast Buenos Aires. Furthermore, fungi belonging to the genus *Fusarium* with ability to produce different mycotoxins, including ZEA, have recently been found in different forage crops in the northeast of Argentina (Ramirez et al., 2012; Ramírez et al., 2014; Salvat et al., 2013a, b).

The aim of this study was to describe and compare the natural ZEA contents found in fodder samples collected in Colonia Benítez (Chaco Province, northeastern Argentina) in the winters of 2012 and 2013, and relate these to the temperature and moisture conditions of the respective preceding autumns (when the forage matures).

Materials and methods

Sampling

Twenty-four forage samples (eleven green pasture samples, eleven deferred sorghum samples, two sorghum silage samples) were collected from fields of the National Institute of Agricultural Technology (INTA) Colonia Benítez Experimental Station (27° 25′ S, 58° 56′ W) in July 2012 and July 2013. One rice straw roll and four dietary

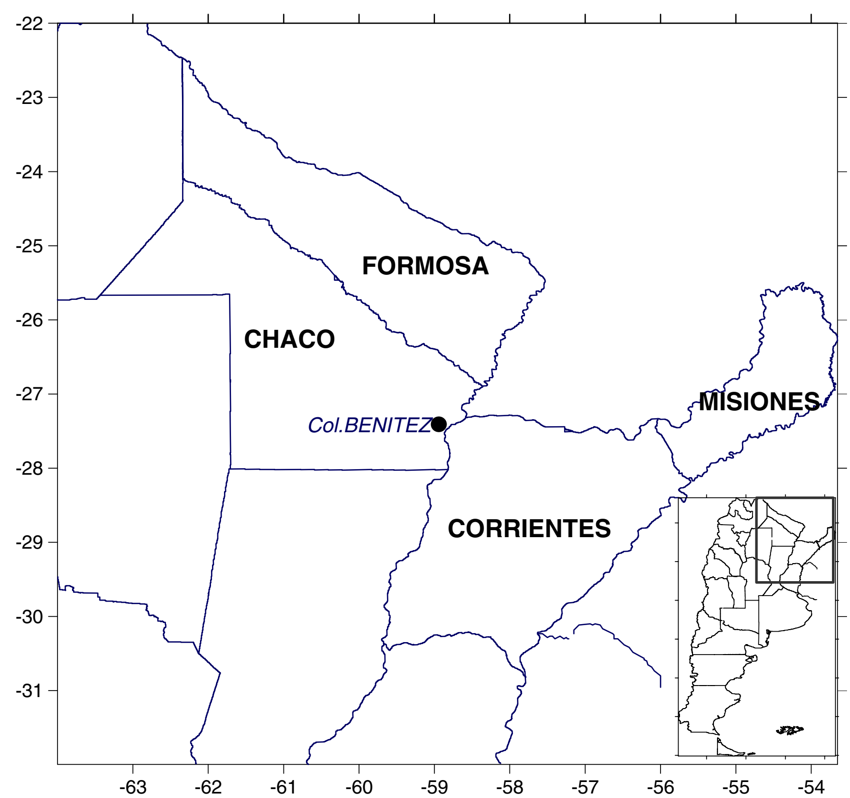

Fig. 1 Northeastern region of Argentina, including four provinces: Formosa, Chaco (where Colonia Benítez is located), Misiones and Corrientes

supplements were purchased from local suppliers, completing a total of 29 samples. Each green pasture sample consisted of approximately 20–30 cuttings taken at a height of 10 cm using a stainless steel knife. The final dry matter content was at least 400 g. The samples of sorghum left for deferred grazing were collected from crops which reached full grain maturity (below 12 % grain moisture) in the field. The samples of sorghum for silage were harvested at the milky dough stage, which is the recommended practice. All samples were placed in paper bags and immediately sent to the laboratory for processing and analysis.

Conditioning of the samples

The fresh material was dried in an oven at 30 °C for seven days and subsampled. One portion was used for the isolation and identification of the *Fusarium* spp., whereas the remaining sample was ground in a TRAPP TRF70® (a fodder crusher with special steel cutting blades, Metalúrgica TRAPP LTDA. Av. Pref. Waldemar Grubba, 117- CXP 106 89,256–500 Jaragua do Sul- SC Brazil, www.trapp.com.br). Both subsamples were kept in paper bags at room temperature, protected from humidity and direct sunlight until the time of processing.

Analysis of ZEA in pasture by high-performance liquid chromatography (HPLC)

ZEA content in pastures was analysed by HPLC at the Institute of Food Technology (CIA, INTA, Castelar, Buenos Aires, Argentina). ZEA was extracted using acetonitrile: water (75:25), with alternating agitation and sonication for 30 min. The mixture was centrifuged and the supernatant purified using immunoaffinity columns R-Biopharm (R-Biopharm Latinoamerica S.A., Buenos Aires, Argentina, www.r-biopharm.com). The purified extract was analysed by HPLC using a fluorescence detector (Waters Alliance System, Milford, Massachusetts, USA) (Schuhmacher et al., 1998; Eskola et al., 2002; De Saeger et al., 2003). Analyses were performed with an injection volume of 30 μl. The chromatographic conditions were: BDS Hypersil C18 column, 150 × 4.6 mm, 3 μm, flux 0.2 ml/min, elusion gradient 0–3 min A 80 % and B 20%, 3–10 min A 20% and B 80%, 10–13 min A 0% and B 100%, 13–20 min A 80 % and B 20%, being the mobile phase: A - water grade HPLC and B - acetonitrile HPLC grade. Detection was performed at λex = 274 nm, λem = 440 nm. The detection limit was 2 μg/kg.

Isolation and identification of *Fusarium* species

Leaves and stems with and without symptoms of disease were sampled. The plant material was surface disinfected with 70% ethanol for 10 s followed by 10% sodium hypochlorite *v*/v and without rinsing the fragments in sterile water. The plant material was dried in a laminar flow hood and aseptically fragmented using sterile tweezers and scissors. From each pasture sample analysed, fragments (5 × 5 mm) were plated on water agar amended with 200 ppm Vancomycin (100 fragments in total). Cultures were incubated for 5 days at 25°C to 30°C under 12 h cycles of near UV (345 to 400 nm) and darkness. The plates were observed under a stereoscopic microscope (Wild M5® Heerbrugg, Switzerland, 6 to 50×) and fungal growth typical of the genus *Fusarium* was aseptically transferred to Carnation Leaf Agar (CLA) and Potato Dextrose Agar (PDA) plates. The cultures were incubated for 14 days at 25°C to 30°C and 12 h cycles of near UV and darkness. Samples were inspected using a microscope after staining with cotton blue (0.1%) containing lactic acid as the mounting fluid. Microscopic observations were made at 3 and 14 days of incubation (Zeiss Axiostar Plus® microscope, Göttingen, Germany, 50 to 1000×). The identification was performed based on colony and spore morphologies according to Gerlach and Nirenberg 1982; Nelson et al., 1983, and Leslie and Summerell 2006.

In vitro analysis of the toxicogenic capacity of *Fusarium* isolates by enzyme-linked immunosorbent assay (ELISA)

To analyse the toxicogenic ability of *Fusarium* isolates, 200 g of white rice was sterilised with 100 ml of water in 1.5L flasks (121°C, 15 min). A loopful of the CLA fungal culture (20 days incubation) was suspended in 5 ml of 0.1% peptone water with 0.05% of Tween 20 (PW). The flasks of rice were inoculated with 5 ml of the PW 10^4 conidial suspension. The flasks of rice were incubated in 12 h daylight at 28°C for 1 month. The flasks were again autoclaved and the contents preserved at −20°C until analysis. The content of ZEA was determined in the Toxicology Laboratory at the Institute of Pathobiology (CICVyA, INTA Castelar), by ELISA Ridascreen® Fast Zearalenon (RBiopharm - Germany). The toxin was extracted using 70% methanol for 3 h with agitation. The extract was filtered through paper (Wathman 11 μm) and diluted 1:1 with distilled water for analysis. Solutions of standards were used at the following concentrations: 0, 50, 100, 200 and 400 μg/kg. The absorbance was determined at 450 nm in a Biotek ELx800 reader (BioTek Instruments, Inc. Winooski, VT, USA, www.biotek.com). The detection limit was 50 μg/kg.

Autumn weather conditions in 2012 and 2013

Readings of daily maximum (xT;°C) and minimum (nT;°C) temperatures, precipitation (Pr; mm) and relative humidity (RH; %: average of the three observations made at 9, 15 and 21 h) were collected from Colonia Benítez INTA meteorological station in 2012 and 2013. Daily average temperature (Td) was calculated as the mean of xT and nT. From these daily data, secondary weather variables were calculated in the autumn (March–May) of both years: **DPr:** total days with precipitation (Pr > 0.2 mm), **AcPr**: accumulated millimeters of daily precipitation, **DPrRH81**: total days with simultaneous occurrence of precipitation (Pr > 0.2 mm) and RH > =81%, **DRH70**: total days with occurrence of RH > =70%, **DwPrRH < 70**: total days without precipitation (Pr < =0.2 mm) and RH < 70%, **DD**: total degree days (°C-days) resulting from accumulating Td (>0°C) in days with nT > 10°C and xT > 27°C, **DD x DwPrRH < 70**: product between DD and DwPrRH < 70, **MxT:** mean maximum temperature (°C), and **MnT**: mean minimum temperature (°C). Departures (anomalies) of the values of these 2012 and 2013 autumn weather variables from the respective long-term (1971–2013) 50th percentile (median) values were calculated. The values of the most relevant autumn weather variables calculated in both years were graphically compared with the 10th, 50th and 90th percentile values of those variables in the 1971–2013 base period.

Assuming that frost occurs on any day when daily minimum shelter temperature is ≤3°C, the number of frost days **(FD)** was calculated not only in the March–May trimester, but also in the April–June and May–June-July trimesters of both years.

Results

The highest concentrations of ZEA were found in the samples collected from sorghum, especially in sorghum stems, which showed much higher values in 2012 compared to 2013 (Table 1). *Cynodon plectostachyus* also had high ZEA values, whereas *Setaria spp.* did not show particularly high values of ZEA. ZEA was also found in other species consumed by animals (Table 2). In 2012 (Tables 1 and 2), ZEA was detected in 94.2% of the samples, of which 47 % had high to very high concentrations of ZEA (310 to 6279 μg/kg), 23.5% had intermediate concentrations (146 to 233 μg/kg), and 23.5% had low concentrations of ZEA (4 to 74 μg/kg). Only one sample, corresponding to sunflower pellets, had no detectable ZEA. In 2013, 41.6 % of the samples had intermediate concentrations of ZEA (131 to 296 μg/kg), whereas 50 % had low concentrations of ZEA

(< 2 to 91 μg/kg). ZEA was not detected in only one sample, but on this occasion that sample was rice straw.

In 2012, a total of 23 isolations of *F. semitectum* (Fig. 2) were made and positively identified in three of the selected sample types: sorghum leaves (cv King Panar), sorghum panicles (line VDH 422), and *Setaria spp.* Typical characteristics of *F. semitectum* were observed in all these cultures (Fig. 3). *Fusarium* was not isolated from sorghum silage. In July 2013, four of the 12 samples collected (two *Cynodon plectostachyus* samples, one sorghum panicle sample and one sorghum silage sample) were cultured to test for presence of *F. semitectum*, but only samples from sorghum panicles appeared to be colonised. Of these, 14 isolates produced <400 μg/kg of ZEA (Table 3).

Negative anomaly values for moisture variables including DPr, AcPr, DPrRH81 and DRH70 were observed in autumn 2012 (Table 4). Lower values of these moisture variables would enhance plant stress due to drought. Conversely, positive to zero anomalies for the same variables were observed in

Table 1 Content of ZEA (μg/kg) in July 2012 and 2013 in various pastures and other livestock food resources in Colonia Benítez, northeastern Argentina

Sample type	ZEA concentration (μg/kg)	
	2012	2013
Sorghum panicle (VDH 422)[a]	1388	212
Sorghum leaves (VDH 422)	4	< 2
Sorghum stems (VDH 422)	6279	31
Chloris gayana (*1*)	< 2.0	< 2
C. gayana (*2*)	-[b]	285
Setaria spp. (*1*)	146	91
Setaria spp. (*2*)	182	-
Cynodon plectostachyus (*1*)	1280	296
C. plectostachyus (*2*)	-	195
Sorghum silage (VDH 422)	37	27

[a] Hybrid

[b] No sample

Table 2 ZEA content (μg/kg) in pastures and other livestock food resource samples collected in July of 2012 and 2013 in Colonia Benítez, northeastern Argentina

Sample type	ZEA concentration (μg/kg)	
	2012	2013
Corn grain	414	-[c]
Sunflower pellets	*ND*[a]	-
Cotton seed	74	-
Hemarthria altísima	58	-
Sorghum stems (Antel Peman)[b]	2611	-
Sorghum leaves (Antel Peman)	233	-
Sorghum stems (King Panar)[b]	2305	-
Sorghum leaves (King Panar)	595	-
Sorghum panicle (King Panar)	310	-
Rice straw roll	-	*ND*
Sunflower pellets	-	33
Leersia hexandra	-	131

[a] *ND* not detected

[b] Hybrid

[c] No sample

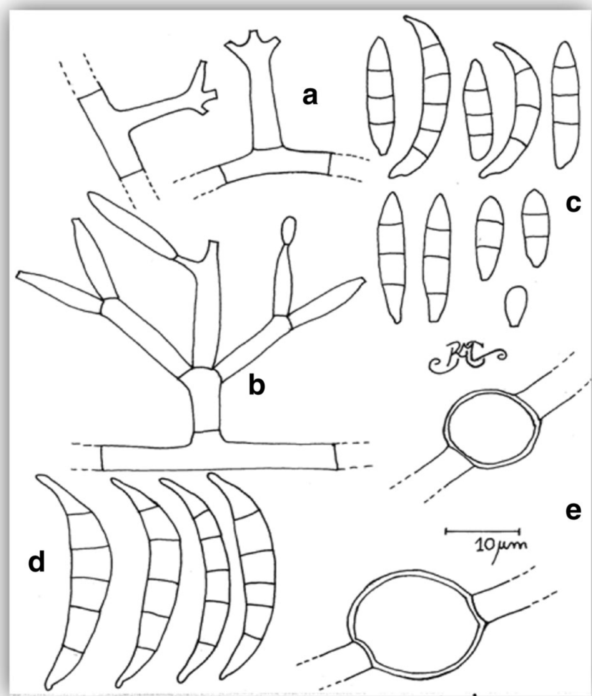

Fig. 2 *Fusarium semitectum*. **a** polyphialides, **b** branched conidiophore with monophialides and polyphialide, **c** mesoconidium and microconidium, **d** macroconidium of sporodochia, **e** chlamydospore

autumn 2013. Another way of expressing the autumn weather conditions leading to water-stressed plants was by estimating variables which accumulate daily temperatures (base temperature: 0°C) over those days with large diurnal temperature range (DD) or by counting the number of dry days (DwPrRH < 70). Positive anomalies for both variables were observed in autumn 2012, contrasting to the zero to negative anomalies in 2013. The same results were obtained when the product between DD and DwPrRH < 70 was analysed. Both the high MxT and low MnT values observed in autumn 2012 agree with the large diurnal temperature range usually accompanying dry days. In contrast, during wet periods, as in the autumn of 2013, low MxT and high MnT occurred (Table 4). In 2012, the moisture and temperature variables analysed sharply deviated from the long-term median values, showing that the autumn period of this year was one of the driest of the 1971–2013 historical series. The autumn of 2013 was clearly average, with weather variable values around the corresponding long-term medians (expected every other year) (Figs 4 and 5). The variable resulting from the product between DD and DwPrRH < 70 reached a value equal to the 90th percentile in 2012 and was below the long-term median value in 2013, showing contrasting autumn weather conditions (Fig. 5).

Daily shelter minimum temperatures were >3°C during the March–May period of both 2012 and 2013 (no frost occurred). When analysing the April–June and May–July periods, the number of days with frost (FD) was clearly different between

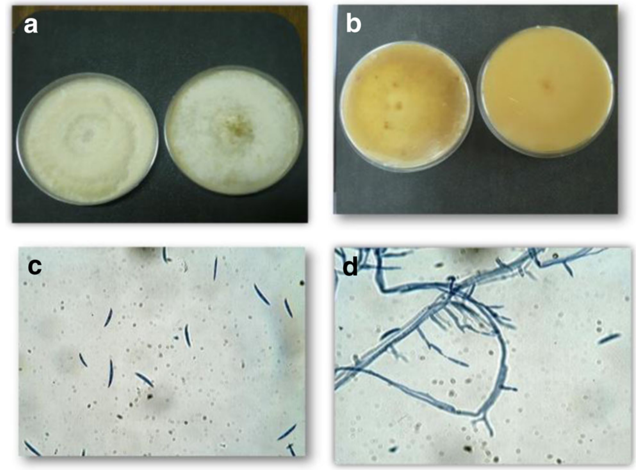

Fig. 3 *Fusarium semitectum*. **a** obverse of a PDA plate, **b** reverse of a PDA plate; **c** macroconidia, **d** polyphialides

both years. FD reached values of 4 and 12 in both periods of 2012, which were above the long-term (1971–2013) median values (3 and 8 frost days in both periods respectively). In contrast, 0 and 5 frost days occurred in both periods of 2013, which were below the long-term median values.

Discussion

The highest concentration of the mycotoxin ZEA in forage samples from Colonia Benítez was recorded in those samples collected in July 2012, contrasting with that found in 2013. Inspections by SENASA (National Service for Agrifood Health and Quality, Argentina) in the same region found the metabolite zeranol in urine samples collected from cattle (SENASA 2010-2011). The synthesis of zeranol derives from ZEA, and if animals ingest ZEA through consumption of contaminated fodder, zeranol is found in the excreted urine together with other metabolites (Fink-Gremmels and Malekinejad 2007; Salvat et al., 2015) It should be mentioned that the use of zeranol-based anabolics is prohibited in Argentina as well as in other countries.

Drought represents one of the most important environmental constraints conducive to plant stress. The present study focused on the identification of weather variables expressing conditions leading to drought-stressed plants over the autumn period preceding the collection of forage samples in July 2012 and July 2013. The high concentration of ZEA found in many of the forage samples collected in July 2012 was preceded by moisture and temperature conditions exceptionally conducive to stressful situations that could result in colonisation of the forage by *Fusarium* spp., and subsequent accumulation of ZEA. The autumn of 2012 was characterised by low moisture values, high temperatures, large diurnal temperature ranges and high and low MxT and MnT respectively. The opposite

Table 3 Total number of isolates of *Fusarium semitectum* isolated, number of toxigenic isolates and production of ZEA in 2012 and 2013 in Colonia Benítez, northeastern Argentina

Sample[a]/year	Fungus	Total N° of isolates	N° of isolates analyzed	N° of toxicogenic isolates	ZEA content (μg/kg)
A/12	*F. semitectum*	12	8	3	*ND* – 100
B/12	*F. semitectum*	10	9	8	*ND* - > 400
C/12	*F. semitectum*	1	1	1	> 400
D/12	*ND*[b]	0	0	0	*ND*
E/13	*ND*	0	0	0	*ND*
F/13	*ND*	0	0	0	*ND*
G/13	*ND*	0	0	0	*ND*
H/13	*F. semitectum*	14	14	14	*> 400*

[a] A: Sorghum leaves (King Panar); B: sorghum panicle (VDH 422); C: *Setaria* spp.; D: sorghum silage; E: *Cynodon plectostachyus*; F: *Cynodon plectostachyus*; G: sorghum silage; H**:** sorghum panicle (VDH 422)

[b] *ND* not detected

trend was found for the weather conditions in autumn 2013. In 2012, the product between total days without precipitation and low relative humidity (<70%) and degree days accumulated (DD x DwPrRH < 70) reached an exceptionally high value, expected only in one every 10 years. This high value would result in high evapotranspiration, which can quickly lead to situations of water deficit and drying (senescence) of plant tissues, and subsequent colonisation by fungal opportunists. Additionally, the process of senescence accelerates when there are more frosts at the end of the autumn and the beginning of winter. The frequency of frosts in April–June and May–July was higher than the long-term median values in 2012, but lower than the medians in 2013.

ZEA in forage appears to be linked to higher autumn activity of ZEA-producing saprophytic fungi (Reed and Moore 2009) that inhabit pasture stubble during decomposition. In general, these fungi take advantage of stressed situations to colonise weaker or nutrient-deficient tissues (Carmona 2008; Simón et al., 2013). *Fusarium semitectum* can be isolated from aerial parts of tropical and sub-tropical plants (Leslie and Summerell 2006) and, in the northeast region of Argentina, it appears to have an ideal habitat to grow and produce toxins, at least during certain periods of the year (Salvat et al., 2013a, b). The results of the current study are in agreement with these previous observations during the summer months (Salvat et al., 2013b).

Besides the effect of the environment on the availability of senescent forages, other factors such as the growth rate of forage species and grazing management may also explain the differences in the concentration of ZEA found among the different forage resources analysed in 2012 and 2013. Laser et al. (2003) found that fungal infection, measured by the concentration of ergosterol, was higher in *Lolium perenne* than in *Festuca arundinacea*, a situation explained by the creeping growth habit of the former. Similarly, in New Zealand, Di Menna and Sprosen (1992) observed differences in the levels of ZEA among the different species of pastures that had been grazed versus those that had not, with strong tendency to increase with the progress of autumn. These authors found that the level of ZEA were almost four times higher in pastures of *Lolium* compared to pastures of *Festuca arundinacea*. Regarding the management of forage resources, Behrendt et al. (2004) found that if the first cut of pastures and crops for silage or hay is delayed, there is greater plant senescence, which significantly

Table 4 Secondary weather variables calculated for March–May 2012 and 2013 and anomalies (A) resulting from the difference between the value of each weather variable for the year analyzed and the corresponding median value from the 1971–2013 series (A = year value - median)

Weather Variable[a]	2012	A	A
DPr	12	−9	2
AcPr	263	−138	141
DPrRH81	7	−10	0
DRH70	58	−25	0
DwPrRH < 70	31	22	0
DD	1306	148	−281
DD × DwPrRH < 70	40,482	30,616	−1799
MnT	15.8	−0.7	0.4
M×T	28.1	1.1	−0.9

[a] Daily maximum (×T;°C) and minimum (nT;°C) temperatures, precipitation (Pr; mm) and relative humidity (RH; %: average of the three observations made at 9, 15 and 21 h). Daily average temperature (Td) was calculated as half the sum of ×T and nT. **DPr:** total days with occurrence of precipitation (Pr > 0.2 mm), **AcPr**: accumulated millimeters of daily precipitations, **DPrRH81**: total days with simultaneous occurrence of precipitation (Pr > 0.2 mm) and RH > =81%, **DRH70**: total days with occurrence of RH > =70%, **DwPrRH < 70**: total days without precipitation (Pr < =0.2 mm) and RH < 70%, **DD**: total degree days (°-days) resulting from accumulating the Td (>0°C) in days with nT > 10°C and ×T > 27°C, **DD × DwPrRH < 70**: product between DD and DwPrRH < 70, **M×T:** mean maximum temperature (°C), and **MnT**: mean minimum temperature (°C)

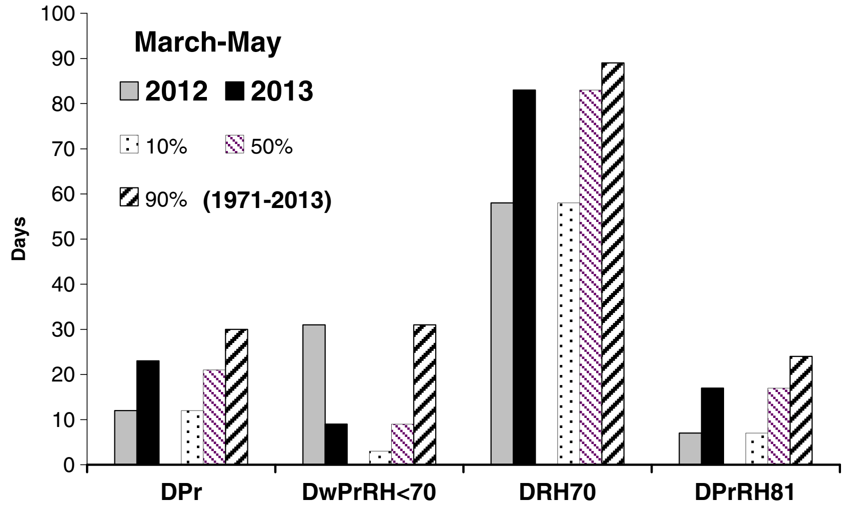

Fig. 4 Comparison between the values of weather-based variables (DPr, DwPrRH < 70, DRH70 and DPrRH81) for March–May 2012 and 2013 and those reached in the 10th, 50th (median) and 90th historical percentiles (1971–2013 series), in Colonia Benítez (Chaco). Precipitation (Pr; mm) and relative humidity (RH; %: average of the three observations made at 9, 15 and 21 h). **DPr:** total days with occurrence of precipitation (Pr > 0.2 mm), **AcPr**: accumulated millimeters of daily precipitations, **DPrRH81**: total days with simultaneous occurrence of precipitation (Pr > 0.2 mm) and RH > =81%, **DRH70**: total days with occurrence of RH > =70%, **DwPrRH < 70**: total days without precipitation (Pr < =0.2 mm) and RH < 70

influences colonisation by fungi and bacteria. In the present study, *C. gayana* and the silage produced from mature sorghum had low concentrations of ZEA, whereas *C. plectostachyus* (which has a creeping habit) showed high concentrations of ZEA (Table 1). However, further studies are needed to reliably verify differences in the levels of ZEA in the different plant species used for livestock feed in the northeast region of Argentina.

We conclude that the anomalous values of moisture and temperature variables (expected approximately once every 10 years) found in March–May 2012 were conducive to drought-stressed plants, which accelerated the senescence of forage plants available for livestock and consequently likely increased its concentration of ZEA (due to greater activity of the ZEA-producing saprophytic fungi including *F. semitectum*). In contrast, when the weather

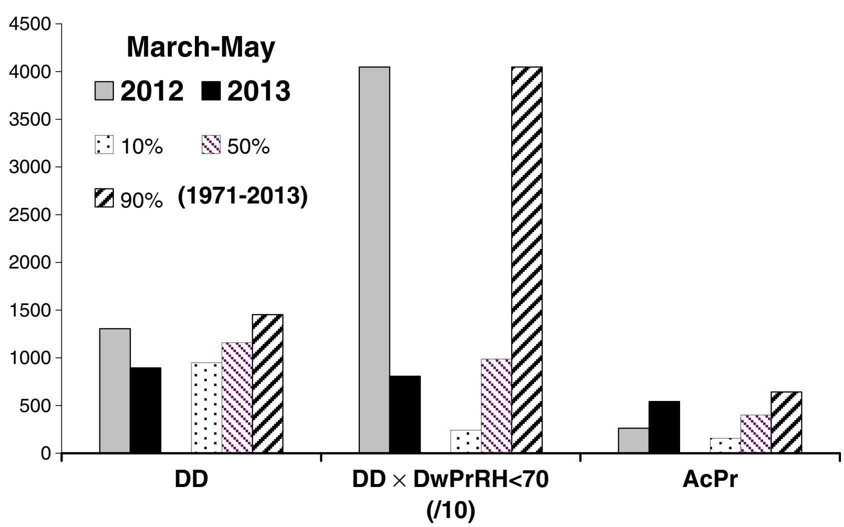

Fig. 5 Comparison between the values of weather-based variables (DD in °days, DD × DwPrRH < 70 in °days x days and AcPr in mm) for March–May 2012 and 2013 and those reached in the 10th, 50th (median) and 90th historical percentiles (1971–2013 series), in Colonia Benítez (Chaco). Daily maximum (×T;°C) and minimum (nT;°C) temperatures, precipitation (Pr; mm) and relative humidity (RH; %: average of the three observations made at 9, 15 and 21 h). Daily average temperature (Td) was calculated as half the sum of ×T and nT. **DD**: total degree days (°-days) resulting from accumulating the Td (>0°C) in days with nT > 10°C and ×T > 27°C, **DwPrRH < 70**: total days without precipitation (Pr < =0.2 mm) and RH < 70%, **DD × DwPrRH < 70**: product between DD and DwPrRH < 70, **AcPr**: accumulated millimeters of daily precipitation

variables were within normal values (approximately the median of the historical series, expected every other year), and as occurred in the autumn of 2013, the pastures and feed collected in the winter showed low to intermediate levels of ZEA. The high frequency of frosts that occurred in late autumn-early winter 2012 also accelerated canopy senescence of the forage plant materials in comparison to 2013. The contrasting autumn environments likely led to different ZEA contents in winter fodder samples.

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