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Maize monoculture causes niacin deficiency in free-living European brown hares and impairs local population development

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Maize (*Zea mays*) is the most produced crop worldwide and the second most important bio-energy plant. Huge maize monoculture is considered a threat to biodiversity in agricultural landscapes and may also contribute to the decline of European brown hares (*Lepus europaeus*, Pallas 1778). Indeed, the intensification of agriculture has been identified as one of the main factors responsible for the decline of brown hare populations. A reason why large maize cultures can be particularly detrimental to animals consuming this plant is its poor nutritional value with respect to niacin. In this study, we investigated the effects of the proportion of area under maize crops on liver concentrations of niacin in free-living hares, on the reproductive output of does (females), and on the development of local populations, at nine study sites in Lower Austria. Hare numbers were estimated from spotlight counts in spring and autumn. Liver samples and uteri were obtained from hares shot in the same areas during regular autumn hunts. Number of offspring born to an individual female during the preceding reproductive period was determined by counting placental scars. Our results show a significant negative effect of the area under maize crops on liver concentrations of niacin of does and on their reproductive output. Further, we found a significant negative effect of the area under maize on the development of a population. Altogether, our findings indicate that high proportions of the area under maize crops contribute to the decline of brown hares by reduced fecundity of does and impaired development of local populations.

KEYWORDS
niacin, placental scars, maize crops, *Lepus europaeus*, monoculture, fecundity, population development

Introduction

Maize (*Zea mays*) is the most produced crop on a global scale (Nuss and Tanumihardjo, 2010), and is, with rapeseed, one of the two main energy crops cultivated in Europe (Klenke et al., 2017). Maize is considered as having the highest yield potential of energy crops grown in central Europe (Amon et al., 2007), and will therefore probably become increasingly important for energy/biogas production (Nielsen and Oleskowicz-Popiel, 2008). However, large-scale cultivation of maize is increasingly threatening biodiversity (Fargione et al., 2009), and affects most farmland animals negatively (Klenke et al., 2017). Low habitat quality, e.g., loss of rotational set-aside or loss of habitat heterogeneity due to maize cultivation, with a landscape structure no longer meeting the ecological requirements of many species, seems to be responsible (Gevers et al., 2011).

Population trends of a typical farmland species, the European brown hare (*Lepus europaeus*, Pallas 1778), show a strong decline in many European countries over the last decades (Mary and Trouvilliez, 1995). As this species also represents an important game species (Pielowski, 1976), long-term trends can be monitored by hunting bag data (Tapper and Parsons, 1984; Langbein et al., 1999). These records show a dramatic population decline throughout Europe [Poland: Pielowski and Raczynski (1976), Denmark: Madsen et al. (1996), Wincentz Jensen (2009), parts of Croatia: Pintur et al. (2006); Popović et al. (2008), Serbia: Ristić et al. (2021), Germany, Austria, Bulgaria, Luxembourg, Netherlands, Slovakia, Switzerland: Mary and Trouvilliez (1995), United Kingdom: Smith et al. (2005)].

A large number of studies tried to identify causes for this decline (reviewed in Smith et al., 2005). Negative effects of predators like a red fox (*Vulpes vulpes*, Linnaeus 1758) on brown hare abundance were reported from Poland, Germany, and the United Kingdom (Reynolds and Tapper, 1995; Panek et al., 2006; Kalchreuter, 2015). However, Weber et al. (2019) showed that populations could grow despite high predator abundance when sufficient habitat structures offering cover to leverets were present. There was no evidence for reduced fertility of brown hare does as a potential cause of the decline (Hackländer et al., 2001; Schai-Braun et al., 2020), but low temperatures and high precipitation during spring increase leveret mortality (Hackländer et al., 2002). Farming practice, particularly the intensification of agriculture, was identified as a significant factor negatively affecting the abundance and population dynamics of European brown hares (Smith et al., 2005), presumably due to poor nutrition caused by low crop diversity and large fields (Tapper, 1987). However, Smith et al. (2005) found no effect of field size *per se* on population densities, but report a negative effect of extensive monocultures on hare abundance. Accordingly, population decline in Bulgaria, for instance, began simultaneously with the increase of monocultures (Petrov, 1976). Increase in maize monoculture was also identified as a potential cause of a population decline in Germany (Sliwinski et al., 2019). A decline in hare densities of up to 90% after a change to maize monoculture was reported by Bertóti (1975).

Altogether, loss of cover and malnutrition are considered important causes of low reproduction, and especially high postnatal mortality of brown hares in Europe (Hansen, 1992; Edwards et al., 2000). The diet of hares is twice as diversified in mixed compared to monocultural farmed landscapes (Frylestam, 1986). There is, however, local variation according to the type of cultivated crops, field size, and alternative field habitats (Reichlin et al., 2006; Petrovan et al., 2012). The brown hare is highly selective in its food consumption and is, to some extent, actively searching for plants rich in fat (Schai-Braun et al., 2015). To meet their nutritional requirements, hares rely strongly on weeds (Reichlin et al., 2006; Schai-Braun et al., 2015), and specific crops such as beets (*Beta vulgaris*), soybean (*Glycine max*), and fodder crops like alfalfa (*Medicago sativa*) or red clover (*Trifolium pratense*). However, most weeds have vanished from farmed landscapes because of the high utilization of herbicides in conventional farming (Wilson et al., 1999; Stoate et al., 2001; Gaba et al., 2016), and fodder crops are nowadays far less cultivated at the expense of energy crops or cereals (Klenke et al., 2017).

An alternative, but not mutually exclusive hypothesis for the negative effects of maize on animals involves its low nutritional value for species consuming this crop. Indeed, although maize is particularly valued for its fatty acid composition (especially adapted for livestock diets), and its high sugar level (for ethanol production), it contains low proportions of many essential micronutrients including calcium, manganese, copper, and iron (Nuss and Tanumihardjo, 2010; INRA et al., 2011). Moreover, maize seeds and leaves are highly deficient in niacin and its precursor tryptophan (Hogan et al., 1955; Henderson et al., 1959; Goss, 1968; Mawson and Jacobs, 1978). Tryptophan (trp) is an essential amino acid for all eukaryotes and even some prokaryotes (de Groot, 1953; Meisinger, 1978; Kantak et al., 1980; Walz et al., 2013). It is the precursor of 5-hydroxytryptophan, an important monoamine neurotransmitter, and of niacin (Kohlmeier, 2003). Niacin can be decomposed into two molecules, nicotinic acid and nicotinamide, essential for the *in vivo* synthesis of nicotinamide adenine dinucleotide (NAD) (Wan et al., 2010). NAD is indispensable for the effective functioning of the Krebs cycle and therefore for cell respiration and ATP synthesis. Animals must obtain trp and most of their niacin from food on a daily basis. Only plants and microorganisms can synthesize trp, and niacin synthesis from trp is very inefficient in many animal species (Baker, 2008). However, in addition to the deficiency of trp in maize, up to 90% of niacin is present as niacytin in mature maize grains, i.e., bound up in a complex with hemicellulose which renders it unavailable to vertebrates (Ammerman et al., 1995; Ball, 2005; Baker, 2008). Deficiencies in trp and its derivatives, especially nicotinamide, lead to dementia, diarrhea, and dermatitis (i.e., skin rashes) in humans and hamsters (Hegyi et al., 2004; Wan et al., 2010; Tissier et al., 2017), the black-tongue syndrome in dogs (Baker, 2008), and aggressiveness and growth retardation in rats (Krehl et al., 1945; Kantak et al., 1980; Walz et al., 2013). Tissier et al. (2017) recently highlighted that niacin deficiency causes high rates of maternal infanticides in European hamsters fed a diet dominated by maize, reducing reproductive success by up to 70%.

Considering the increase in field size and the amount of maize cultivation, as well as the fact that brown hares select the maize plant as a food source in certain periods of the year (Schai-Braun et al., 2015), and the negative effects of maize dominated diets, we expected with increasing proportions of maize crops in the agricultural landscape

1. Negative effects on niacin concentrations in the liver of brown hares where "*de novo* biosynthesis" of nicotinic acid solely takes place (Yang and Sauve, 2016), and reserves of this vitamin are stored (Podlogar and Smollich, 2019).
2. Negative effects on hare densities due to detrimental effects of niacin and trp malnutrition on survival and reproductive output of does.

Materials and methods

We gathered data at nine study sites in Lower Austria during 2017 and at one site also in 2018 (for more detailed information about each study site, see **Supplementary Table 1**). From these sites, we selected 226 does for tissue sampling from a total of 1,054 hares shot during regular autumn hunts. Selected animals were shot during our presence. The selection was made to guarantee high-quality, and fresh samples. Of these 226 carcasses, only 117 had intact uteri. We assumed random shooting and hence considered local hunting bags as representative samples of hare populations. Carcasses were sexed by optical inspection of secondary sexual characteristics by experienced biologists. After the first examinations in the field, carcasses were cooled to 2°C. Liver samples (1 g of tissue) and uteri were stored at −18°C until further analyses.

In the laboratory, we determined in liver samples three forms of niacin, free nicotinic acid, free nicotinamide, and nicotinamide moiety of NAD+/NADP+, by gas chromatography with flame ionization detection (Hämmerle et al., 2020). Total niacin concentrations were expressed as the sum of these three forms in μg per g of liver tissue. Reproductive output of does during the preceding season of reproduction was determined by counting placental scars (Hackländer et al., 2001). *In situ* age classification was verified by the weight of dried eye lenses (Suchentrunk et al., 1991).

Population sizes (individuals per 100 ha) were estimated by spotlight counts (Langbein et al., 1999; Schai-Braun et al., 2013) in spring (late March/early April) and autumn (October) at the nine study sites in Lower Austria during years 2002–2015.

The proportion of arable land cultivated with maize crops was obtained for each study site and year from Statistik Austria (2018).

Statistical analyses

All statistical analyses were performed using R v4.2.1 (R Core Team, 2021). If necessary for obtaining the normal distribution of residuals, we log-transformed variables. We investigated the effects of the area under maize crops, and of liver vitamin B3 concentrations, on the difference between spring and autumn counts of hares with linear mixed effects models [lme, package "nlme"; Pinheiro et al. (2021)]. The study area was included in these models as a random effect to account for repeated measurements at one site. To identify a potential effect of maize monoculture on the reproductive output of does, independent of the effect of niacin concentration in the liver, we calculated a generalized linear mixed effects model with family "poisson" (glmer, package "lme4; Bates et al. (2015). The response variable was in this model the number of uterine scars. The concentration of niacin in the liver and proportion of area under maize crop were entered as fixed effects, study area as random effect. Plots were generated using the "ggplot2" package (Wickham, 2016) with a back transformation of logarithmized values.

Results

The proportion of area under maize crops of total arable land at our study sites had a significant negative effect on the development of the local hare populations (**Figure 1**, $p < 0.005$, t-value = −2.872, $R^2 = 0.063$). For every 10% increase of the area under maize crops, the difference between spring and autumn counts of hares decreased on average by 2.2 hares per 100 ha. To some degree, this negative influence was caused by an increasingly lower fecundity of does. The more maize was cultivated at a study site, the lower the number of uterine scars found in does shot there (**Figure 2**, glmer: $p = 0.002$, z-value = −3.097, $R^2 = 0.083$).

We further found that the proportions of arable land used for growing maize had a significant negative relation with niacin concentrations in liver samples of does living there (**Figure 3**, $p = 0.013$, t-value = −2.497, $R^2 = 0.182$), with a decrease of on average 0.97 μg/g with every 10% increase of the area under maize crops. In line with these results, we also found a negative association between niacin concentrations in livers and the population development of

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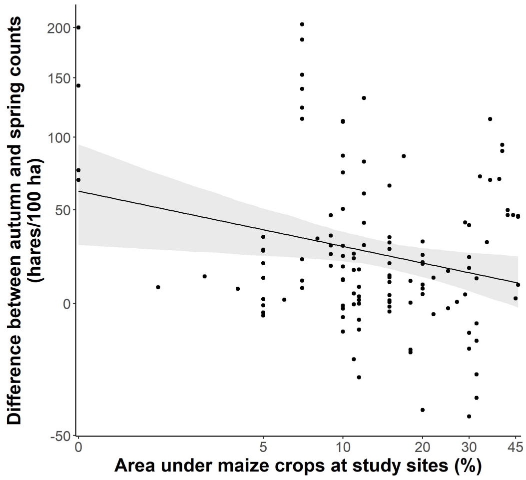

FIGURE 1
Proportion of maize of total arable land and differences between autumn and spring population counts of hares per 100 ha (light shaded: 95% confidence interval). Both variables were transformed with natural logarithm for analysis and back-transformed for the plot.

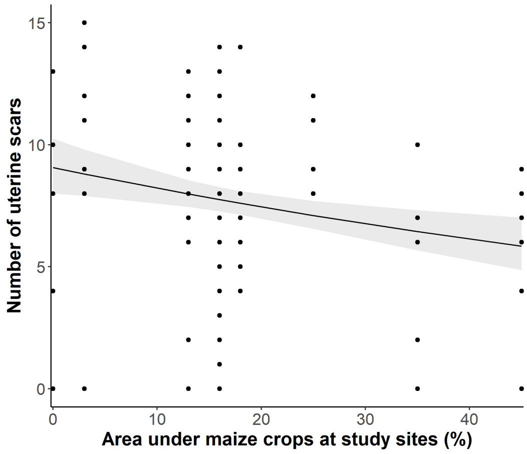

FIGURE 2
Proportion of maize of total arable land and numbers of uterine scars as a measure of fecundity, i.e., total reproductive output of a female during the preceding period of reproduction (light shaded: 95% confidence interval).

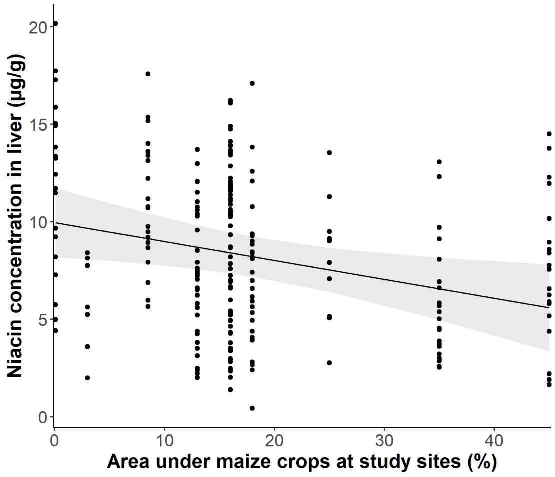

FIGURE 3
Proportion of maize of total arable land and concentrations of niacin in liver samples (light shaded: 95% confidence interval).

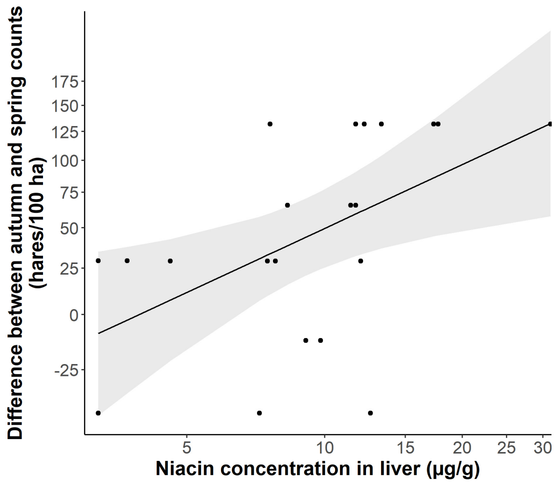

FIGURE 4
Differences between autumn and spring population counts of hares and its relation to the liver concentrations of niacin found at a study site (light shaded: 95% confidence interval). Both variables were transformed with natural logarithms for analysis and back-transformed for the plot.

local hare populations (**Figure 4**, $p = 0.018$, t-value = 2.627, $R^2 = 0.257$). However, a direct effect of niacin deficiency on the number of uterine scars, independent of other potential effects of maize abundance, was statistically not detectable (partial regression coefficient −0.008, z-value = −0.934, $p = 0.346$). Conversely, the negative effect of the proportion of arable land used for maize production on the reproductive output of does remained significant when adjusting for a direct effect of niacin (partial regression coefficient −0.01, z-value = −3.186, $p = 0.001$), suggesting that other aspects of maize cultivation were also detrimental, on top of an undersupply of niacin.

Discussion

In this study, we discovered several important effects of maize monoculture on local brown hare populations. Our results show that populations rather declined from spring to autumn, or grew less, the higher the proportion of maize crops on total arable land was. A similar negative effect of maize cultures on brown hare abundance has been reported by Mayer and Sunde (2020). Potential causes could be temporal migration of hares and a shift or increase in their home ranges after harvest events, as described by Ullmann et al. (2018) and Schai-Braun and Hackländer (2014). However, a recent study by Ullmann et al. (2020) showed, based on telemetry data, that hares specifically shift their home ranges toward maize fields after the harvest of maize. These areas are selected because of potential cover and forage (Mayer et al., 2018). It therefore seems unlikely that dispersal contributes significantly to the change of hare abundance in a given area from spring to autumn. Instead, our findings suggest that the detrimental effects of maize monoculture on survival and reproduction are responsible.

Effects of maize monoculture on survival of hares

Brown hares select plants rich in fat and protein and strongly rely on weeds (Reichlin et al., 2006). Similar feeding behavior was also observed in the Mountain hare (*Lepus timidus*, Linnaeus 1758) (Dingerkus and Montgomery, 2001), in the Arctic hare (*Lepus arcticus*, Ross 1819) (Klein and Bay, 1994), in the Italian hare (*Lepus corsicanus,* de Winton 1898) (Rizzardini et al., 2019), and the Granada hare (*Lepus granatensis,* Rosenhauer 1856) (Paupério and Alves, 2008). Therefore, brown hares likely suffer malnutrition when living in areas with monocultures poor in plant biodiversity (Schai-Braun et al., 2015). It is well known that malnutrition negatively impacts body condition and hence the survival of brown hares (Hansen, 1992; Edwards et al., 2000). Further, malnutrition impairs reproductive success, because lactating does experience high energetic costs. Particularly for nursing litters in spring, does rely to a certain degree on energy reserves stored in body fat depots (Parkes, 1989; Valencak et al., 2009). Female hares nurse their offspring only once a day (Broekhuizen and Maaskamp, 1980). Further, leverets lack shelters like burrows and are therefore exposed to unfavorable weather conditions, particularly in spring (Andersen, 1952; Broekhuizen and Maaskamp, 1980; Hackländer et al., 2002; Karp and Gehr, 2020). It is therefore pivotal for leverets to obtain milk with a high energy content. To meet this requirement, does produce milk with a high-fat content, which in spring is even fatter than average (30% vs. 20%, Valencak et al., 2009). Sufficient plant biodiversity is absent in areas of maize monoculture even after harvest in autumn and winter (Fuchs et al., 2021), presumably resulting in malnutrition of does during the period when they accumulate body fat for subsequent reproduction. This reduces the survival chances of leverets, particularly in spring litters, which are most important for population development (Olesen and Asferg, 2006).

Effects of maize monoculture on fecundity

We also found a decrease in reproductive output of does with the proportion of maize in arable land. Our measure of reproductive output is the number of scars found in the uteri of does shot during the autumn hunting season. These numbers reflect the total number of juveniles born by a doe during the preceding season of reproduction (Hackländer et al., 2001), and are therefore a measure of female fecundity. Numbers of uterine scars could be affected either by direct negative effects of the area of maize monoculture on brown hare nutrition, and therefore body condition, or by general detrimental effects of monoculture. However, a more likely explanation for the effects on uterine scars is changes in the age structure of a population, because we did not find a direct effect of niacin stored in the liver on the number of uterine scars. Schai-Braun et al. (2021) showed that heavier does have larger litters. The same study reported that primiparous does, which are lighter, had smaller litters than an adult does. Unfortunately, we have no reliable information about the age structure of females at our study sites to confirm that a higher number of juvenile does in the bag was responsible for lower numbers of uterine scars. Alternatively, the direct effects of niacin stores on the reproductive output of does could have remained undetected due to the small sample size. From 223 uteri gathered, only 117 were suitable for analysis, and few were obtained from areas with no maize monoculture or with high proportions of maize fields (n = 5 and n = 10 respectively).

Maize monoculture and niacin deficiency

For the first time, we identified here that maize monoculture is detrimental to brown hare populations because it leads to niacin deficiency. The concentration of this vitamin in liver tissue of does decline with the area under maize crops. The concentration of niacin is very low in maize. Further, niacin in maize is mainly present in its bound form, rendering it inaccessible to vertebrates (Ammerman et al., 1995; Ball, 2005; Baker, 2008). In addition to the low content of niacin, maize is also poor in its precursor tryptophan (Carpenter, 1983). Consumption of maize therefore also impairs the potential, for an inefficient pathway of biosynthesis of niacin from tryptophan. Fragments of maize plants were found in the stomach contents of brown hares even at low proportions of areas under maize crops, showing that brown hares readily consume maize plants as well as corn (Steineck, 1978; Reichlin et al., 2006).

Diets with maize as a major component can lead to severe health problems. In humans, for instance, such a diet causes the pathology of "pellagra"

(WHO [World Health Organization], 2000). In European hamsters (*Cricetus*, Linnaeus 1758), less than 12% of pups born to mothers fed a niacin deficient diet survived a 30-day period (Tissier et al., 2017). As a reason for infanticide and insufficient maternal care, Tissier et al. (2017) suggested low levels of oxytocin concentrations in the blood of the mothers, caused by niacin deficiency, as this peptide, which acts as a neurotransmitter, plays an important role in maternal and sexual behavior. Stronger effects of maize diets on niacin deficiency on the reproductive output of hamsters could be a result of less pronounced "caecotrophy" compared to hares (National Research Council [NRC], 1995; Weinhold and Kayser, 2006; Miśta et al., 2015). If niacin deficiency has similar effects in hares, does could show abnormal maternal behavior and give up nursing. As a result, offspring survival would be jeopardized because leverets strongly rely on milk supply during the first three weeks of life (Hackländer et al., 2002). Keeping in mind that hare offspring require daily nursing with milk rich in fat, as described above, additional negative effects of niacin deficiency on the fat content of milk, as reported by Havlin et al. (2017), could have further impaired offspring survival.

Conclusion

Our study clearly identified the negative impacts of maize monoculture on population dynamics and reproductive output of European brown hares. Most importantly, we discovered that niacin deficiency, resulting from the consumption of maize, is a likely, so far unknown mediator. However, whether a maize-dominated diet results in hares in similar severe health issues and impairment of reproduction like in other mammals remains an open question. In contrast to humans and mice, brown hares regularly consume soft feces with caecum content, a nutritional pathway making nutrients and vitamins produced by caecal microorganisms available to the host. Therefore, brown hares may be less affected than other species by consuming a maize-dominated diet, a hypothesis that needs to be investigated experimentally.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because samples analysed in this study were taken from legally shot animals during seasonal hunts according to the hunting laws in Austria.

Author contributions

WA and MT conceived the study. AS performed field work. WA and AS analyzed the data. AS, WA, and MT wrote the manuscript. All authors approved the final version and are accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1017691/full#supplementary-material

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