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Effects of contamination of forage with explosives on feeding behaviour in sheep

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English summary

The common military explosives TNT, RDX and HMX are distributed in many military training areas, and are thus encountered by large herbivores. We present the first scientific study of large herbivores' voluntary intake of explosives. Using an indoor, experimental setup we examined if contamination of forage by the compounds affect intake by sheep. Data were analyzed using a general mixed linear model. The results clearly demonstrate that contamination by any of the three explosives reduce forage intake in sheep; in order of increasing avoidance: RDX < TNT < HMX. The avoidance implies a reduced, but still relevant, risk of poisoning of sheep and other large herbivores in military training areas. However, since the results are not directly transferable to rangeland conditions, an outdoor experiment was performed closely mimicking natural conditions in order to investigate responses of sheep to TNT and HMX contamination of growing pasture – on a fine scale. No statistical significant difference was found between grazing in areas contaminated with explosives and grazing in clean areas. The results indicates that sheep can react by avoidance when explosives are present in the forage, but not in a degree that should be given any weight in risk assessment.

Sammendrag

Eksplosivene TNT, RDX og HMX, som er de mest benyttede eksplosivene i ammunisjon, finnes som forurensninger i mange skyte- og øvingsfelt. Eksplosivene er giftige og kan utgjøre en risiko for beitedyr i området. Målet med dette studiet er å undersøke om smak eller lukt av eksplosiver gjør at beitedyr vil forsøke å unngå forurensede områder, eller i motsatt fall om lukt eller smak av eksplosiver tiltrekker beitedyr. Dette er viktig informasjon i en risikovurdering. Ved å benytte et innendørs forsøk med forurensning av for, samt et utendørs forsøk med forurenset beitemark, ble effekter på inntaket av for studert. Resultatene i innendørsforsøket viser at forurensning av for reduserer forinntak hos sau hvor RDX gir minst reduksjon, og HMX gir størst reduksjon. I utendørsforsøket hvor sauene kunne velge mellom forurenset og rent beite ble det ikke funnet noen signifikant forskjell på forinntak i forurensede og rene områder. Den delvise unngåelsen kan indikere en redusert risiko for at beitedyr eksponeres for eksplosiver, men er ikke signifikant nok til at den bør tas hensyn til i en risikovurdering.

Content

Preface

This study was performed under the project 108903, Munitions; pollution, environmental risk and remediation. The study was performed under a cooperation between FFI and the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås. The authors Geir Steinheim, Øystein Holand and Tormod Ådnøy work at the Nowegian University of Life Sciences.

1 Introduction

Military training with heavy weaponry takes place in remote areas often used by wild or domestic herbivores. When detonations of devices are incomplete, fragments of explosives are distributed and may contaminate vegetation, soil and water. Ungulates using such areas are then potentially exposed to the pollutants and risk being affected. The three most common explosives are TNT (2-methyl-1,3,5-trinitrobenzene), RDX (1,3,5-trinitroperhydro-1,3,5-triazine) and HMX (1,3,5,7-tetranitro-1,3,5,7-tetrazocane). All may have negative effects on fauna, including vertebrates (e.g., TNT: Tan et al., 1992; Honeycutt et al., 1996; Reddy et al., 2000; Lewis et al., 2004, RDX: Smith et al., 2009; Zhang and Pan, 2009; Bruchim et al., 2005, HMX: Brunjes et al., 2007; Johnson et al. 2009).

Large herbivores grazing training ranges are exposed to the pollutants mainly through voluntary (salt licks) or inadvertent (contaminated forage) soil ingestion. Grazing ruminants ingest appreciable amounts of soil, though estimates vary greatly with weather conditions and herbage scarcity (Field and Purves, 1964; Healy, 1968; Thornton, 1974; Abrahams and Thornton, 1994; Beyer et al., 1994; Thornton, 2002). Contaminant particles may also stick to plant surfaces in the short time frame. Large herbivores' behavioral responses to TNT, RDX and HMX have not been studied previously.

Clearly, animals have no evolutionary relationships with these contaminants, but bovine rumen microbes are still able, to some degree, to metabolize RDX (Eaton et al., 2009) and TNT (Fleiscmann et al., 2004; Smith et al., 2008). Preference or avoidance may arise from post-ingestive feedback and learning from adverse effects on wellbeing, or initially from novelty of the bitter taste (pers.obs.), or from responses to intoxicating properties (e.g. Stone et al., 1969). We expected sheep to show indifference, or some degree of avoidance, as demonstrated in quails by Johnson et al. (2005).

2 Materials and Methods

Explosives were collected at Hjerkinn shooting range by gathering particles after low order detonations of different grenades (Figure 1). There were no grenades that contained pure RDX. Grenades containing Comp B was used instead, which is a mixture of TNT (ca. 40 %) and RDX (ca. 60 %). Hence, when RDX is referred to in the report it is actually residues of Comp B.

Figure 1 Sampling of explosive particles from a low order detonation of a 155 mm grenade at Hjerkinn, Norway.

2.1 Study animals and experimental setup for the indoor experiment

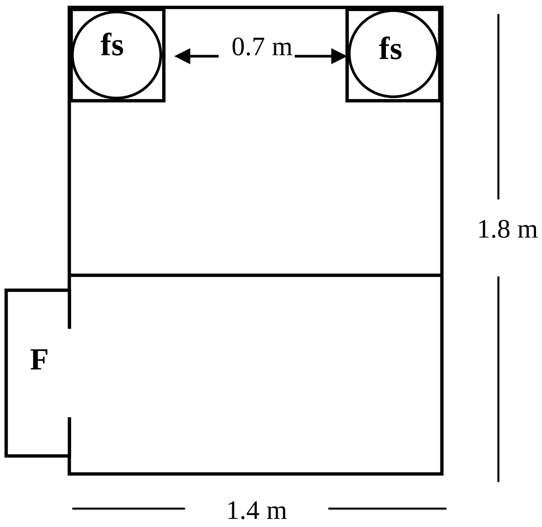

Figure 2 *Extended metabolism cage:* ***fs*** *= experimental forage station with silage, of which one was contaminated (with TNT, HMX or RDX) and one was clean. Stations changed places halfway during the day.* ***F*** *= forage trough for "breakfast" portion (not contaminated).*

None of the study animals suffered ill effects during or after the study that were noticeable. The experiment was approved by the Norwegian Animal Research Authority (NARA).

Nine young rams (≈ 8 months old), mean weight 49.6 kg (sd= 3.2 kg), of the composite breed Norwegian White sheep were used as study animals. The rams were placed in separate metabolism pens, each extended with a larger pen where two food stations were placed (see Figure 2 for details, and Figure 3). The sheep were allowed three days to get familiar with the setup before the experiment started. The experiment started in early April 2009 and consisted of three experimental periods of three days each. A sheep was offered the same contaminant throughout a 3-day period; order of contaminants offered was balanced across animals. Between each period the rams had 7 days of rest and detoxification, staying together as a group in an outside paddock with free access to forage, water and shelter.

Figure 3 Sheep in extended metabolism pen (Photo: Kristin B. Bruun, UMB)

Clean and contaminated grass silage (2.5 g RDX, 5.0 g TNT or 5.0 g HMX; spread evenly on top of 3.9 kg forage portions) was offered in buckets placed within forage stations designed to keep faeces and urine away. Clean and contaminated forage station changed places halfway through the day (start at 1000, swap at 1300, and stop at 1600 hours). Sheep number 7 and sheep number 8 were mistakenly given each other's treatment during the second period; these animals thus received no HMX + two RDX treatments, and no RDX + two HMX treatments, respectively.

The quantity of forage offered in each station was enough that animals could choose to eat from only one of the stations throughout the day. Forage was weighed in the morning before start-up, at swapping of clean/contaminated positions, and at the end of the day. The pens were placed ≈ 1 m apart in two well ventilated rooms; there were no barriers excluding sight, sound or smell between pens. Early in the morning the rams were offered a small "breakfast" portion (1.1 kg) of silage in the forage trough. They had free access to clean drinking water throughout their stay in the pens and were offered a small quantity of salt (NaCl) each day (outside of experimental sessions).

2.2 Study animals and experimental setup for the outdoor experiment

Four circular fenced test area were laid out on a homogeneous field of grasses and herbs, mainly *Poa* spp. , *Agrostis* spp., and *Trifolium* spp., i.e. quite attractive species, with a suitable sward height of approximately 6-8 cm (Figure 4).

The areas were circular with a diameter of 8.0 m. To make observations easier the centre of each circle was covered with a circular tarp with diameter 2 m. Each circle was then divided into eight 45° sectors, each of ≈ 5.8 m^2. In the first circle, sector 1 was randomly assigned as "clean", "HMX" or "TNT", if 1 was "HMX" sector 2 would then be "clean", sector 3 was "TNT", sector 4 was “clean”, sector 5 “HMX” – and so on. Position of treatments was then varied systematically between circles. For an example of the layout, see Figure 5.

30 g of contaminant was mixed with approximately 60 g of dry soil before being spread inside the designated 5.8 m^2 sector. The “clean” sectors received 90 g of soil without contaminants. A total of 12 ewes were used in the study. Groups of three ewes entered the circle and were observed for 11 min – each 10 seconds activity and which sector they were positioned in was recorded (in total 64 observations per animal and observation bout); one observer per animal. With the short period between observations the time-use will accurately index the quantities eaten (Martin & Bateson, 1995).

Figure 4 Sheep in a circular fenced test area (Photo: FFI).

When not being observed the animals spent their time on a pasture with low-quality forage - to ensure (some) grazing during observations. Each group went through all circles twice during the experiment, in a random order.

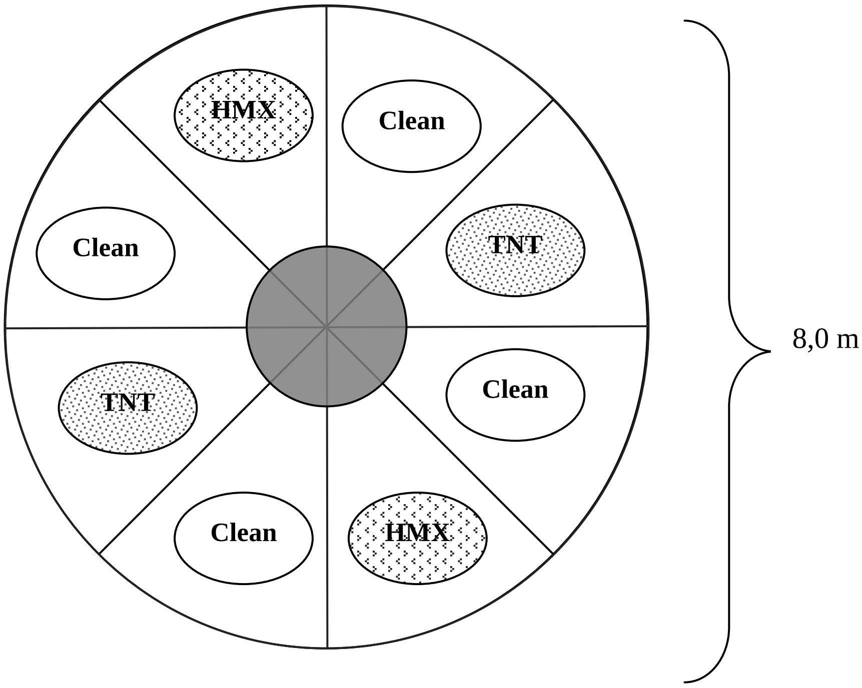

Figure 5 Example of layout of the experimental circles. Two sectors contained TNT and two HMX. The explosives were pulverized and evenly spread within the sectors. Clean= sector without contamination.

2.3 Statistical analysis for the indoor experiment

The mixed procedure in SAS version 9.1 was used for data handling and statistical analysis (SAS Institute Inc., 2004). Means (± SE) were computed. To investigate if contamination had an effect on choice of forage we used a general mixed linear model, with period by session by day defined as a random effect to account for dependency within the data.
The current model was used:

Intake_diff = treatment + sheep + session + <u>*period*session*day*</u> + <u>*e*</u> ,

where **Intake_diff** is a sheep's difference in intake (kg) between contaminated and clean silage during one session (i.e., intake of contaminated minus intake of clean, in kg), **treatment** is either RDX, HMX or TNT, **sheep** is one of the nine study animals, **session** is first (1000-1300) or second (1300-1600) part of the day, <u>***period*session*day***</u> is the random effect of period (3 periods á 3 days) by session (first or second) by day (3 days). Finally, <u>***e***</u> is the residual variation. The Satterthwaite (SAS Institute Inc., 2004) option was used to control denominator degrees of freedom and obtain correct tests; fixed effects were tested against the period by session by day error term. The two interaction effects sheep by treatment and treatment by day were tried in the model but omitted in the final analysis as effects were small and far from significant.

The response variable is the difference in intake between contaminated and clean forage; the test of effect of treatment was thus performed by testing if least square means (lsmeans) for treatment were different from zero (and not by the type 3 F-test). Differences between pairs of lsmeans were then tested to see if the effect on the difference between contaminated and clean was affected by treatment.

2.4 Statistical analysis for the outdoor experiment

Total numbers of foraging observations were recorded, per sector category. For analysis we used the general linear mixed model:

Foraging = **treatment** + **group** + **day** + **ewe** + **e**

where **foraging** is number of observations of foraging of a treatment class (clean, HMX or TNT) during one 11 min observation bout, **treatment** is effect of treatment (clean, HMX or TNT); group is one of the 4 groups of ewes, **day** is one of the 3 days, **ewe** is the effect of the individual ewe (1, 2, .., or 12) – defined as random to account for dependency within animal, and **e** is the residual variation.

3 Results

3.1 Results from the indoor experiment

Mean quantities (± SE) of forage consumed by one ram, divided into treatment and during a 3-hours long session was 0.88 (± 0.045), 0.41 (± 0.048), 0.33 (± 0.053) and 0.18 (± 0.038) kg for treatments clean, RDX, TNT and HMX, respectively, indicating a clear preference for the non-contaminated forage: the second most ingested forage – with RDX – was eaten less than half as much as clean forage. Means per individual sheep confirms the picture (Figure 6).

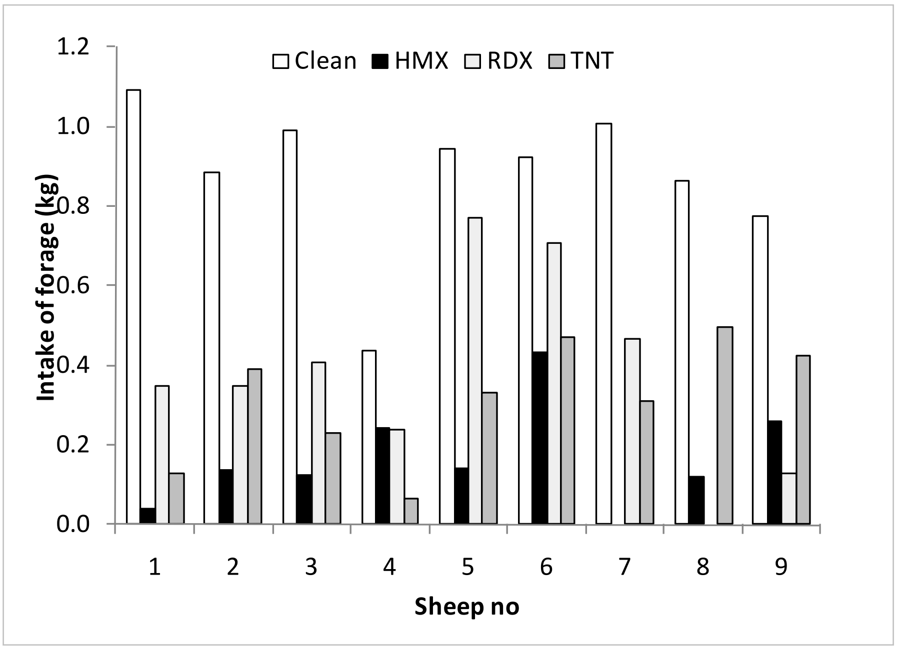

Figure 6 Intake of clean forage (control) and forage contaminated with HMX, RDX, and TNT by the different sheep

All lsmeans for treatment (Figure 7) were significantly different from zero: HMX (df= 75.3, t= -6.72, p < 0.0001), TNT (df= 69, t= -5.26, p= < 0.0001) and RDX (df= 76, t= -3.82, p= 0.0003); for all contaminators intake of forage was significantly reduced compared to clean forage. Comparisons between the lsmeans gave a significant difference between HMX and RDX in how contaminants affected the difference in intake between contaminated and clean forage (df= 134, t= -2.14, p= 0.035).

The factor ‘sheep’ did not affect the difference in (contaminated - clean) intake (df= 8, ddf= 134, F= 1.37, p= 0.215), while session did (df= 1, ddf= 16, F= 20.48, p= 0.0003), mainly because sheep ate more clean forage during the first than the second session while the intake of contaminated forage was relatively stable between session one and two.

The random effect of period by session by day had a variance estimated to 0.034 (± 0.033) kg^2; this is not significantly different from zero (Z= 1.04, p= 0.15). The residual error term had a significant effect (Z= 8.19, p < 0.0001) and was estimated to 0.508 (± 0.062) kg^2.

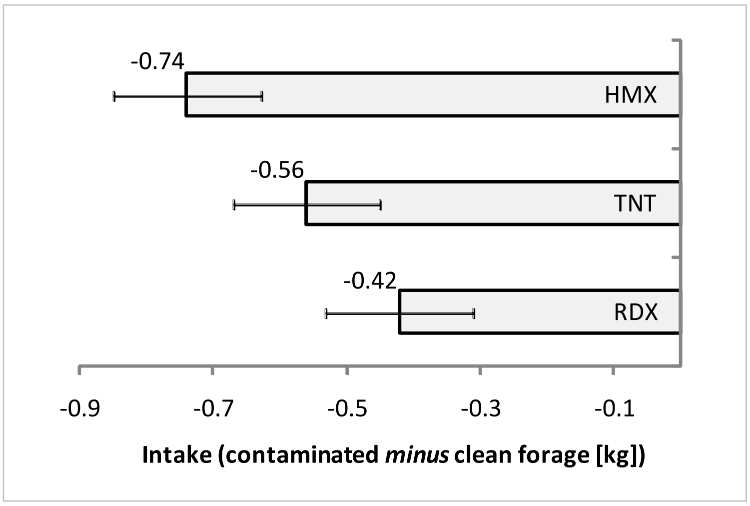

Figure 7 *Least square means for intake of forage (kg) minus least square means for intake of clean forage.*

3.2 Results from the outdoor experiment

Total number of foraging observations for sectors containing clean pasture was 1175 (2351 observations, divided by 2 as area of "clean" was twice that of HMX/TNT); for the contaminants totals of 1159 and 1277 observations were recorded, for TNT and HMX, respectively. An even distribution would give 4788*0.25= 1197 for HMX/TNT, and 0.5*4788= 2394 (/2 = 1197) for clean pasture. The descriptive statistics thus do not indicate any deviations from random foraging.

If sectors were contaminated with TNT or HMX or if they were clean did not influence how much the animals grazed within them (F= 0.99, P= 0.37); in fact a weak tendency was for ewes to graze *more* on TNT contaminated grass than on clean grass (least square means for clean grass and TNT were 13.0 [SE 0.94) and 13.4 [SE 0.96], respectively. Further, the ewes grazed more on HMX contaminated (lsmeans: 14.6 [SE 0.96]) than TNT contaminated grass. These small differences were far from significant, and attributable to chance. The only significant effect was that of circle (F= 3.43, P= 0.02); for some reason the ewes grazed less in circle D than in the other test areas (in D, we recorded a total of 895 grazing observations, whilst in circle B the ewes grazed the most [1367 observations]).

4 Discussion

In the indoor study it was shown that sheep reduce eating of forage contaminated with the explosives RDX, HMX and TNT. From direct observations it seemed that the sheep disliked the taste of the explosives. It is then somewhat surprising that they went back again and again to eat from the contaminated forage throughout the days and periods. There was no clear difference in intake of contaminated forage between first and second session, while the sheep ate substantially more clean forage in the first compared to the second session.

It is interesting that the sheep found the HMX to be the most repelling contaminant while RDX was the least avoided. Both belong to the same class (nitroamines) of explosives, while the nitroaromatic TNT came in midway between the two. A quite strong avoidance of HMX was also found in Northern bobwhite quail (*Colinus virginianus*) by Johnson et al. (2005). It must be noted that the effects found in this study is from an experimental setup where contaminants were placed on top of grass silage and thus were more conspicuous than in most rangeland situations, and where the clean and the contaminated "food patches" were small, close by each other, and clearly separated by an area without any food. The animals were also restricted to a small area, whereas free-ranging sheep would have to move about a lot during a few hours time. In summary, our sheep were presented with the contaminants in a situation where avoidance was easy and did not generate appreciable costs.

The observed avoidance was substantial, but far from absolute. Our results do indicate a reduced danger of poisoning of sheep and other ruminants on rangeland training areas, but the effect will depend on how animals choose between clean/dirty foraging patches on different spatial scales when free-ranging on natural pastures. Importantly, we need to establish how strong cues that are needed for sheep to reduce eating. Will a typical situation on military training ranges with explosives being present mainly in the ground layer affect foraging in sheep comparably to what was found in this study?

In the outdoor study we investigated if sheep differentiate between clean grass and grass contaminated by TNT or HMX – on a fine scale. An animal could cross one sector in a few steps, and were typically observed 3-4 times in a row within the same sector (i.e., ½ to 1 min). On this scale the contamination did not have an effect. For further studies a central focus will be designing experiments that allow animals to select foraging patches also on a larger scale. Free-ranging sheep may choose not to forage close to contaminated patches, and leave the entire clean-contaminated mosaic for continuous clean areas. In the present study the animals did not have this option.

We conclude that sheep do not differentiate between clean pasture and TNT/HMX contaminated pasture – on a fine scale. The experimental setup may, however, have confounded the effect of contamination. HMX and TNT may to some extent have been transported by the animals across sector borders. We did not, however find an effect of the interaction between day and treatment (unpublished, from an extended model: $F= 0.22$, $P= 0.93$). A possible source of error was that the sheep sometimes grazed along the outer border of the circles and may have reached some forage outside the contaminated sectors.

The overall effect of the presence of explosives on the feeding behaviour was not significant enough to give it any weight in risk assessments of grazing animals in shooting ranges.

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