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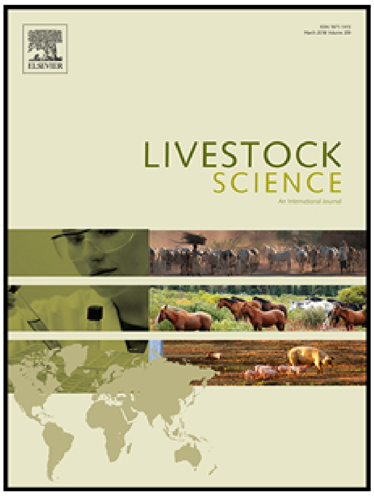

Willow leaves as a cobalt supplement for weaned lambs

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Highlights

- Willow leaf cobalt concentrations met the requirements for lambs; grass did not.
- Lambs readily consumed willow leaves.
- Willow supplemented lambs had significantly higher plasma vitamin B12 concentration.
- Willow could be used by producers to improve vitamin B12 status.

Willow leaves as a cobalt supplement for weaned lambs

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Short title: Cobalt supplementation of lambs with willow

This work was carried out in compliance with ARRIVE guidelines and in accordance with UK Animals (Scientific Procedures) Act, 1986.

Abstract

Cobalt is an essential trace element in sheep for the synthesis of vitamin B12 in the rumen to support growth and prevent clinical deficiencies (Pine). Willow (Salix spp.) leaves contain a high concentration of cobalt (~6 times the requirement of lambs). The aim of this study was to determine the efficacy of supplementary willow leaves in improving plasma vitamin B12 status of sheep. Weaned lambs (n=24) on ad libitum grass were randomly allocated to one of three groups: Willow (offered up to 300 g of fresh willow leaves per lamb each day); Drench (2.8mg oral cobalt sulphate heptahydrate drench on day 0); Control (received no treatment). Plasma B12 concentration was evaluated for all lambs using blood samples taken on days 0 and 14. Values for plasma B12 concentration were log transformed prior to statistical analysis. At day 14 the mean (± s.d.) concentrations, on the $\log_{10}$ scale, were 3.00 (±0.155) pmol/L, 2.61 (±0.146) pmol/L and 2.55 (±0.214) pmol/L for Willow, Drench and Control, respectively. The corresponding back transformed means were 990 pmol/L, 407 pmol/L, 351 pmol/L, respectively. The value for the Willow treatment was significantly higher than each of the other treatments, which did not differ significantly. Supplementary willow can be used by producers to improve vitamin B12 status in lambs.

Keywords

Sheep, Silvopasture, Vitamin B12, Minerals, Nutrition, Agroforestry

Introduction

Cobalt is an essential trace element in sheep. Microorganisms within the rumen require cobalt for the synthesis of vitamin B12, which is involved in the production of propionate in the rumen and facilitation of energy metabolism in the liver (Strobel, 1992; Wang et al., 2010).

Cobalt / vitamin B12 deficiency, or ill-thrift, is more common in weaned lambs in which it causes reduced daily live weight gain and non-specific immunosuppression (Silk, 2016; Ulvund and Pestalozzi, 1990; Vellema et al., 1996). Production losses may equate to £10 to £15 per cobalt-deficient lamb based on delays to market caused by poor growth (Scott, 2016). The APHA (2019) reported that at post-mortem through Oct-Dec of 2019 9.9% of sheep tested had cobalt deficiency.

It is well known that there is seasonal variation in the cobalt concentration of grass pasture. Research on predominantly perennial ryegrass pasture (which included the site of this trial) found cobalt concentration to range from 0.05 mg/kg DM during Summer and early Autumn to 0.4 mg/kg DM through Winter (Kendall et al., 2017). Dietary cobalt requirements for lambs, 0.2 mg/kg DM (calculated from NRC, 2007), are infrequently met from grazing pasture alone and cobalt supplementation is important. Current methods for supplementing cobalt have some disadvantages for producers. Administering drenches and boluses can be time-consuming especially on large-scale systems. Recent research has shown that weekly cobalt drenching is more effective than two-weekly drenching for preventing vitamin B12 deficiency, though the often recommended two-weekly drenching protocol was more effective than the control treatment (Hession et al., 2021). Mineral blocks and concentrate feeds cannot guarantee consistent intakes as some animals may consume anything from many times the required intake to nothing at all (McDowell, 1992).

Previous research by Kendall et al. (2021) has shown that leaves from willow trees (Salix spp) collected in June and September had cobalt concentrations that exceeded lamb dietary requirements by a factor of 6. Willow has a long historical precedent of being fed to livestock; goat willow (Salix caprea) is named after its traditional use as goat fodder, though the practice has largely died out. This suggests it may be feasible to use willow leaves as a supplementary source of cobalt. The aim of this research was to investigate if willow leaves are an effective cobalt supplement.

Methodology

Experimental Design

Aberfield cross lambs (n=24) aged 82 to 103 days and weighing 23.5 to 28 kg were split into 6 pens by restricted randomisation based on sex and weight on day -4. Each group of lambs was subsequently allocated one of six pens made using three-wire electric fencing in a permanent predominantly perennial ryegrass pasture field. A pen size of 25 m x 30 m was chosen so as to provide ad libitum grazing based on 1 kg DM/day intake (based on lamb weight and growth of 200 g/day (NRC, 2007)) of 4 lambs and an initial sward height of 11 cm.

All of the lambs were treated with an ivermectin oral drench (Chanelle Pharma, Hungerford, UK) and a bolus containing selenium and iodine (Animax, Bury-St-Edmonds, UK).

Individuals in the Drench groups (2x4) received a single dose (5ml) of cobalt sulphate heptahydrate (Sigma-Aldrich, Gillingham, Dorset, UK) delivered as an oral drench (560 mg Co/l) on day 0. This dosage was calculated based on required cobalt intake (20 kg lamb growing at 200 g/day = 0.16 mg/day, 30 kg lambs growing at 200 g/day = 0.22 mg/day (NRC, 2007)) of 0.2 mg/day (background not included) for 14 days; so 2.8 mg was the total requirement for the two weeks of the trial.

Individuals in the Willow groups (2x4) were offered supplementary leaves (including petioles) stripped from nearby goat willow (Salix caprea) each morning. Previous research showed the DM of willow to be ~330 g/kg and provide 10-times the cobalt requirement for sheep (Kendall et al., 2021). For this reason, the intention was to offer lambs in the Willow groups 300 g per lamb of fresh leaves so that it would make up ~10% of their DM intake. Uneaten willow from the Willow groups pens from the previous 24 hours was combined and weighed the following morning to provide a percentage eaten the previous day. Lambs in the Control groups (2x4) had neither cobalt drench nor supplementary willow.

Issues with lambs escaping from the fenced pens and lack of enough replacement electric netting led to the amalgamation of the willow fed lambs into a single group. From day 4, the lambs in the Willow groups were contained together within a pen measuring 50 m x 30 m with the other lambs all amalgamated and left with the rest of the field area, there were no subsequent escapes. Prior to day 4 willow consumption was observed and willow removed from the trial areas when no observation was possible, after day 4 willow was left within the willow pen.

Measurements

Representative grass samples from across the field were taken at the beginning and end of the trial using w-transect sampling. Willow was collected from four locations close to the trial field and composite samples from each collection location were analysed for cobalt concentration. Grass and leaf samples underwent milling and microwave acid digestion before inductively coupled plasma mass spectrometry (ICP-ms) was used to determine cobalt concentration (Kendall et al., 2021).

Blood samples were taken from each lamb on days 0 and 14 by jugular venepuncture using a 20 g x 1" Vacuette needle (Greiner Bio-One, Stonehouse, Gloucestershire, UK) into one 9 ml lithium heparin (LH) Vacuette tube (Greiner Bio-One, Stonehouse, Gloucestershire, UK). Blood samples were centrifuged at 2000 g for 15 minutes (Allegra x22, Beckman Coulter, High Wycombe, UK) and the plasma was removed for analysis of vitamin B12 concentration. Analysis was carried out by Axiom Veterinary Laboratories (Newton Abbot, UK) using a Siemens Immulite 2000XPI solid-phase, competitive chemiluminescent vitamin B12 enzyme immunoassay.

Statistical Analysis

A t-test was used to evaluate the differences in observed cobalt concentration between grass and willow (MINITAB v17.2.1 (Minitab, Coventry, UK)). The values for plasma concentration of vitamin B12 on day 14 were $\log_{10}$ transformed prior to analysis using the general linear model (ANOVA) procedure on MINITAB v17.2.1 with treatment as the main effect and plasma cobalt ($\log_{10}$ transformed) at day 0 as covariate. A normal probability plot was used to verify that the residuals were normally distributed. Means (on the log scale) were presented with ± s.d.; back-transformed means were also presented to aid interpretation.

Results

Lambs in the Willow group readily consumed the willow leaves offered to them from day 3 onwards (Fig. 1). As noted in the methodology, there were multiple escapes from pens between days 0 and 4 which meant that during this time it was not always possible to offer the full 300 g of willow per lamb as desired. The mean percentage of total willow offered that was eaten between days 5 and 14 was 92.6% (±5.7).

The mean cobalt concentration of willow (1.08 (±0.163) mg/kg DM) was significantly greater ($P < 0.001$) than that of the grass on offer (0.14 (±0.025) mg/kg DM).

On day 14 the log plasma vitamin B12 concentration for the lambs on the Willow treatment was significantly ($P<0.001$) higher, 3.00 (±0.155) $\log_{10}$(pmol/L), than the Drench or Control treatments 2.61 (±0.146) $\log_{10}$(pmol/L), 2.55 (±0.214) $\log_{10}$(pmol/L) respectively (Fig. 2). The corresponding back transformed means were 990 pmol/L, 407 pmol/L, 351 pmol/L, respectively. All lambs in the Willow treatment had plasma vitamin B12 concentrations above the marginal threshold (400 pmol/L), however this was not the case for the Drench or Control groups.

Discussion

Initially, fencing problems and perhaps a hesitancy to eat from buckets meant that it took until day 5 before the Willow group began to eat nearly 300 g of willow per lamb. While previous research found the concentration of cobalt in the willow leaves to exceed lamb dietary requirements by a factor of 6 (Kendall et al., 2021), measurements in this trial were closer to a factor of 5. The grass did not meet this requirement. There was more variability in the cobalt concentration of willow leaves than that of the grass. This variability may be due to the use of willow leaves from different sites while the sampled grass was all taken from the same field.

Assuming that daily DM intake was 1 kg per lamb (based upon lamb weight and growth of 0.2 kg/day (NRC, 2007)) we can estimate that cobalt intake was ~0.141 mg/kg DM for lambs in the Control and Drench groups and ~0.235 mg/kg DM for lambs in the Willow groups based upon cobalt concentration of willow and grass samples, and the amount of willow eaten in the latter half of the trial. Only the Willow group had a daily dietary cobalt intake in excess of the required 0.2 mg/kg DM (calculated from NRC, 2007). When the willow was given as a supplementary food source – making up ~10% of dry matter intake – more cobalt was available for rumen microflora to synthesise sufficient vitamin B12 and to improve the lambs vitamin B12 status in a way that was not seen with the Control or Drench groups. As was discussed by Williams et al. (2017) a single dose of cobalt drench showed poor efficacy over a two week period.

More research into the application of willow as a supplemental source of cobalt is required. Willow would be most useful for producers during August/September when cobalt concentrations in perennial ryegrass pastures are at their lowest. A silvopasture (integration of trees and livestock) approach could provide the benefits of cobalt supplementation to lambs alongside the other known benefits such as: reducing livestock greenhouse emissions (Stoate et al., 2021), improved shelter and shade from the wind, rain and sun (Karki and Goodman, 2010), prevention of soil erosion (Wilkinson, 1999), and provision of fodder that grass pasture may not always be able to fulfil, such as during periods of drought (Gabriel, 2018).

Willow leaves, when consumed by weaned lambs, are an effective means of providing supplementary cobalt and significantly raise plasma vitamin B12 concentration.

We accept the suggestion that our manuscript should be resubmitted as a Short Communication. Thank you for your comments – we have revised the manuscript as suggested.

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Declarations of interest

none

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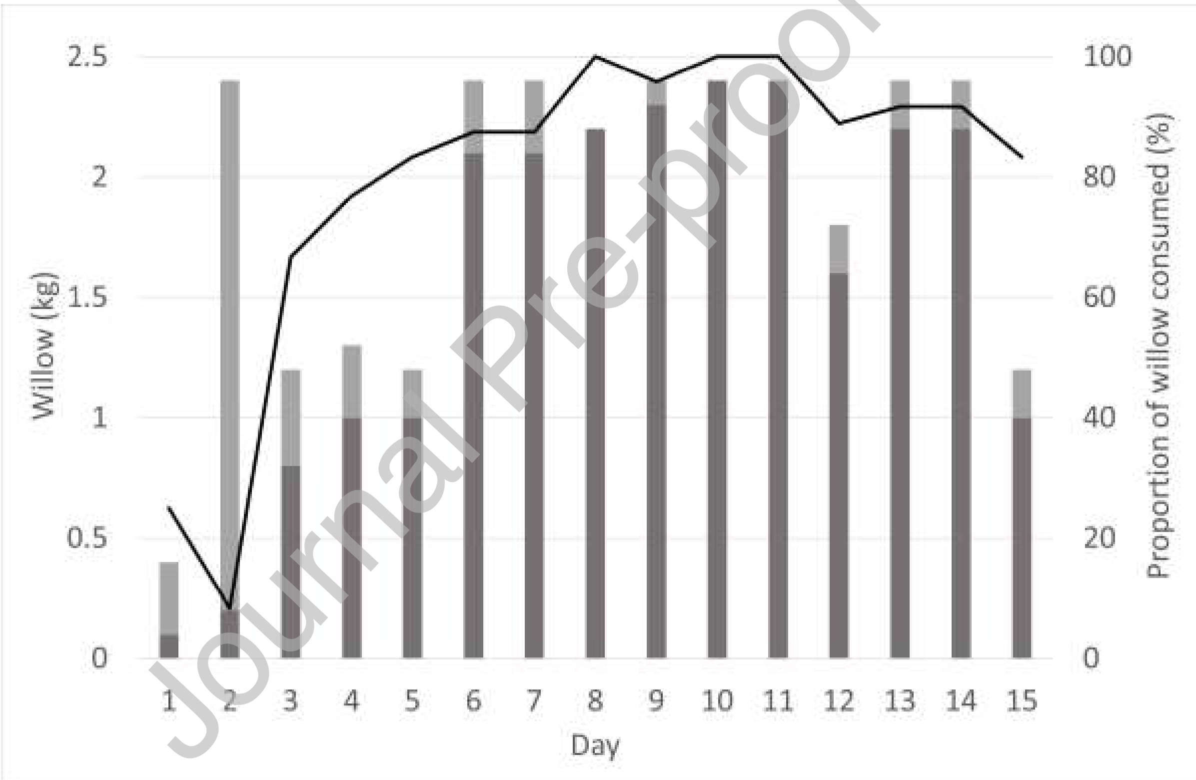

Figure 1. Stacked graph showing the total amount (kg) of willow leaf offered to the Willow groups each day that was eaten (black) or left uneaten (grey). The solid black line (-) shows the percentage of available willow leaf that was eaten each day.

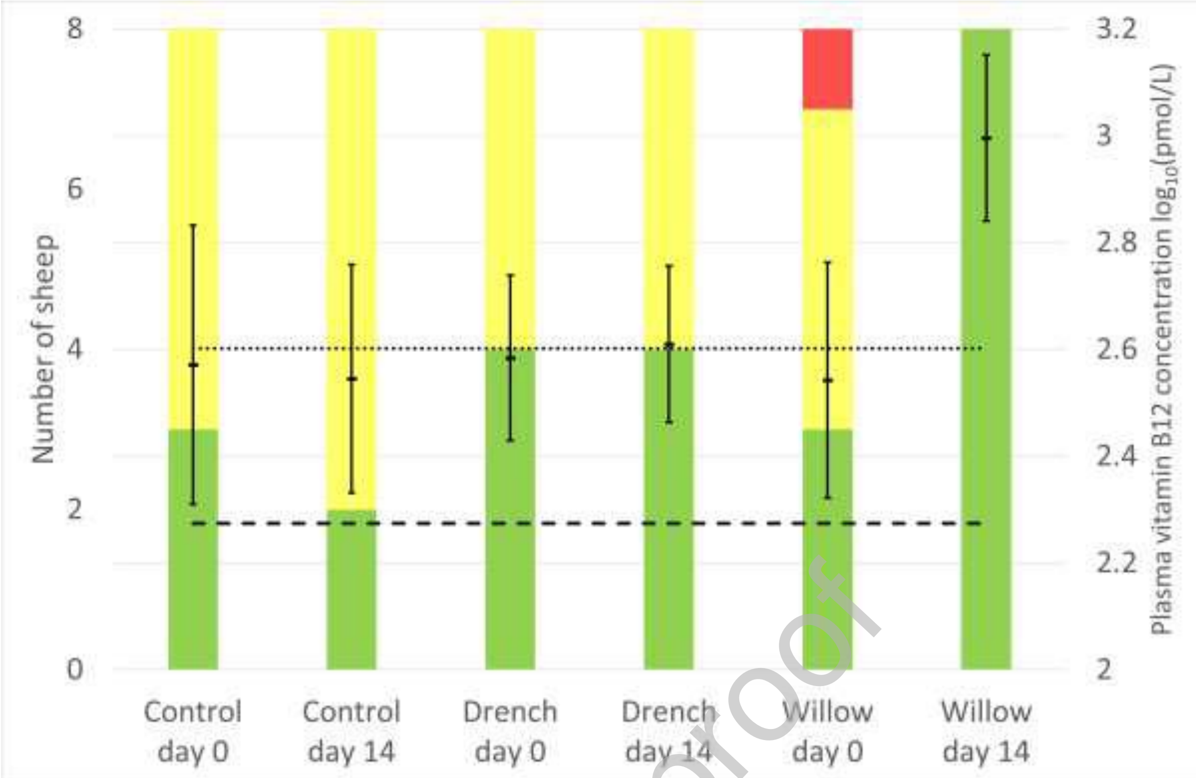

Figure 2. Mean (± s.d.) plasma vitamin B12 concentration (pmol/L) on a $\log_{10}$ scale for each treatment on days 0 and 14. The dotted lines represent marginal (2.60 $\log_{10}$(pmol/L) = 400 pmol/L) and low (2.27 $\log_{10}$(pmol/L) = 188 pmol/L) thresholds. The stack graph shows the number of individual lambs with normal (green) (>400 pmol/L), marginal (yellow) (188-400 pmol/L) and low (red) (<188 pmol/L) plasma vitamin B12 concentration.