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Initial evaluation of willow (*Salix acmophylla*) irrigated with treated wastewater as a fodder crop for dairy goats

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ABSTRACT

This research aimed at providing a first evaluation of willow tree (*Salix acmophylla*) irrigated by secondarily-treated wastewater as a fodder for goats. The nutrient and mineral contents in stems and leaves of two willow types (termed "red" and "white" for their bark color) and the concentration of secondary compounds were established. The adaptation of naïve goats to willow forage and its effect on the milk composition of late lactating goats were also studied. Willow fodder was composed of 45% leaves and 55% stems, on a DM basis; the weighted content of CP, ME, NDF, Ca, and P were (on a DM basis): 13.6%, 1.8 Mcal, 44.9%, 1.1%, and 0.2%, respectively. Lead, nickel and cadmium were found below the detection threshold in willow fodder and the concentrations of metals did not exceed the recommended ranges in feed. Intake was higher for red than for white willow fodder. The adaptation rate to white willow was low (27 g/d) but steady and intake was similar to that of clover hay after one week. White willow contained 1.2-fold more salicin ($P < 0.05$), 1.5-fold more gallic acid ($P < 0.01$) and 1.8-fold more kaempferol than red willow. The two types of willow did not differ in their concentrations of salicylic acid, hyperin, salidroside and helicon. No evidence was found that willow at the amounts consumed in this study – up to 500 g/d during the adaptation period and *ca.* 600 g/d in late lactation – could be harmful to goats, as neither of the liver enzymes increased in blood, following exposure to willow. When Baladi (Mamber) goats at their third lactation at 240 days *post-partum* were provided with willow after grazing hours for a period of 10 days, milk yield was not affected. Somatic cell counts soared from $1.3*10^6$ to up to $2.9*10^6$ in the control group, but did not change in the group that was fed willow. These initial results imply that willow that is irrigated with treated wastewater can serve as medium-quality forage for dairy goats. Specific compounds contained in willow fodder may be of significant value in maintaining and improving the health and welfare of dairy goats.

1. Introduction

In Mediterranean semi-arid areas, the quality of annual herbaceous pastures decreases during summer and autumn because of water deficit (Svoray et al., 2008). During the dry season, woody plants contribute greatly to ruminant nutrition and offset the shortage of herbaceous material (Kababya et al., 1998).

The willow tree (*Salix* spp.) grows naturally in riparian landscapes. It roots easily and enhances landscapes amenities (Kuzovkina and Quigley, 2005). Willow combines rapid growth (Wilkinson, 1999), high rates of evapotranspiration (Ebbs et al., 2003), and tolerance to saturated soils that lead to oxygen shortage in the root zone (Kuzovkina et al., 2004a). The tolerance of willow for metals is so high that it is used for soil phyto-remediation (Kuzovkina et al., 2004b), in particular, of cadmium (Robinson et al., 2000).

In temperate regions such as New Zealand, willows provide forage for livestock during the summer (Douglas et al., 1996). Moore et al. (2003) reported that willow has potential as drought fodder for cattle. McWilliam et al. (2005) showed that supplementing ewes grazing in droughted pasture with willow trees increased both dry matter (DM) intake and the number of lambs born per ewe. Feeding willow to parasitized weaned lambs reduced nematode worm burdens in the

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abomasum (Mupeyo et al., 2011). McCabe and Barry (1988) hypothesized that willow could be used most effectively if fed to goats, followed by deer, with sheep as the least efficient targeted species.

Willows contain phenolic glucosides and in particular, salicylates. Salicin, the most common phenolic glucoside found in willows, is converted into the nonsteroidal anti-inflammatory drug (NSAID), acetylsalicylic acid (commercially known as aspirin) (Kammerer et al., 2005; Lindroth and Pajutee, 1987). Salidroside contained in willow attenuates inflammatory responses by suppressing nuclear factor-κB and mitogen activated protein kinases activation in lipopolysaccharide-induced mastitis in mice (Li et al., 2013). Sodium salicylate given in early lactation has beneficial effects throughout the whole period of lactation in cows (Farney et al., 2013).

In Israel, more than 90% of urban wastewater is recycled and used for irrigation (Tal, 2006). However, in small communities, significant amounts of water for reuse are treated only to the secondary level of purification (Avnimelech, 1993; Inbar, 2007), i.e., organic matter is reduced but some minerals are high, which prevents using the water for direct irrigation of plants that will be consumed by humans. Therefore, it is a challenge to find a use for locally available effluents of low quality. Using willow as green fodder could be sustainable, as long as the nutritional quality is satisfactory and the minerals and metals accumulated in the fodder do not jeopardize animal health and welfare.

This study is the first assessment of willow nutritional values for dairy goats grown exclusively with treated wastewater.

2. Materials and methods

2.1. Study site and vegetation

The study was conducted at the Ramat Hanadiv nature park, located on the southern end of the Carmel mountain ridge, Israel (32° 33′N, 34° 56′E) with average annual rainfall of 570 mm. The pasture is dominated by *Phillyrea latifolia* trees and *Pistacia lentiscus* shrubs. In February 2014, a 0.1-ha plot was planted with 300 willows *(S. acmophylla)* of six ecotypes collected from different regions in Israel. Trees were irrigated with effluent (treated wastewater − TWW) from a local wastewater facility (the Ramat Hanadiv visitors' center) treated at the secondary level, encompassing removal of settling solids and floating materials and aerobic microbial treatment to decrease biodegradable organic matter, but without additional treatment for metals. TWW allocation to the field averaged 15 cubic meters per day. Although the willows originated from a number of different geographic areas within Israel, we characterized them into two different lines of three ecotypes each, based on whether stem color was red or green at the height of summer (Newsholme, 1992).

2.2. Nutritional attributes of willow fodder predicted by NIRS (Near infra-Red spectroscopy)

2.2.1. Nutritional attributes

In order to evaluate nutritive value, 44 samples were collected from 11 randomly selected trees. From each tree, four samples were collected: upper and lower leaves and upper (3–5 mm in diameter) and lower stems (more than 5 mm in diameter). After clipping, samples were dried at 60 °C for two days in an aerated oven. Assays included: percentage of dry matter of ash (after combustion at 550 ° C for four hours); calcium and phosphorus content according to standard method of SM 3500 Ca-B and SM 4500 P-E respectively; crude protein (CP), using an automated Kjeldahl method according to procedure 976.05 of AOAC (1990); acid detergent fiber (ADF) and neutral detergent fiber (NDF, after α-amylase) by standard method (Ankom; according to Goering and Van Soest (1970); *in vitro* dry matter digestibility (IVDMD) according to Tilley and Terry (1963).

For tests of minerals and metals concentrations in samples of wastewater effluents and forage, dry leaves were ground, digested in nitric acid (10%) in triplicate, and incubated at room temperature for at least 24 h before measuring concentration using the ICP after suitable dilution (Varian 720-ES, Varian medical system Inc.). Samples of wastewater effluents were assayed without dilution.

2.2.2. NIRS procedures

NIRS calibrations were developed with the 44 above-cited samples in order to determine the nutritional value of willows as described by Landau et al. (2006).

2.2.2.1. Sample preparation. Willow samples were re-dried at 60 °C for 1 h, allowed to equilibrate in a desiccator at ambient temperature for 1 h, packed into sample cells with a near-infrared-transparent quartz cover glass, and scanned at wavelengths from 1104 to 2492 nm in 2-nm increments with a Foss NIRSystems (Hoganas, Sweden) model 5000 NIR reflectance monochromator spectrometer in order to collect NIR spectra as log (1/R) where R = reflectance.

2.2.2.2. NIRS calibration equation development. Before calibration equations were developed, raw spectral data were transformed with the Standard Normal Variance (SNV) and detrend procedures to remove the non-linearity caused by light scattering (Barnes et al., 1989). Mathematical treatments used to enhance spectral differences where "1, 4, 4, 1" or "2, 6, 6, 1", in which the numbers represent the derivative, the gap width over which the derivative is calculated, the number of points in a moving average, i.e., first smoothing procedure, and the number of nm over which the second smoothing is applied, respectively (ISI, 1999). A modified partial least squares regression (Martens and Naes, 1987) was used to develop calibration equations in which stored NIRS spectra from fecal samples were the independent variables, and nutritional attributes were the dependent reference data.

The calibration precision was evaluated according to the multiple coefficient of determination (R^2) and the standard error of calibration (SEC). The calibration accuracy was evaluated by cross-validation and expressed as the standard error of cross-validation (SECV; Naes et al., 2002).

2.3. Secondary compounds in red and white willow

2.3.1. Total phenolics and tannins

The content of polyethylene glycol-binding-tannins (PEG-b-T), condensed tannins (CT) and total phenolics were determined using NIRS without extraction, according to Landau et al. (2004).

2.3.2. Analysis of volatile content of fresh white and red salix leaves using GCMS

Solid-phase micro-extraction: Frozen powder (1 g) was homogenized with 20% NaCl solution (7 mL). The homogenate and NaCl powder (1g- to inhibit enzymatic reactions) were poured into 20 mL. The vials sealed and kept at 4 °C until analysis. As a control, we used vials containing 20% NaCl. The injector temperature was 250 °C and the detector temperature was 280 °C. The oven was set to 50 °C for 1 min, and then the temperature was increased to 200 °C at a rate of 5 °C min^{-1}. The detector temperature was 280 °C. Mass range was recorded from 41 to 350 *m/z*, with electron energy of 70 eV.

The identification of the compounds was performed by comparing their relative retention indices and mass spectra with those of authentic standards or with those found in the literature and supplemented with NIST 98 and QuadLib 2205 GC–MS libraries. A mixture of straight-chain alkanes (C7-C23) was injected into the column under the above-mentioned conditions for retention indices calculation.

2.3.3. Analysis of salicylic acid and derivatives in fresh cut salix leaves using LC-TOF-MS

2.3.3.1. Sample preparation. three healthy leaves from each tree were collected into a tube and frozen in aluminum foil in liquid nitrogen.