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In vivo assessment of an industrial waste product as a feed additive in dairy cows: Effects of larch (*Larix decidua* L.) sawdust on blood parameters and milk composition

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ABSTRACT

When larch (*Larix* spp.) is processed in the wood industry, the sawdust is currently disposed of as waste or used as combustible material, even though it is rich in biologically active compounds. In this study the effect of larch sawdust supplementation on blood parameters as well as milk composition was examined in healthy mid-lactating dairy cows. Twenty-four multiparous Italian Friesian dairy cows were assigned to groups receiving either 300 g/day/cow of larch sawdust or a control diet, and treatments were continued for a 20 day period.

Milk parameters were unaffected by treatment. A lower plasma total protein concentration was observed and can be attributed to a decrease in globulin concentration. A lower plasma urea concentration was also detected in the larch group. Moreover, biomarkers of liver function were influenced by the treatment. Total bilirubin was lower in larch-treated animals, and cholesterol tended to be lower. In addition, an interaction between day and treatment was observed for very low density lipoprotein. The concentration of other parameters, including reactive oxygen metabolites, superoxide dismutase, glutathione peroxidase and nitrotyrosine, did not differ between treatments. The observed benefits, together with the good palatability, make larch sawdust a promising candidate for the development of beneficial feed supplements for livestock. Further studies will be useful, particularly to evaluate its efficacy in different health conditions.

Introduction

Larch wood (*Larix decidua* L., Pinaceae) is well known for its high content of biologically active compounds: arabinogalactans (Willför et al., 2005), lignans (mainly secoisolariciresinol and lariciresinol) (Pietarinen et al., 2006), flavonoids (mainly taxifolin and dihydrokaempferol) (Medvedeva et al., 2010) and diterpenes (larixyl acetate and larixol) (Ostroukhova et al., 2012).

Arabinogalactans, abundant in the genus *Larix*, are an excellent source of water-soluble prebiotic fibre (Fitzpatrick et al., 2004). They have been approved as a source of dietary fibre by the US Food and Drug Administration (FDA) and were included in the European Union (EU) Novel Food Catalogue (Reg. 258/97). Moreover, arabinogalactans are reported to enhance immune defences (Kelly, 1999). The larch lignan secoisolariciresinol has a higher antioxidative potency than the synthetic antioxidant butylated hydroxyanisole (BHA), an efficient radical scavenging capacity compared to the antioxidant Trolox (Willför et al., 2003; Pietarinen et al., 2006) and an antioxidant activity higher than vitamin E (Prasad, 2000). Furthermore, the lignans lariciresinol and isolariciresinol possess significant anti-inflammatory activities (Saleem et al., 2005). Among larch flavonoids, taxifolin (also known as dihydroquercetin) has the strongest antioxidant activity as evaluated in vitro (Teselkin et al., 1996; Burda and Oleszek, 2001; Willför et al., 2003; Khairullina et al., 2006; Pietarinen et al., 2006; Medvedeva et al., 2010) and in vivo (Wang et al., 2006; Weidmann, 2012).

In the wood industry, the sawdust from larch wood is mainly used as combustible pellets. The potential bioactivity of this waste material was investigated by the EU-funded research project SAFEWASTES (Franz et al., 2008), whose main goal was to evaluate the physiological and environmental consequences of using organic wastes in diets for livestock and humans. The working hypothesis of this project was that such organic waste material still contains potential health-beneficial compounds, such as pectins,

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polyphenols, and flavonoids, and it can therefore be further used to produce high added-value products with possible specific activities. The wastes tested in this project were chosen in accordance with the requirements of EU legislation in order to protect human health, animal products, animal health and the environment.

Among the organic wastes tested during the SAFEWASTES project, larch sawdust showed the most interesting results from in vitro trials. A series of diterpenes and diterpene acids isolated from a lipophilic extract of larch sawdust was found to inhibit prostaglandin and leukotriene formation, indicating their anti-inflammatory potential (Pferschy-Wenzig et al., 2008; Bauer et al., 2010). Furthermore, immunomodulatory activity was exhibited on ovine neutrophils activated with phorbol 12-myristate 13-acetate (PMA) to reproduce the response to inflammation or pathogen injury. The hydroethanolic extracts of larch sawdust strongly blocked neutrophil adhesion in a dose-dependent manner and inhibited superoxide production from activated neutrophils (Farinacci et al., 2008). An ethanolic extract of larch sawdust exhibited high antioxidative activity, most likely due to the high amounts of lignans and flavonoids present (Stockhammer et al., 2009). In the preliminary assessment for safe use of this organic waste in a diet for ruminants, no negative effects were observed on the rumen microflora (Tedesco et al., 2007).

These findings have motivated the hypothesis that larch sawdust can be used in ruminant diets. Because this is the first time that larch sawdust has been tested in dairy cows, the supply was verified in clinically healthy mid-lactation dairy cows, which were not exposed to drastic physiological changes or to environmental stress and were kept under standard dairy farm conditions. The aim of the study was to investigate the influence of larch sawdust supplementation on blood parameters, milk production and milk composition.

Materials and methods

The study was carried out according to the requirements of the Italian legislation on animal welfare (DL 116/1992) and the local ethics committee, and it was conducted with the informed consent of the animals' owner.

Animals and treatment

Twenty-four healthy Italian Friesian dairy cows in mid-lactation were used in a randomised complete block design with repeated measures. The cows came from a commercial dairy herd in northern Italy with 700 lactating cows. One week before the experimental period, cows were selected according to their health condition: milk production (32.57 ± 1.98 kg/day), parity 2–3, days in milk (DIM) 115 ± 25, body condition score (BCS) 3.3 ± 0.4 (5-point scale), and milk somatic cell count (SCC) < 200,000 cells/mL of milk.

Animals were blocked according to milk production, parity, and DIM and randomly placed into two groups. The treated group (larch) was given 300 g/day/cow of larch sawdust milled to a particle size between 0.5 and 1.5 mm. The dose was established considering the animal species, the weight of the animals and the rumen impact. Larch (*Larix decidua* L., Pinaceae) sawdust was provided by Jannach Lärchenholz GmbH. The chemical composition of larch sawdust is presented in Table 1.

The material had been phytochemically characterised by high-performance liquid chromatography (HPLC) by the SAFEWASTES research group (E.D. Tzika et al., unpublished data). The material used as feed supplement in the present study contained 0.7% taxifolin and 0.7% of the related dihydroflavonol dihydrokaempferol. To guarantee the dosage, the larch sawdust was mixed with 1 kg of total mixed ration (TMR) administered prior to the morning feeding. The control group (control) received 300 g/day/cow of wheat straw, mixed with 1 kg of TMR. The treatment lasted for 20 consecutive days.

Table 1
Chemical composition (% of dry matter) of larch sawdust.

Component	
NDF	88.71
ADF	76.29
Lignin	24.07
CP	0.45
Fat	0.87
Ash	0.10

ADF, acid detergent fibre; CP crude protein; NDF, neutral detergent fibre.

Cows were housed in two separate sections (treated and control groups) of the free-stall barn, had free access to water and were milked three times daily. Animals received the TMR (Table 2) twice daily at 08.00 and 16.00 h. Feed was offered to achieve 5% refusals. The amounts of feed offered and refused were recorded for each treatment group. Weekly samples of TMR were analysed for dry matter (DM; method 930.15; AOAC, 1999), crude protein (CP; method 990.03; AOAC, 1999), ether extract (method 920.39; AOAC, 1999), minerals (method 985.01; AOAC, 1999), neutral detergent fibre (NDF) and acid detergent fibre (ADF; Van Soest et al., 1991). Milk production was electronically recorded for the whole trial period. Animals were monitored daily by herd personnel and a veterinarian to evaluate their general physical condition and health status.

Sample collection and analysis

Milk samples were collected from each cow using an automatic sampler at 0, 7, 14, and 20 days at each milking, approximately at 03.30, 11.00 and 17.00 h, proportional to the milk yield, and the three samples were combined to give one sample for each cow on each sampling day. Samples were preserved with Bronopol, stored at 4 °C and analysed within 24 h to determine milk composition. Milk samples were analysed by the laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER, Brescia, Italy) in order to determine the concentrations of fat, protein, casein and lactose (MilkoScan 605, Foss Electric), urea (CL 10, EUROCHEM), and SCC (Fossomatic 360, Foss Electric).

Blood samples were collected from the jugular vein at days 0 and 20 before the morning feeding. Plasma was obtained from Vacutainer tubes containing lithium-heparin (Venoject, Terumo) by centrifugation at 500 *g* for 15 min at 10 °C and stored frozen at –20 °C. Serum was obtained from tubes without anticoagulant by centrifugation at 500 *g* for 15 min at 10 °C and stored frozen at –20 °C.

The following blood assays were performed with commercial kits, according to the manufacturer's instructions: total protein (Dye reagent, Bio-Rad), albumin (Bromocresol green Albumin Assay Kit, Sigma-Aldrich), globulin (determined by subtracting the albumin from the total protein), total cholesterol (Esterase/oxidase method, Alfa Wassermann), triglycerides (Sigma-Aldrich), β-hydroxybutyrate (BHBA; Sigma-Aldrich), nonesterified fatty acids (NEFA; Enzycolor, Boehringer-Mannheim), glucose (glucose oxidase, GOD/PAP method, Roche Diagnostics), urea (Alfa Biotech), aspartate transaminase (AST; International Federation of Clinical Chemistry method, IFCC Alfa Wassermann), alkaline phosphatase (ALP; IFCC Alfa Wassermann), γ-glutamyl transferase (GGT; IFCC Alfa Wassermann), lactate dehydrogenase (LDH; Lactate Dehydrogenase Activity assay kit, Sigma-Aldrich), total bilirubin (Bilirubin Assay Kit,

Table 2
Ingredients and chemical composition (% of dry matter) of the diet.

Item	
Ingredients	
Corn silage	30.86
Alfalfa hay	14.06
Italian ryegrass hay	3.94
Ground corn grain	14.96
Ground barley grain	6.43
Cottonseed	2.01
Protected fat[a]	1.75
Concentrate[b]	21.61
Sodium bicarbonate	0.84
Propylene glycol	1.14
Vitamin and mineral premix[c]	0.6
Magnesium oxide	0.17
Salt	0.41
Calcium carbonate	0.65
Dicalcium phosphate	0.57
Chemical composition	
CP	17.6
NDF	32.52
ADF	19.51
Ca	0.95
P	0.46
NEL, Mcal/kg	1.72

[a] Same as Megalac.
[b] The ingredients (% of total mixed ration) of concentrate: 13.72 soybean meal, 2.44 decorticated sunflower meal, 2.03 canola meal, 1.22 wheat bran, 1.22 beet pulp, 0.98 molasses sugarcane.
[c] Formulated to provide (per kg of premix) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 10,000 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3000 mg of Fe, 40 mg of Co, 3000 mg of Mn, and 3000 mg of Cu.
NEL, net energy of lactation.