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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.

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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.

Sigma-Aldrich), very low density lipoprotein (VLDL; Friedewald et al., 1972) and high density lipoprotein (HDL; Biogamma).

The concentration of reactive oxygen metabolites (ROM) was measured using a d-ROMs test (Diacron). The d-ROMs test assesses the concentration of hydroperoxides (R–OOH) in a biological sample. Superoxide dismutase (SOD) activity was measured using the superoxide dismutase assay kit of Cayman Chemical (Vinci-Biochem). SOD activity was expressed as units/mL. One unit of SOD is defined as the amount of the enzyme needed to exhibit 50% dismutation of the superoxide radical. The glutathione peroxidase (GPx) activity was measured indirectly by a coupled reaction with glutathione reductase (GR), using a glutathione peroxidase assay kit (Vinci-Biochem). GPx activity was expressed as nmol of NADPH oxidised/min/mL of sample.

Nitrated protein levels (μM) in plasma samples were measured by ELISA. Plasma samples were diluted (1:300, 1:1000, 1:3000, 1:9000, and 1:20,000) with coating buffer (7 mM Na_2CO_3, 17 mM $NaHCO_3$, 1.5 mM NaN_3, pH 9.6) and incubated in the wells of a microtitre plate overnight at 4 °C. Standard curves were obtained by serial dilutions of nitrated bovine serum albumin (BSA). The nitrotyrosine (N-Tyr) was detected by incubation with rabbit anti-N-Tyr antibody (1:1500 dilution in 130 mM NaCl, 20 mM Tris–HCl, 0.05% Tween 20, pH 7.3, supplemented with 0.25% BSA; 1 h, 37 °C), followed by goat anti-rabbit IgG–horseradish peroxidase linked (GAR–HRP) diluted 1:3000 as the primary antibody. Colour development was monitored at 492 nm, as previously described (Spagnuolo et al., 2003).

Statistical analysis

Data from the experiment were analysed statistically as a randomised block design with a repeated measures treatment structure using the MIXED procedure of SAS version 9.2 (SAS Institute). The model included the effects of treatment, day, and a treatment × day interaction as fixed effects and animal within treatment as a random effect. Day was included in the model as a repeated measure. SCC was linearised by taking $\log_{10}$ SCC. For blood parameters, baseline values measured on day 0 were used as covariates if significant. No repeated measure effects were included in the analysis of blood parameters. For ROM, the distribution was non-normal as assessed by the Shapiro–Wilk test, and no transformations met the assumptions of normality (Box–Cox test). ROM variables were analysed using the generalised linear mixed model procedure of SAS (GLIMMIX procedure). The variance–covariance matrix structure was chosen for each statistical model in a process wherein the best fit was chosen based on the Schwarz's Bayesian criterion. The Bayesian information criterion (BIC) was used to indicate the goodness of model fit, where lower values indicate a better fit. Degrees of freedom were calculated using the Kenward–Roger option. Least squares means and SEM for each milk and blood samples were obtained and used for multiple comparisons using the Tukey adjustment. $P < 0.05$ was used as a threshold for significance, with P values >0.05 and <0.10 considered a 'trend'.

Results

Average milk yield and milk profile responses to larch treatment are presented in Table 3. Milk parameters were unaffected by treatment. However, several parameters were affected by time, depending on the progress in lactation. We observed an interaction of day and treatment ($P = 0.03$) due to decreased concentration of milk urea nitrogen (MUN) in the larch-treated animals.

Cows from control and treated groups remained healthy throughout the trial, and larch sawdust was well tolerated by the animals and completely ingested without refusal and without affecting feed intake.

In Table 4, measurements of blood metabolites are presented. Intra-assay and inter-assay coefficients of variation (CV) for blood assays were 5% and 7.5%, respectively. The total protein was significantly affected by the interaction between day and treatment ($P = 0.0001$), and there was a significant change in protein concentration from day 0 to day 20 ($P = 0.0003$). The lower protein level can be attributed to the decrease in globulin concentration ($P = 0.005$) in the cows given the larch diet. A lower urea concentration was found in the larch-treated group ($P = 0.03$). Glucose tended to be lower ($P = 0.07$) in the larch group, and an interaction between day and treatment ($P = 0.03$) was observed.

Biomarkers of liver function were also influenced by the treatment. Total bilirubin was significantly lower in larch-treated animals ($P = 0.03$). We also observed that cholesterol tended to be lower in animals given the larch diet ($P = 0.06$). The concentrations of the other parameters did not vary significantly with diet, but an interaction of day and treatment was observed for VLDL ($P < 0.001$).

As reported in Table 4, the concentration of ROM and the activities of enzymatic antioxidants SOD and GPx were not affected by treatment. N-Tyr level did not significantly differ between the two groups.

Discussion

We have investigated larch sawdust supplementation in vivo in clinically healthy mid-lactation dairy cows, which were not exposed to drastic physiological changes or to environmental stress, in order to investigate the effects on blood parameters, milk production and milk composition. The safety of larch sawdust as a feed additive was in accordance with the EU legislation (absence of contaminants, evidence for the absence of toxicological properties and microbial contamination).

Larch treatment did not affect milk yield, milk composition or feed intake. Overall, although the blood parameters measured were within the reference ranges of healthy dairy cows in both the control and treated animals (Cozzi et al., 2011), several biochemical changes induced by the supplementation of larch sawdust were observed. Total protein decreased significantly in animals given the larch diet, mainly due to a decrease in globulin concentration. Because total globulin concentration can provide an indication of an animal's

Table 3
Effect of larch sawdust treatment on milk composition.

Parameter[a]	Treatment		SEM[b]	*P* value[c]		
	Control	Larch		TRT	Day	TRT × Day
Yield, kg/day						
Milk	32.48	32.61	1.78	0.96	0.0004	0.36
ECM	35.90	36.04	0.95	0.92	0.95	0.98
Fat	1.33	1.35	0.09	0.88	0.02	0.42
Protein	1.09	1.10	0.05	0.88	0.007	0.46
Lactose	1.62	1.62	0.09	0.95	0.001	0.55
Casein	0.85	0.85	0.04	0.88	0.004	0.38
Milk composition						
Fat, %	4.12	4.13	0.13	0.94	0.0003	0.24
Protein, %	3.38	3.39	0.07	0.96	<0.0001	0.26
Lactose, %	4.97	4.98	0.04	0.83	0.25	0.28
Casein, %	2.63	2.63	0.06	0.95	<0.0001	0.13
MUN, mg/100 mL	11.66	11.32	0.48	0.62	<0.0001	0.03
SCC × 1000 cells/mL	163.05	197.31	43.38	0.59	0.72	0.95
$\log_{10}$ SCC	1.99	2.09	0.10	0.52	0.93	0.96

[a] ECM, energy corrected milk (0.327 × milk yield + 12.95 × fat yield + 7.65 × protein yield); MUN, milk urea nitrogen; SCC, somatic cell count.
[b] Least squares means are presented with the pooled standard error of the mean (SEM).
[c] TRT, treatment; TRT × Day, interaction between TRT and day.

Table 4
Effect of larch sawdust treatment on blood parameters.

Parameter[a]	Day of treatment				SEM[b]	*P* value[c]		
	0		20					
	Control	Larch	Control	Larch		TRT	Day	TRT × Day
Protein and energy markers								
Total protein (g/L)	76.88	79.13	81.16	73.97	0.59	0.0003	0.60	0.0001
Globulin (g/L)	39.13	36.71	39.59	34.61	0.61	0.005	0.29	0.008
Albumin (g/L)	37.75	39.14	39.97	38.17	0.35	0.17	0.18	0.03
Urea (mmol/L)	6.22	6.38	6.26	5.75	0.08	0.03	0.01	0.03
Glucose (mmol/L)	3.70	3.78	3.84	3.65	0.03	0.07	0.91	0.037
NEFA (mmol/L)	0.18	0.16	0.16	0.14	0.005	0.25	0.09	0.91
BHBA (mmol/L)	0.53	0.45	0.44	0.52	0.02	0.17	0.54	0.11
Triglyceride (mmol/L)	0.10	0.11	0.17	0.17	0.008	0.81	<.0001	0.77
Hepatic and lipid markers								
AST (IU/L)	63.25	64.14	65.16	60.67	0.98	0.13	0.61	0.13
ALP (IU/L)	31.88	31.43	33.42	31.37	0.67	0.30	0.46	0.31
GGT(IU/L)	35.75	34.50	35.39	33.86	0.38	0.16	0.36	0.18
LDH (IU/L)	2.17	2.21	2.34	2.18	0.03	0.14	0.19	0.13
Total bilirubin (mmol/L)	2.11	2.26	2.63	1.86	0.11	0.03	0.72	0.02
Cholesterol (mmol/L)	7.59	8.22	8.25	7.63	0.11	0.06	0.84	0.05
VLDL (mM)	3.51	2.94	2.70	3.55	0.12	0.13	0.51	0.0008
HDL (mM)	36.62	37.14	35.20	39.50	1.19	0.17	0.81	0.28
Oxidative status markers								
d-ROMs (mmol H_2O_2)	1.13	1.12	1.14	1.13	0.03	0.65	0.67	0.88
SOD (U/mL)	1.90	1.77	1.65	1.85	0.11	0.89	0.60	0.33
GPx (nmol/min/mL)	130.99	107.86	120.36	151.16	10.93	0.50	0.36	0.46
Plasma nitrotyrosine (μM)	1.33	1.39	1.77	1.61	0.16	0.70	0.20	0.53

[a] NEFA, nonesterified fatty acids; BHBA, β-hydroxybutyrate; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; VLDL, very low density lipoprotein; HDL, high density lipoprotein; ROM, reactive oxygen metabolites; SOD, superoxide dismutase; GPx, glutathione peroxidase.
[b] LS means are presented with the pooled standard error of the mean (SEM).
[c] TRT, treatment; TRT × Day, interaction between TRT and day.

humoral immune status or response, the observed effect could be further investigated in challenged animals.

In this study, we found that blood urea concentration decreased in the treated group. In dairy cows, the pattern of blood urea concentration over time should reflect the rumen ammonia N concentration because the liver essentially removes all net portal absorption of ammonia N when hepatic function is not impaired (Huntington, 1990). The observed effect cannot be due to the nutritional characteristics of the administered diets, which were low in protein content. Therefore, in the future, identifying the interactions between larch sawdust and the rumen environment could explain the pathway of rumen nitrogen utilisation.

In healthy animals, larch sawdust supplementation improved liver function by reducing total bilirubin, and it tended to reduce cholesterol. VLDL, which is involved in lipid transport from the liver, tended to increase. These effects should be verified in peripartum, when dairy cows experience moderate to severe fatty liver (Grummer, 1993). The effects of taxifolin on blood lipid concentration and liver function have been studied extensively. It is known to act like a statin drug, and it has hepatoprotective characteristics (Igarashi et al., 1996; Teselkin et al., 1998; Theriault et al., 2000; Polyak et al., 2010; Weidmann, 2012; Liang et al., 2013).

In our previous works (Tedesco et al., 2004a, 2004b), we suggested that the observed hepatoprotection of silymarin could be also related to its taxifolin content, as taxifolin is the major contributor to the antioxidant activity of silymarin (Kevin and Saleh, 2013). We hypothesise that the reduction of bilirubin and cholesterol and the effect on VLDL observed in the larch-treated group in this study demonstrate an influence on hepatic metabolism that could be due to the high amounts of taxifolin in larch sawdust. Taxifolin is a dihydroflavonol present in large amounts in *Larix* spp. The material used as feed supplement in the present study has been found to contain approximately 0.7% taxifolin (E.D. Tzika et al., unpublished data). In dairy cows, the ruminal degradation of taxifolin was found to be 59%, and the total digestibility was assumed to be 100% (Křížová et al., 2011). The degradation of taxifolin by *Eubacterium oxidoreducens* (Krumholz et al., 1986), *Eubacterium ramulus* (Schneider et al., 1999; Braune et al., 2001) and *Butyrivibrio* spp. (Cheng et al., 1969) resulted in the formation of 3,4-dihydroxyphenylacetic acid, a small metabolite better absorbed in the intestine (Selma et al., 2009). In terms of biological activity, this metabolite has been reported to exert an antioxidant activity greater than that of α-tocopherol (Dueñas et al., 2011), and it could have contributed to the hepatoprotection observed in this trial.

Although larch wood contains several compounds with strong antioxidant activity (Teselkin et al., 1996; Burda and Oleszek, 2001; Willför et al., 2003; Khairullina et al., 2006; Pietarinen et al., 2006; Wang et al., 2006; Medvedeva et al., 2010; Weidmann, 2012), the activities of enzymatic antioxidants, the level of ROM and the concentration of the oxidative stress markers N-Tyr did not differ between the control and treatment groups. This might be because we analysed cows in mid-late lactation, a state that has been reported to be associated with greater efficiency of the antioxidant defence system (Cigliano et al., 2014).

Conclusions

This preliminary study reports for the first time the effects of larch sawdust on blood parameters, milk production and milk composition in healthy dairy cows. Milk parameters did not indicate an influence on milk production and composition. Larch sawdust supplementation improved liver function. These effects are promising and motivate further investigation of whether larch sawdust can be beneficial in livestock during challenges such as the peripartum period and weaning, as well as in disease states. Further studies should be carried out to evaluate the effect of larch sawdust in different health conditions and in long-term feeding studies. Furthermore, the potential influence of larch sawdust supplementation on animal products needs to be assessed and the pharmacokinetics of active larch sawdust constituents should be elucidated to

evaluate their bioactivity and metabolic effects, with consideration of safety for animal product consumers. The study emphasises that the use of organic wastes as beneficial feed supplements is an interesting alternative for increasing waste recycling and promoting the sustainable use of plant-derived waste materials.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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