Valorization of waste by-products of rose oil production as feedstuff phytonutrients

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Highlights

• Identified 30 polyphenol antioxidant components in dry rose petals.

• Found 6 new glycosides of gallic acid except glycosides of kaempferol and quercetin.

• Supplementation of 545 mg DDRP/kg/d can be successfully used in pig's husbandry.

• This DDRP supplementation isn't effective for growth performance of broilers or lambs.

Abstract

The aim of this study was to evaluate possibility for valorization of the dry pressed distilled rose petals (DDRP) as a feedstuff phytonutrients in animal husbandry. In connection with the chemical composition and radical scavenging activity of polyphenol complex in rose waste products were studied. The polyphenol composition in dry rose petals, DDRP and waste water after distillation was identified and quantified. The chromatographic analysis was conducted by HPLC-PDA and LC-MS. The experiments were conducted with bird, monogastric and ruminants representatives. A total of 40 one days old chicks from the hybrid combination Ross 308 were distributed into two groups each containing of 20 birds fed for 49 days. The control group C fed with basal diet vs. experimental group R fed with the same fodder plus 40 mg DDRP/kg/d. A total of 72 Danube White 155 days old fattening pigs (both sexes - 36 male and 36 female) of

72.500 ± 1.937 kg were distributed into three groups (one control C and two experimental R1 and R2 supplemented with 0.255 g DDRP/kg/d or 0.545 g DDRP/kg/d). Each one group (24 piglets) was fed for 45 days. A total of 30 numbers of 65 days old lambs from the Synthetic population of Bulgarian milk sheep breed lambs (both sexes - 15 male and 15 female) with average life weight 20.500 ± 1.039 kg were divided into two groups (control C and experimental R) each one containing of 10 animals fed for 50 days. The experimental group was fed with combined feed for lambs granules and ground alfalfa supplemented either 0.545 g DDRP/kg/d. Animals were assessed for average daily weight gain and feed conversion ratio. Thirteen glycosides of kaempferol, ten glycosides of quercetin, six glycosides of gallic acid and the two flavonol aglycones have been identified in dry rose petals. Those polyphenols possess high antioxidant activity and positively influence the growth performance of pigs. The supplementation of pig's feed with 545 mg DDRP/kg/d increased ($p < 0.05$) with 6.73% the total feed consumption and with 27.05% the average daily weight gain, and decreases with approximately 16% the feed conversion ratio comparing to control group (C) pigs fattening without DDRP supplementation. The studied concentrations of DDRP were not effective in small ruminants and poultry husbandry.

Keywords: dry pressed distilled rose petals; polyphenols; feedstuff supplements; livestock; growth performance

Abbreviations: ADG, average daily weight gain; AFC, average feed consumption; DDRP, dry pressed distilled rose petals; DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl; FCR, feed conversion ratio; FRAP, ferric ion reducing antioxidant power; FWHM, full width at half maximum; HCD, higher-energy C-trap dissociation; HPLC, high-performance liquid chromatography; HPLC-PDA, high-performance liquid chromatography-photodiode array; HRMS, high-resolution mass

spectrometer; GAE, gallic acid equivalents; LC-MS, Liquid chromatography–mass spectrometry; PDA, photodiode array; NCE, normalized collision energy; SD, standard deviation; TE, Trolox equivalents; UHPLC, ultrahigh- performance liquid chromatography; UHPLC-MS/MS, ultra-high-performance liquid chromatography-tandem mass spectrometry.

1. Introduction

Oilseed rose (*Rosa damascena* Mill.) processing is widely distributed on the Balkan Peninsula countries Bulgaria (Rusanov et al., 2014) and Turkey (Erbas and Baydar, 2016). The rose oil and rose water are highly valued in perfumery and cosmetics (Aggarwal and Kaur, 2017). Recently Özkan et al. (2004) have been reported the strong antioxidant capacity and antibacterial activity of *Rosa damascena* fresh and spent flower extracts against 15 pathogenic bacterial species. The data of Baydar and Baydar (2013) confirm the high antiradical activity and antioxidant capacity of several phenolic compounds discovered in hot and cold methanolic extracts of oil-bearing rose (*Rosa damascena* Mill.). They suppose that oil-bearing rose by-products and wastes can be discussed as natural antioxidant sources. In the literature can be found only few publications discussing the optimisation of technology and the extraction procedure of rose by-products and waste with the purpuse of their utilization. An original approach for hydrothermal gasification of *Rosa damascena* residues was published by Akgül et al. (2014). They concluded that the rose wastes have potential to be a future source for hydrogen production. It has been offered industrial wastes of *Rosa damascena* to be regarded as a source of water-soluble pectic extracts (Slavov et al., 2016). In connection with this a method for recovery of biologically active substances from rose (*Rosa damascena* Mill.) by-products was proposed (Slavov et al., 2017).

Schieber et al., (2005) first have analyzed the flavonol glycosides extracted from distilled rose petals of oil-bearing rose. Twenty two major compounds were identified including kaempferol and quercetin glycosides, quercetin 3-O-galactoside and quercetin 3-O-xyloside. It has been shown that the kaempferol glycosides comprise about 80% of the quantified compounds. By LC-MS analysis were indentified thirteen kaempferol and eleven quercetin glycosides in 30% v/v water-ethanolic extract from industrially distilled rose petals (Shikov et al., 2008).

The by-products of Taif rose (Rosa *damascena trigintipetala* Dieck) have been shown to be a source of natural antioxidants with strong antioxidant activity (Abdel-Hameed et al., 2012) too. Their free radical scavenging potential correlated with the biochemical components content in red rose petals (Pal et al., 2018). Rusanov et al. (2014) have reported the presence of flavan-3-ols, flavanones, flavonols and flavones in rose oil distillation wastewater. Due to their pronounced antioxidant and antibacterial properties, rose by-products or wastes have found many applications in various branches of the food industry (Slavov et al., 2017). They were suggested as colour stabilizers of strawberry beverage (Mollov et al., 2007) and texture-improved canned strawberries (Shikov et al., 2012), and as natural antioxidants added to meat and sausages (Oswell et al., 2018). The potential of the rose oil industry wastes was disscussed to find application in liqueurs preparations (Vasileva et al., 2019) and in probiotic lactic acid bacteria dairy products (Dimitrova et al., 2019). The dry distilled rose (*Rosa damascena* Mill.) petals were used for enrichment of broiler's feed (Balev et al., 2015). The utility model relating to the composition of the feed supplement for livestock and poultry, based on dry distilled rose petals was registred, as well (Vlahova-Vangelova et al., 2018).

When consumed regularly by humans, polyphenols have been associated with a reduction in the incidence of diseases such as cancer, obesity, diabetes and heart disease (Koch, 2019). The ability of these natural antioxidants, to scavenge several oxygen and nitrogen free radicals has been associated to the health benefits of diets rich in polyphenols (Abdel-Hameed et al., 2012; Baydar and Baydar, 2013; Pal et al.,2018). From this point of view the objectives of this research were to determine the possibilities for dry pressed distilled rose petals (DDRP) valorization as a feedstuff phytonutrients in animal husbandory, to study the chemical composition and radical scavenging activity of polyphenol complex in rose (*Rosa damascena* Mill.) waste products, to identify and quantify the polyphenol composition in dry rose petals, dry pressed distilled rose petals and waste water (liquid aqueous phase after distillation).

2. Materials and methods

2.1. Rosa damascena Mill. Sampling

Dry rose petals and distilled waste by-products such as dry pressed distilled rose petals (DDRP) and waste water (liquid aqueous phase after distillation) were analyzed. The waste by-product was collected after distillation column of the installation of rose oil extraction by the company Bulattars Productions (Skobelevo village, Bulgaria). It was compressed for 12 h at room temperature at a pressure of 303.975 KPa. The obtained presses were dried with hot air (60°C, 6 h) to equilibrium humidity. The dry matter in dry distilled rose petals was 97.92 ± 0.19 g/100 g and 97.98 ± 0.21 g/100 g in dry pressed distilled rose petals, respectivelly. The dry residues were ground in a ball mill.

The liquid aqueous phase from the rose petals distillation was collected after the distillation column of the rose oil extraction installation. It was concentrated by evaporation during the

boiling in open pots. A concentrate was obtained from the liquid aqueous phase of the distilled rose petals.

The distilled rose petals were dehydrated by presses and then was dried. The distilled liquid phase was concentrated by evaporation of the water contained therein.

2.2. Experimental procedures

2.2.1. Polyphenol indices and antioxidant activity

2.2.1.1. Determination of total anthocyanins

The amount of total monomer anthocyanins was determined by the pH differential method (Giusti and Jing, 2007) based on the property of anthocyanin pigments to change the color with pH.

2.2.1.2. Determination of total polyphenols

The content of total polyphenols was determined by the method of Singleton and Rossi (1965). The results obtained are presented as gallic acid equivalents (GAE).

2.2.1.3. Determination of antioxidant activity using DPPH• free radical

The DPPH• radical scavenging ability was determined by the method of Brand-Williams et al. (1995). The results obtained are presented as Trolox (TE) equivalents.

2.2.1.4. Determination of ferric ion reducing antioxidant power (FRAP-test)

The FRAP was determined by the method of Benzie and Strain (1996). The results are presented as Trolox (TE) equivalents.

2.3. Chromatographic analysis

2.3.1. Ultrasound-assisted extraction of polyphenols

0.2 g powder of dry rose petals and dry pressed distilled rose petals samples were weighed. Five instances of every sample were prepared. The waste rose water was injected directly in the

HPLC device after filtration. The polyphenols were analyzed in their glycoside form (and therefore no hydrolyzed plant extracts were prepared) by extracting the samples with 10 ml 70% (v/v) aqueous methanol in an ultrasound bath for 40 min at room temperature (25°C). The extracts were filtrated under reduced pressure. The volume of the samples was adjusted to 10 ml and passed through a membrane filter 0.45 μm prior to HPLC analysis.

2.3.2. HPLC-PDA analysis of polyphenols

The instrumentation used for HPLC analysis consisted of quaternary mixer Smartline Manager 5000, pump Smartline 1000 and PDA 2800 detector (Knauer, Germany). Separation of polyphenol components was performed on Kromasil C18, 15 cm × 4.6 mm i.d. 5μm particle size (Supelco, USA. The chromatography was carried out using as mobile phase A mixture from 95 parts 2% formic acid in water and 5 parts mobile phase B. As solvent B was used mixture from 10 parts 2% formic acid in water and 90 parts 2% formic acid in acetonitrile. The polyphenols were eluted with a gradient system as follow: 0-15 min, 100 % - 90 % A (0 - 10 % B), 15 - 25 min, 80 % A (20 % B), 25 - 55 min, 50 % A (50 % B), 55 - 60 min, 0 % A (100 % B).

Mobile phase flow rate was set by 1.0 ml/min; sample volume was 20μl. The polyphenols were monitored at 320 nm, 340 nm, 352 nm and 280 nm.

The spectral characteristics of eluting peaks of each sample, scanned with a PDA detector (λ=200–400 nm), were compared with those of authentic standards, gallic acid, rutin, quercetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin and kaempferol (Sigma Aldrich). The identification of compounds was made by summarizing the data for retention times, UV and MS spectra of standards and the peaks in the samples, and previously published information. The MS/MS data for the molecular mass and the fragmentation of deprotonated molecular ions $[M-H]^-$ were worked up by assigning the structure of compounds.

Quantification of main polyphenol components was performed by using the data from the fingerprint profiles obtained from HPLC-PDA analysis at 280nm and 352nm.

The calibration curves were prepared from stock solutions of analytical standards at a concentration of 1000 $mg.L^{-1}$ in methanol by successive dilution until the optimal range of application for each compound. The calibration standards and the samples were injected in duplicate.

2.3.3. LC-MS analysis

The ground powder of dry rose petals (10 g) was extracted with 500 ml ethylacetate: methanol (1:1 v/v) in an ultrasonic bath for 40 min at room temperature (25°C). The extract was filtered under reduced pressure. The liquid was evaporated in a rotary evaporator at 40°C to give a residue (1.1426 g), which was dissolved in water and successively partitioned among hexane, chloroform and ethyl acetate, each (10 x 30 ml). The obtained fractions were evaporated to dryness. The chloroform and ethyl acetate fractions were dissolved in 4 ml methanol each and were subjected to UHPLC-MS/MS analysis (Bojilov et al., 2017).

The ethyl acetate (23.67%) and chloroform (3.79%) fractions obtained after liquid–liquid extraction of the aqueous solution of dry rose petals crude extract were analyzed. The LC-MS analysis was performed on a Q Exactive Plus high-resolution mass spectrometer (HRMS) with heated electrospray ionization source (HESI-II) (Thermo Fisher Scientific, Inc., Bremen, Germany) equipped with a Dionex Ultimate 3000RSLC ultrahigh-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific, Inc.). Operating conditions for the HESI source in negative ionization mode were: 2.5 kV spray voltage, 320°C capillary and probe heater temperature, sheath gas flow rate 38 units, auxiliary gas flow 12 units (units refer to arbitrary values set by the Exactive Tune software) and SLens RF level 50.0. Nitrogen was used

for sample nebulization and collision gas in the HCD cell. The LC–MS method was operating in hull scan-ddMS2/Top5 with the following settings: 70,000 FWHM resolution (at m/z 200), AGC target 3e6, max. IT 100 ms and mass range m/z 100-1500 were chosen, while ddMS2 conditions were set to resolution 17,500 FWHM (at m/z 200), AGC target 1e5, max. IT 50 ms, isolation window 2.0 amu and step normalized collision energy (NCE) was set to 10, 20 and 30. The UHPLC separations were performed on a Kromasil Eternity XT C18, 1.8 μm, 2.1×100mm column (AkzoNobel, Sweden) with a binary mobile phase consisting of solution A: 0.1% formic acid in water and solution B: 0.1% formic acid in acetonitrile. The following step gradient program was used: 0 min, 95% A; 0.5 min, 95% A, 6 min, 86% A. 12 min, 76% A; 26 min, 48% A; 28 min, 10% A; 30 min, 10% A; 30.5 min, 95% A. Equilibration time was 4.5 min prior to injection, the flow rate was 0.3 mL/min and the sample volume was 1 μL. The column compartment temperature was set to 40°C. The data acquisition was accomplished with Xcalibur (Thermo Scientific) software ver. 4.0. The calculation of the exact masses and mass measurement errors, prediction of molecular formulas and simulation of monoisotopic profiles were carried out with Xcalibur ver. 4.0 or FreeStyle ver. 1.5 software (Thermo Scientific).

2.4. Design of experiments for valorization of DDRP as phytonutrient

The experiments were conducted in accordance with Art. 14 of Part V. Breeding and Livestock Units form European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Commission Recommendation 2007/526/EC and Council Regulation (EC) No 1099/2009. The experiments were approved by the Bulgarian Scientific Ethics Committee and requirements of the Council Directive 2010/63/EC were met. During the fattening period, the animals were reared in accordance with the requirements of Bulgarian Ordinance No 21 of December 14, 2005.

2.4.1. Pigs

A total of 72 Danube White 155 days old fattening pigs (both sexes - 36 male and 36 female) of 72.500 ± 1.937 kg (mean ± SD) were used. They were housed in barn equipped with individual pens with feeders and drinkers, in facilities at the Experimental farm of Agricultural Institute, Shumen, Bulgaria. During the experiment, the temperature was between 19 to 27°C. Feed was available ad libitum. Water was provided by nipples drinkers ad libitum. The pigs were randomized by origin, age, sex, weight and were distributed into three groups (one control and two experimental), each containing of 24 animals fed for 45 days with two different levels of DDRP supplementations. The control group (C) was fed basal diet (Table 1) supplied by Vasil Kostov feed factory, Lyuben Karavelovo village, Varna district, Bulgaria. The other two experimental groups were fed with the same diets containing either 0.255 g DDRP/kg/d (R1) or 0.545 g DDRP/kg/d (R2). Residual feed was monitored daily and was weighed and subtracted from of the daily amount of feed consumed. Individual daily doses of the supplements were calculated according to previous weighing of animals, mixed with feed and given with the morning feeding. Pigs were weighed every two weeks. At the end of the experiment, the age of the pigs was 200 days and the average live weight was at about 110.300 ± 2.274 kg.

2.4.2. Lambs

A total of 30 numbers of 65 days old lambs from the Synthetic population of Bulgarian milk sheep breed lambs (both sexes - 15 male and 15 female) with average life weight 20.500 ± 1.039 kg (mean ± SD) were used. They were housed in a total indoor barn in facilities at the Experimental Farm of Agricultural Institute, Shumen, Bulgaria. The lambs were divided into three groups (one control and two experimental), each containing of 10 animals fed for 50 days. During the experiment, the temperature was 19 - 25°C. Feeding the lambs was ad libitum in

group boxes, with access to water and salt. Feed and water were available. Individual daily doses of the supplements were calculated according to previous weighing of animals, mixed with feed and given with the morning feeding. The control group (C) was fed combined feed for lambs granules and ground alfalfa (grower and finisher basal diets Table 1) supplied by Vasil Kostov feed factory, Lyuben Karavelovo village, Varna district, Bulgaria. The experimental group (R) was fed with the same diet supplemented either 0.545 g DDRP/kg/day. Daily control of the amount of combined feed consumption during the experiment was made. Residual feed was weighed and subtracted from of the daily amount of feed consumed. Lambs were weighed every two weeks. At the end of the experiment, the lambs were 115 days old with the average live weight at about 36.415 ± 0.849 kg.

2.4.3. Broilers

A total of 40 one days old chicks from the hybrid combination Ross 308 were purchased from the Bovans Bulgaria hatchery, Chirpan, Bulgaria. They were housed in a breeding room at the Experimental farm of Agricultural University, Plovdiv, Bulgaria. During the experiment, the temperature was between 19 to 25°C. Feed and water were available ad libitum. The chicks were distributed into two groups each containing of 20 birds fed for 49 days. The control group (C) was fed basal diet (Table 1). The combined feed was purchased from Viand Ltd, Sofia, Bulgaria. The experimental group (R) was fed with the same fodder containing either 40 mg DDRP/kg/d. A DDRP supplementation was made after its pre-grinding. Individual daily doses of the supplements were calculated according to previous weighing of broilers, mixed with feed and given with the morning feeding. The body weight of broilers was controlled at seven days intervals. Residual feed was monitored daily and was weighed and subtracted from of the daily

amount of feed consumed. Feeding and watering the broilers was done ad libitum. At the end of the experiment the live weight of the broilers was at about 3.460 ± 0.089 kg.

2.5. Determination of total feed consumption and growth performance

The total feed consumption and growth performance was determined by weight measurements.

2.6. Feed conversion ratio calculation

The feed conversion ratio (FCR) was calculated as a ratio between the average feed consumption (AFC) and the average daily growth (ADG) of the animals or birds. The

FCR was calculated by the formula (1):

FCR = AFC/ADG (1), where:

AFC - average feed consumption, kg;

ADG - average daily growth, kg;

2.7. Statistical Analysis

Five repetitions of every sample were analyzed. Data are expressed as means ± standard deviation (± SD), n = 5. Microsoft Excel Office Professional Plus 2010 was used to process the statistics.

3. Results

To achieve the fullest possible utilization of valuable rose biomass our attention was directed to define the possibility for valorization of waste products after isolation of essential oil from *Rosa damascena* as a feedstuff in the animal husbandory.

3.1 Basic polyphenol indices and antioxidant activity of DDRP

Hence, at first a careful study of polyphenol complex of the waste products was undertaken with special attention on dry pressed distilled rose petals (DDRP) regarded as a much

suitable feedstuff for animal's feeding. The basic polyphenol indices as the content of total polyphenols and anthocyanins reveal their high amount in the dry pressed distilled rose petals. DDRP contains total polyphenols 7504.00 ± 24.00 mg GAE/100 g and 92.10 ± 0.25 mg anthocyanins/100 g dry matter. This is a reason DDRP to have a strong radical scavenging acitivity against DPPH• radical (39138.90 µmol TE/100 g) and to expresse high ferric ion reducing antioxidant power (FRAP = 35550 µmol TE/100 g).

3.2. Identification and quantification of polyphenol components in dry rose petals and waste after distillation of essential oil

On Figure 1 are presented the fingerprint chromatographic profiles of dry rose petals, dry pressed distilled rose petals (DDRP) and waste water at 280nm and 352nm. In the three fingerprint profiles the UV spectra from PDA detector show mostly peaks of gallic acid (2min – 20min), quercetin (24min-26min) and kaempferol (27min-36min) derivatives. The chromatographic profiles of dry rose petals and distilled waste by-products DDRP and waste water contain components with the same spectra, hence no qualitative differences in the polyphenol composition were observed. The careful chromatographic analysis revealed the abundance of polyphenolic compounds in the dry rose petals and DDRP. Different proportions of three main groups of polyphenolic compounds were found in dry rose petals, in dry pressed distilled rose petals and in waste water after isolation of essential oil from *Rosa damascena* (Table 2). In the waste water was established the lowest amount of all components. The DDRP contains significant amount of glycosides as well as kaempferol (Table 2). Approximately 50% from all polyphenolic compounds determined in rose petals were found in DDRP. The DDRP was characterized with the highest content of kaempferol. On contrary, the waste water profile was leaking of kaempferol and in general the polyphenol components were in lower amount.

The fractionation of dry rose petals leaded to a small chloroform fraction (3.79 %) in which both flavonol aglycones quercetin and kaempferol were identified. In the ethylacetate fraction (23.67%) only glycosides were ascertained (Figure 2). In summary 30 polyphenol components were identified in dry rose petals as presented in Table 3. Mainly three groups of polyphenolic compounds in following order comprise the polyphenol complex of *Rosa damascena* dry petals: glycosides of kaempferol > glycosides of quercetin > glycosides of gallic acid (Table 3). Thirteen glycosides of kaempferol, 10 glycosides of quercetin, 6 glycosides of gallic acid and the two flavonol aglycones are identified. The MS/MS data reveal the predominant number of kaempferol glycosides in the polyphenol complex of *Rosa damascena* petals. The fragmentation of glycosides by low collision energy is leading to decomposition of the glycosil bond and loss of 162 Da, 146 Da, 152 Da, 132 Da и 42 Da, which are hexose, (glucose, galactose, rhamnose), galloyl group, pentose and acetyl group. The ESI-MS/MS spectra show the ions of quercetin (*m/z* 301), kaempferol (*m/z* 285) and gallic acid (*m/z* 169) (Table 3). Our data indicate the presence of flavonol glycosides mainly kaempferol and quercetin glycosides in the dry rose petals with exception of six glycosides of gallic acid (1-6) and additional isomers of quercetin galloyl hexoside and kaempferol disaccharide, which are identified for the first time. The galloyl glycosides undergo fragmentation with elimination of galloyl group (152 Da) and hexose (162 Da). The characteristic ions for gallic acid are *m/z* 125 and *m/z* 107 (Table 3).

3.3. DDRP as feed supplement

Three types of experiments for valorization of DDRP were conducted concerning feeding pigs, broilers and lambs with addition of rose waste to the basal diet. Similar *in vivo* investigations are scarce therefore the results are with high practical value.

3.3.1. Pigs

It was found the addition of 0.545 g DDRP/kg/d (Experimental group R2) to the forage of fattening pigs contributes for an increasing of the total feed consumption with 6.73% ($p < 0.05$) and of the average daily growth with 27.05%, comparing to control group (C) (Table 4). Therefore, the feed conversion ratio in pigs from experimental group (R2) was with approximately 16% lower than those in pigs from control group (C) (Table 4). The conclusion was made that the supplementation of feed with 0.545 g of DDRP/kg/d can intensify the Danube white pig's growth performance comparing with pigs from control group (C).

3.3.2. Chickens

The 40 mg DDRP/kg/d supplementation of feedstuffs for broilers has no enough significant effect ($p \geq 0.05$) on the total feed consumption, average daily growth and feed conversion ratio in broilers (Table 4).

3.3.3. Lambs

Similarly, the 545 mg DDRP/kg/d supplementation of feedstuffs for lambs does not have a effective sufficient ($p \geq 0.05$) impact on their growth performance, the total feed consumption, average daily growth and ultimately on the feed conversion ratio (Table 4).

4. Discussion

In the last decade the increased essential oil production generates huge amount of waste containing bioactive components. Waste waters and the solid biomass are retained and in most cases simply discarded after the distillation process. Various approaches for rose waste valorization have been proposed during the last years with purpose to utilize the valuable biomass (Slavov et al. 2017). The determined high amount of total polyphenols and anthocyanins in the dry pressed distilled rose petals confirm the results presented by Özkan et al. (2004) in residues of spent flowers of *Rosa damascena* Mill. after steam distillation and by Dina et al. (2018) in the rose hydrodistillation byproducts remaining as aqueous extract. The final extract

has a significant antioxidant activity and could be find application on the market of cosmetics, nutraceuticals or phytotherapeuticals (Dina et al., 2018).

Polyphenols are polar compounds containing more than one hydroxyl group, hence for their extraction 70% methanol is recommended as the most selective and appropriate solvent for HPLC analyses with high precision (Dagnon et al., 2018). Proper identification and quantification of compounds is needed to ascertain the polyphenol composition demanding to develop HPLC-PDA fingerprint chromatographic profiles. For this reason, by the analysis of specific extract, the analysis time, the type of acid in the mobile phase and its concentration, and the slope of the gradient require optimization. The fingerprint profile was accepted when it contained maximum number of good separated peaks (Dagnon et al., 2018). In the case of *Rosa damascena* the best separation of both quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside, which are very similar, was achieved on Kromasil column by the described mobile phase and gradient.

Similarly to our results the presence of flavonol glycosides in distilled rose petals has been reported by Schieber et al. (2005). They have identified 22 kaempferol and quercetin glycosides unlike our data that are identified 30 polyphenolic compounds: 13 kaempferol glycosides, 10 quercetin glycosides, 6 glycosides of gallic acid and 2 flavonol aglycones. The data in our study are leading to suggest that most of the bioactive flavonoid glycosides are retained unchanged in DDRP after distillation comprising predominantly kaempferol derivatives. Nevertheless the amounts of quercetin and kaempferol glycosides are quite the same.

Similarly to us Abdel-Hameed et al. (2012) reported that the residues of Taif rose possess radical scavenging activity, antioxidant capacity and reducing power activity. Abdel-Hameed et

al. (2012) confirm the phenolic compounds, especially flavonols are the major antioxidant active components in the Taif rose residues.

The found increasing of the total feed consumption the average daily growth and decreasing of feed conversion ratio in pigs supplemented with 0.545 g DDRP/kg/d can be explained with antioxidative and antimicrobial actions of their phytogenic compounds which can promote the animal health (Windisch et al., 2008). As the polyphenols in DDRP probably have ability to stimulate the digestive tract they may be increase the appetite of pigs (Frankič et al. 2009) and can find an application as growth promoters in pigs' production (Valenzuela-Grijalva et al., 2017). Yang et al. (2015) suggested that the established increase in feed palatability could be due to the antioxidative effects of DDRP polyphenols, which might contribute to preserving release of unfavorable odors and a good quality of diets. On the other hand, the investigated phytonutrients exert a specific effect on the gut functions, stabilizing microbial eubiosis (Jamroz et al., 2006). They show an antibacterial activity against 15 bacteria species (Özkan et al., 2004). Thus they improve of nutrient digestion and absorption (Valenzuela-Grijalva et al., 2017). Last but not the least, discussed photogenic compounds may have anti-inflamatory effect too (Pitman and Blumberg, 2000). Superior feed conversion ratios of finisher pigs can also be achieved through not mixing rations on the operation (Losinger, 1998).

Our results indicate that the supplementation enrichment of broiler feed with 40 mg DDRP/kg/d is not sufficiently effective to increase the total feed consumption, average daily growth and to decrease the feed conversion ratio. Additional studies are needed for determining the effective doses of DDRP supplementations in broilers because the respective experimental *in vivo* evidences are still quite limited (Hashemi and Davoodi, 2011). Breeding and genetics are important factors for 6 weeks of age chickens housed in groups (Leenstra and Pit, 1987). When

planning further research it has to pay attention on individual selection for feed conversion. Probably changes in body composition and in growth pattern contribute to the favorable feed conversion ratio from 21 to 42 days of age chickens (Pasternak and Shalev, 1983). Next important factor we have to pay attention in future research it is heat stress and connected to it body temperature (Cooper and Washburn, 1998). Those authors shown the individual gain, feed consumption, and feed conversion ratio from 28 to 49 d directly depend on a heat stress environment temperature (32°C). This means that should be avoided to perform the experiments during the summer months, when the air temperature normally reached similar values.

Those findings confirm that the addition of 545 mg DDRP/kg/d to lambs' feed did not have a significant effect on the total feed consumption, average daily gain and feed conversion ratio. The explanation of those finding were likely due to the fact that these amounts of DDRP could not improve the condition and feed intake because they are not modulators of ruminal fermentation in small ruminants (Surai, 2014) and have no anabolic activity on target tissues (Bahadoran et al., 2013).

4. Conclusions

In conclusion the dry distilled rose petals contain three groups of polyphenolic compounds: glycosides of kaempferol > glycosides of quercetin > glycosides of gallic acid. Therefore the DDRP characterizes with significant amount of polyphenols and high antioxidant activity.

The DDRP in the investigated concentrations showed limited potential for application as feed supplements in small ruminants and poultry husbandry. On contrary supplementation of feed with 0.545 g of DDRP/kg/d improved Danube white pig's performance.

Conflict of interests

The authors declare no conflict of interests.

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564 Table 1

565 Ingredients, chemical composition and energy values of basal diet

566

Components of combined feed for pigs	**Grower for pigs**, g/kg	**Finisher for pigs**, g/kg	**Components of combined feed for lambs**	**Grower for lambs**, g/kg	**Finisher for lambs**, g/kg	**Components of combined feed for broilers**	**Starter for chicks**, g/kg	**Finisher for broilers, g/kg**
Formulations								
Maize	150.00	130.00	Maize	450.00	250.00	Maize	400.00	450.00
Barley	250.00	100.00	Barley	-	150.00	Wheat	200.00	200.00
Wheat	270.00	500.00	Wheat	200.00	170.00	Soybean meal	150.00	150.00
Wheat bran	80.00	70.00	Oats	135.00	170.00	Sunflower pomace	100.00	100.00
Bio-concentrate BC14	250.00	–	Sunflower pomace	100.00	200.00	Vegetable fat	100.00	50.00
Bio-concentrate BC16	–	200.00	Vitamin-mineral premix	50.00	50.00	Vitamin-mineral premix including phosphate, lysine, methionine, enzymes, coccidiostatic, antitoxic substances	40.00	40.00
			Chalk	10.00	10.00	Chalk	7.00	7.00
			Alfalfa	100.00	100.00	Salt	3.00	3.00
Calculated chemical compositions, g/kg								
Crude protein	157.50	150.20	Crude protein	180.00	165.00	Crude protein	180.00	160.00
Crude fats	28.10	24.20	Crude fats	26.50	33.00	Crude fats	25.00	60.00
Crude ash	46.80	43.90	Crude ash	74.10	79.00	Crude ash	48.00	54.50
Dietary fibers	47.90	38.40	Dietary fibers	89.00	93.00	Dietary fibers	40.00	36.00
Amino acids, g/100g								
Lysine	0.80	0.72	Lysine	0.85	0.75	Lysine	0.92	0.88
Methionine			Methionine	0.40	0.33	Methionine	0.33	0.35
Minerals, g/100g								
Calcium	1.31	1.26	Calcium	1.30	1.50	Calcium	0.94	1.33
Phosphorus	0.85	0.31	Phosphorus	0.66	0.70	Phosphorus	0.78	0.82
			Sodium	0.40	0.54	Sodium	0.15	0.12
			Cupper	0.0105	0.0095	Cupper	0.0007	0.0010
			Magnesium	0.00011	-			
Energy intake, MJ/kg								
Digestible energy	13.46	13.72	Digestible energy	12.90	13.33	Digestible energy	12.87	13.80
Exchangeable energy	12.92	13.18	Exchangeable energy	12.34	12.82	Exchangeable energy	12.35	13.26

567

568 [a] The quantities of Siberian larch dihydroquercetin or dry distilled rose petals added as supplements to the
569 diets were calculated as 3.5 mg dihydroquercetin/kg/d (D1); 7.5 mg dihydroquercetin/kg/d (D2); 0.255 g dry
570 distilled rose petals/kg/d (R1); or 0.545 g dry distilled rose petals/kg/d (R2).

571 [b] The bio-concentrate BC14 contents: 312.10 g/kg crude protein, 10.70 g/kg crude fat, 153.00 g/kg crude ash,
572 38.10 g/kg crude fibers, 5.88 g/100 g lysine, 2.79 g/100 g methionine, 7.80 g/100 g calcium, 2.69 g/100 g

phosphorus, 268 mg/kg copperas sulphate, 670 mg/kg dl-α-tocopherol, 93800 UI/kg vitamin A, 16080 UI/kg vitamin D3, 1975.845 kcal/kg total energy

[c] The bio-concentrate BC16 contents: 348.00 g/kg crude protein, 17.40 g/kg crude fat, 165.00 g/kg g crude ash, 108.30 g/kg crude fibers, 2.26 g/100 g lysine, 0.67 g/100 g methionine, i.e. 1.25 g/100 g methionine + cystine, 1.31 g/100 g threonine, 3.66 g/100 g calcium, 0.95 g/100 g phosphorus, i.e. 0.67 g/100 g absorbable phosphorus, 0.78 g/100 g sodium, 560.00 mg/kg iron, 545.00 mg/kg zinc, 195.00 mg/kg manganese, 100.00 mg/kg copper, 4.10 mg/kg iodine, 1.50 mg/kg selenium, 0.40 g/100g antioxidants, 320.00 mg/kg vitamin E, 32500 UI/kg vitamin A, 6000 UI/kg vitamin D3.

[d] Vitamin-mineral premix for lambs contents: E672 Vitamin A (Retinal acetate) - 8000 IU/kg, Vitamin E (α-Tocopherylacetat) - 40.00mg/kg, E671 Vitamin D3 (Cholecalciferol) - 2000 IU/kg, E1 Iron (Iron sulfate) - 170.26 mg/kg, E2 Iodine (Potassium iodate) - 1.10 mg/kg, E3 Cobalt (Cobalt carbonate) - 0.30 mg/kg, E4 Copper (Copper oxide) - 5.65 mg/kg, E5 Manganese (Manganese oxide) - 95.61 mg/kg, E6 Zinc (Zinc oxide) - 108.86 mg/kg, E8 Selenium (Sodium selenite) - 0.40 mg/kg.

Table 2
Content of polyphenolic compounds in dry rose petals, dry pressed distilled rose petals and waste water after isolation of essential oil from *Rosa damascena*

Compounds		**Waste products after isolation of essential oil from oil-bearing Bulgarian rose (*Rosa damascena*)**		
	HPLC, t_R, min	Dry rose petals, mg/g	Dry pressed distilled rose petals, mg/g	Waste water, µg/ml
Gallic acid glycosides	2 - 20	2,89 ± 0,07	1,43 ± 0,04	27,64 ± 0,83
Quercetin-3-*O*-galactoside	24.9 ± 0.6	1.64 ± 0.33	0.96 ± 0.18	19.72 ± 1.01
Quercetin 3-*O*-glucoside	25.5 ± 0.6	1.35 ± 0.21	0.79 ± 0.10	21.36 ± 0.56
Sum of Quercetin glycosides	24-26	2,92 ± 0,06	1,98 ± 0,05	49,96 ± 1,22
Sum of Kaempferol glycosides	27-36	2,81 ± 0,06	1,97 ± 0,05	47,53 ± 1,19
Kaempferol	43.5 ± 1.2	traces	0.22 ± 0.03	-

[a] Gallic acid glycosides are determined as gallic acid, Sum of Quercetin glycosides as quercetin, and Sum of Kaempferol glycosides as kaempferol

Table 3

Chromatographic and spectral data of polyphenolic compounds in the ethylacetate and chloroform fractions of dry rose petals of *Rosa damascena*

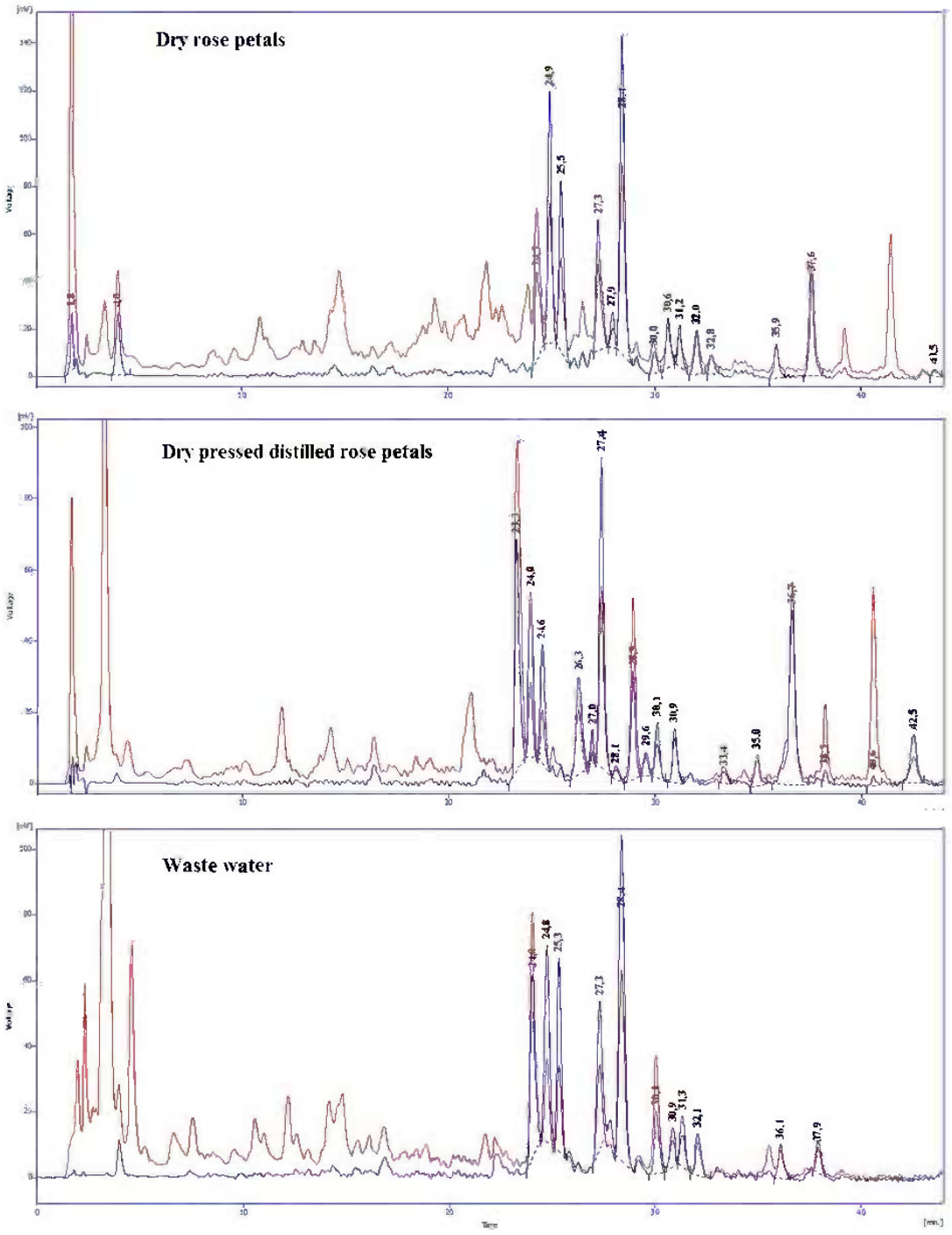
No	t_R	Compounds	[M-H]-	MS/MS
1	1,79	Digalloyl hexoside	483	483, 331, 313, 169, 125, 107
2	2,71	Digalloyl hexoside	483	483, 331, 313, 169, 125, 107
3	3,48	Digalloyl hexoside	483	483, 331, 313, 169, 125, 107
4	5,17	Digalloyl hexoside	483	483, 331, 313, 169, 125, 107
5	5,93	Three galloyl hexoside	635	635, 483, 465, 313, 169, 125, 107
6	7,87	Methyl galloyl-galloyl hexoside	497	497, 447, 345, 334, 313, 183, 169
7	10,25	Quercetin galloyl hexoside	615	463, 301, 151
8	10,41	Quercetin galloyl hexoside	615	463, 301, 151
9	10,64	Quercetin 3-*O*-rutinoside	609	609, 301, 179
10	10,78	Quercetin 3-*O*-galactoside	463	463, 301, 300, 179, 151
11	10,99	Quercetin 3-*O*-glucoside	463	463, 301, 300, 179, 151
12	11,43	Quercetin galloyl hexoside	615	463, 301, 179, 151
13	11,49	Quercetin pentoside	433	433, 301
14a	11,74	Kaempferol hexoside	447	285, 151
14b		Kaempferol disaccharide	593	593, 447, 285, 151
15	11,92	Quercetin pentoside	433	433, 301 179, 151
16	12,15	Kaempferol hexoside	447	447, 285, 284, 151
17	12,18	Kaempferol hexoside	447	447, 285, 284, 151
18	12,24	Kaempferol hexoside	447	447, 285, 284, 151
19	12,64	Kaempferol galloyl hexoside	599	599, 447, 285, 151
20	12,93	Kaempferol pentoside	417	417, 285, 284, 151
21	13,08	Kaempferol disaccharide	593	593, 285
22	13,21	Kaempferol pentoside	417	417, 285, 179, 151
23	13,64	Kaempferol deoxyhexoside	431	431, 285
24	13,84	Quercetin acetyldisaccharide	651	651, 609, 301, 179, 151
25	14,66	Quercetin disaccharide	609	609, 463, 301, 179, 151
26	15,12	Kaempferol acetyldisaccharide	635	635, 593, 285, 257
27	15,93	Kaempferol disaccharide	593	593, 285
28	16,20	Kaempferol disaccharide	593	593, 285
29	15,38	Quercetin	301	301, 273, 179, 151, 121, 107
30	17,86	Kaempferol	285	285, 257, 213, 151, 107

Table 4

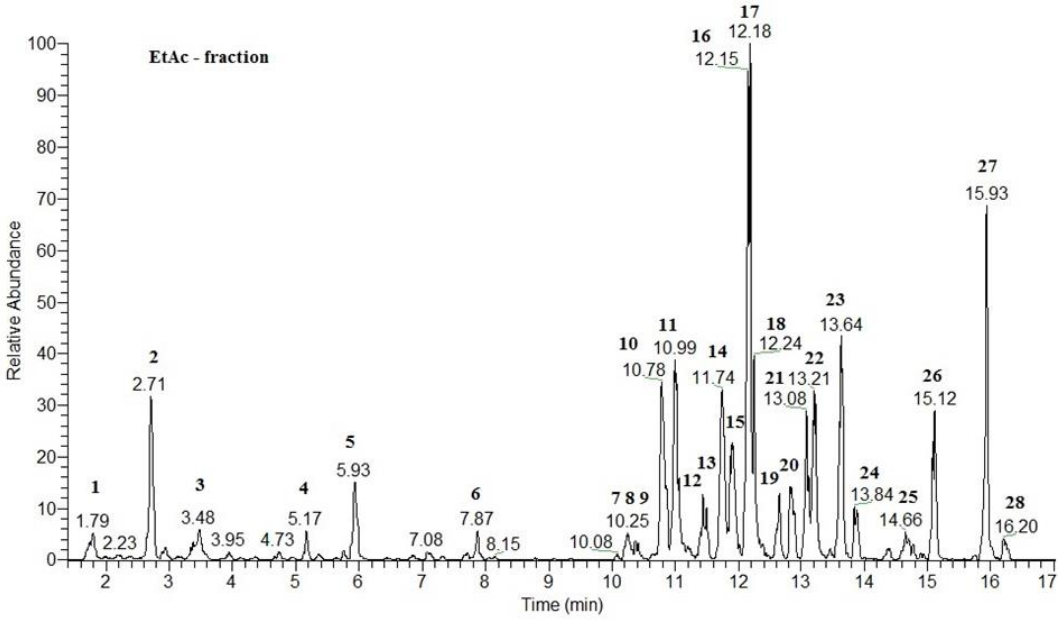
Overall total feed consumption, average daily growth and feed-conversion ratio in experimental animals

Parameters	**Pigs**			**Lambs**		**Broilers**	
	Control group (C)	Experimental group (R1)	Experimental group (R2)	Control group (C)	Experimental group (R)	Control group (C)	Experimental group (R)
Total feed consumption, kg	2.941±0.110^{b}	3.099±0.079^{b}	3.139±0.064^{bc}	2.182±0.044^{a}	2.172±0.057^{a}	6.780±0.101^{d}	6.980±0.114^{d}
Average daily growth, kg	0.717±0.062^{c}	0.828±0.051^{c}	0.911±0.036^{cd}	0.319±0.027^{b}	0.318±0.030^{b}	0.073±0.012^{a}	0.066±0.011^{a}
Feed-conversion ratio (FCR)	4.102 ± 0.291^{c}	3.743 ± 0.311^{bc}	3.446 ± 0.300^{b}	6.841± 0.672^{d}	6.831± 0.655^{d}	2.270± 0.334^{a}	2.220 ± 0.357^{a}

Fig 1. HPLC-PDA fingerprint profile of polyphenolic compounds of dry rose petals (*Rosa damascena*). Peak 4 – quercetin 3-*O*-galactoside (t_R=24.9 min), peak 5 – quercetin 3-*O*-glucoside (t_R=25.5 min), Peak 16- kaempferol (t_R=43.5); λ at 280nm and 352nm



637 Fig. 2. Total ion current (TIC) of ethylacetate fraction of dry rose petals by UHPLC-MS/MS
638
639
640



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