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

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Abstract

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The rose (*Rosa damascena* Mill.) flowers characterized with strong antioxidant capacity and antibacterial activity. Because of that it has been suggested the oil-bearing rose by-products and wastes can be discussed as natural antioxidant sources. From this point of view the objectives of this research were to determine the possibilities for dry pressed distilled rose petals valorisation as feed stuff phytonutrients in animal husbandry, 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). The polyphenol composition in dry rose petals, dry pressed distilled rose petals and waste water after distillation was identified and quantified. By HPLC-PDA and LC-MS 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 depending on the dose and length of fattening is expected to have a positively influence on the growth performance of pigs, broilers and lambs.

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

Abbreviations: DPPH*, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric ion reducing antioxidant power; FWHM, full width at half maximum; HCD, higher-energy C-trap dissociation; 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.
Introduction

Oilseed rose (*Rosa damascena* Mill.) processing is widely distributed on the Balkan Peninsula countries Bulgaria (Rusanov et al., 2014) and Turkey (Erbas & Baydar, 2016). The rose oil and rose water are highly valued in perfumery and cosmetics (Aggarwal & 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 fifteen pathogenic bacterial species. The data of Baydar & 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 purpose 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-xylloside. It has been shown that the kaempferol glycosides comprise about 80% of the quantified compounds. By LC-MS analysis were identified 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 tringintipetalata* 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 discussed 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 registered, 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 & 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 valorization as a feedstuff phytounitrients in animal husbandry, 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).

Materials and Methods

*Rosa damascena* Mill. sampling

Dry rose petals and distilled waste by-products such as dry pressed distilled rose petals 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, respectively. 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 were dried. The distilled liquid phase was concentrated by evaporation of the water contained therein.
Experimental procedures

**Polyphenol indices and antioxidant activity**

*Determination of total anthocyanins*

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

*Determination of total polyphenols*

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

*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.

*Utrasound-assisted extraction of polyphenols*

Two hundred mg powder of dry rose petals and dry pressed distillate 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 hydrolysed 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 filtered 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.

**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.e. 5 μm 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 20 μl. The polyphenols were monitored at 280 nm, 340 nm, 352 nm and 280 nm.

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

The calibration curves were prepared from stock solutions of analytical standards at a concentration of 1000 mg/l in methanol by successive dilution until the optimal range of application for each compound. Calibration standards and the samples were injected in duplicate.

**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 has 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 ml 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 3000RSRLC 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 nebulisation 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
Statistical analysis

The experiment was conducted in accordance with the 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.

Results and Discussion

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

Basic polyphenol indices and antioxidant activity of dry pressed distilled rose petals

Hence, at first a careful study of polyphenol complex of the waste products was undertaken with special attention on dry pressed distilled rose petals 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. Dry pressed distilled rose petals contains total polyphenols 7504.00 ± 24.00 mg GAE/100 g and 92.10 ± 0.25 mg anthocyanins/100 g dry matter. Probably this is a reason the dry pressed distilled rose petals to have a strong radical scavenging activity against DPPH* radical (39138.90 μmol TE/100 g) and to express high ferric ion reducing antioxidant power (FRAP = 35550 μmol TE/100 g).

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 valorisation 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 by-products 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).

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

The fingerprint chromatographic profiles of dry rose petals, dry pressed distilled rose petals and waste water at 280 nm and 352 nm are presented on Figure 1. In the three fingerprint profiles the UV spectra from PDA detector show mostly peaks of gallic acid (2 min – 20 min), quercetin (24 min-26 min) and kaempferol (27 min – 36 min) derivatives. The chromatographic profiles of dry rose petals and distilled waste by-products – dry pressed distilled rose petals 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 dry pressed distilled rose petals. 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 1). In the waste water were established the lowest amount of all components. The dry pressed distilled rose petals contain significant amount of glycosides as well as kaempferol (Table 1). Approximately...
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 ($t_R=43.5$); $\lambda$ at 280nm and 352 nm
Table 1. Content of polyphenolic compounds in dry rose petals, dry pressed distilled rose petals and waste water after isolation of essential oil from Bulgarian rose (Rosa damascena Mill.)

| Compounds                          | Waste products after isolation of essential oil from oil-bearing Bulgarian rose (Rosa damascena Mill.) |
|------------------------------------|-----------------------------------------------------------------------------------------------------|
|                                    | HPLC, \( t_p, \text{ min} \) | 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                           | –             |

*Gallic acid glycosides are determined as gallic acid, sum of quercetin glycosides as quercetin, and sum of kaempferol glycosides as kaempferol*

50% from all polyphenolic compounds determined in rose petals were found in dry pressed distilled rose petals. The dry pressed distilled rose petals were characterized with the highest content of kaempferol. On contrary, the waste water profile was lacking 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 thirty polyphenol components were identified in dry rose petals as presented in Table 2. Mainly three groups of polyphenol compounds in following order comprise the polyphenol complex of Rosa damascena dry petals: glycosides of kaempferol > glycosides of quercetin > glycosides of gallic acid (Table 2). Thirteen glycosides of kaempferol, ten glycosides of quercetin, six 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 glucosyl bond and loss of 162 Da, 146 Da, 152 Da, 132 Da and 42 Da, which are hexose, (glucose, galactose, rhamnose), galloyl group, pentose and acetyl group. The ESIMS/MS spectra show the ions of quercetin (\( m/z \) 301), kaempferol (\( m/z \) 285) and gallic acid (\( m/z \) 169) (Table 2). Our data indicate the presence of the 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 2).

Polyphehnols 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). Exactly for these reasons we optimize the processes of extraction, identification and quantification of components in the polyphenol composition.

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. Our data indicated the presence of the same compounds in the dry rose petals with exception of 6 glycosides of gallic acid (1-6) and additional isomers of quercetin galloyl hexoside and kaempferol disaccharide, which are identified for the first time. 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, espe-
Flavonols are the major antioxidant active components in the Taif rose residues.

**Dry pressed distilled rose petals as feed supplement**

Three types of experiments for valorization of dry pressed distilled rose petals were conducted concerning feeding pigs (Ivanova et al., 2020), broilers (Balev et al., 2015) and lambs (Stancheva et al., 2020) with addition of rose waste to the basal diet. Similar in vivo investigations are scarce therefore the results are with high practical value.

It was found (Ivanova et al., 2020) the addition of 0.545 g dry pressed distilled rose petals/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). Therefore, the feed conversion ratio in pigs from experimental group (R2) was with approximately 16% lower than those in pigs from control group (C). 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) (Ivanova et al., 2020).

The 40 mg dry pressed distilled rose petals/kg/d supplementation of feedstuffs for broilers has no enough significant effect (p ≥ 0.05) on the total feed consumption, average daily growth and feed conversion ratio in broilers (Balev et al., 2015).

Similarly, the 545 mg dry pressed distilled rose petals/kg/d supplementation of feedstuffs for lambs does not have an effective sufficient (p ≥ 0.05) impact on their growth performance, the total feed consumption, average daily growth and ultimately on the feed conversion ratio (Stancheva et al., 2020).

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 dry pressed distilled rose petals/kg/d (Ivanova et al., 2020) 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 dry pressed distilled rose petals probably have ability to stimulate the digestive tract, they may be increasing 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 dry pressed distilled rose petals polyphenols, which might contribute to preserving release of unfavourable odours 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-inflammatory effect too (Pitman & Blumberg, 2000). Superior feed conversion ratios of finisher pigs can also be achieved through not mixing rations on the operation (Losinger, 1998).

The results indicate that the supplementation enrichment of broiler feed with 40 mg dry pressed distilled rose petals/kg/d is not sufficiently effective could contribute to increase the total feed consumption, average daily growth and to decrease the feed conversion ratio (Balev et al., 2015). The additional studies are needed for determining the effective

| No | t<sub>r</sub> | 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| 11.92       | Kaempferol disaccharide          | 593     | 593, 447, 285, 151         |
| 15 | 12.15       | Kaempferol pentoside             | 447     | 447, 285, 284, 151         |
| 16 | 12.18       | Kaempferol hexoside              | 447     | 447, 285, 284, 151         |
| 17 | 12.24       | Kaempferol hexoside              | 447     | 447, 285, 284, 151         |
| 18 | 12.64       | Kaempferol galloyl hexoside      | 599     | 599, 447, 285, 151         |
| 19 | 12.93       | Kaempferol pentoside             | 417     | 417, 285, 284, 151         |
| 20 | 13.08       | Kaempferol disaccharide          | 593     | 593, 285                   |
| 21 | 13.21       | Kaempferol pentoside             | 417     | 417, 285, 179, 151         |
| 22 | 13.64       | Kaempferol deoxyhexoside         | 431     | 431, 285                   |
| 23 | 13.84       | Quercetin acetyldisaccharide     | 651     | 651, 609, 301, 179, 151    |
| 24 | 14.66       | Quercetin disaccharide           | 609     | 609, 463, 301, 179, 151    |
| 25 | 15.12       | Kaempferol acetyldisaccharide    | 635     | 635, 593, 285, 257         |
| 26 | 15.93       | Kaempferol disaccharide          | 593     | 593, 285                   |
| 27 | 16.20       | Kaempferol disaccharide          | 593     | 593, 285                   |
| 28 | 15.38       | Quercetin                        | 301     | 301, 273, 179, 151, 121, 107 |
| 29 | 17.86       | Kaempferol                       | 285     | 285, 257, 213, 151, 107    |
doses of dry pressed distilled rose petals supplementations in broilers because the respective experimental in vivo evidences are still quite limited (Hashemi & Davoodi, 2011). Breeding and genetics are important factors for six weeks of age chickens housed in groups (Leenstra & 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 favourable feed conversion ratio from twenty one to forty two days of age chickens (Pasternak & Shaley, 1983). Next important factor we have to pay attention in future research it is heat stress and connected to it body temperature (Cooper & Washburn, 1998). The individual gain, feed consumption, and feed conversion ratio from 28 to 49 d are directly depended from a heat stress environment temperature (32°C) (Cooper & Washburn, 1998). 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 dry pressed distilled rose petals/kg/d to lambs’ feed did not have a significant effect on the total feed consumption, average daily gain and feed conversion ratio (Stancheva et al., 2020). The explanation of those finding were likely due to the fact that these amounts of dry pressed distilled rose petals 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).

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 dry pressed distilled rose petals characterizes with significant amount of polyphenols and high antioxidant activity. In dry rose petals are identified 30 polyphenol antioxidant components including six new glycosides of gallic acid except glycosides of kaempferol and quercetin.

The dry pressed distilled rose petals in the investigated concentrations showed limited potential for application as feed supplements in small ruminants and poultry husbandry. On contrary supplementation of feed with 545 mg dry pressed distilled rose petals /kg/d improved Danube white pig’s performance and can be successfully used in pig’s husbandry.

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