

Chemical composition, *in vitro* gas production and relative feed value of rose flower wastes (*Rosa damascena* Mill.) from conventional and organic production

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Abstract

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The oil-bearing rose is one of the main essential oil crops grown in Bulgaria. During the processing of rose flowers rose oil, rose concrete, rose absolute, and rose water are obtained. Waste rose flowers after the distillation of the essential oil still have a limited usage. The objective of the present study is to determine the chemical composition, *in vitro* gas production, digestibility and relative feed value of waste flowers of *Rosa damascena* Mill. and their suitability for use as forage. The rose flowers were collected from six fields located in the Kazanlak valley, Southern Bulgaria: three of them are with organic rose production and three are conventional. After their distillation the chemical composition of rose wastes was determined: crude protein, crude fat, crude fibre, ash and nitrogen-free extracts (NFE). Comparative analysis of the chemical composition, structural fibre components: neutral detergent fibre (NDF) and acid detergent fibre (ADF), *in vitro* gas production and relative feed value (RFV) of rose wastes was made under the conditions of the two production systems.

Keywords: *Rosa damascena* Mill.; rose wastes; chemical composition; detergent fibre; *in vitro* gas production; relative feed value

Introduction

The industrial cultivation of the oil-bearing rose in Bulgaria includes predominantly the species *Rosa damascena* Mill. due to the high content and quality of rose oil. The main products extracted from the rose flowers are rose oil, rose concrete, rose absolute and rose water. A small amount is used as a raw material in the food industry – syrup, jam, etc. The main consumers of rose oil and concrete are the large perfumery, cosmetics and pharmaceutical companies (Kovacheva et al., 2010). It is known that extracts of *R. damascena* as well as rose water contribute to the proper

functioning of the digestive system (Gholamhoseinian et al., 2008). It is therefore not surprising that in addition to food products containing rose flowers, food supplements with beneficial effect on the digestive system are produced and marketed to control tension, stress and discomfort.

R. damascena flowers contain volatile components (basically terpenes), phenolic compounds: tannins, anthocyanidins, flavonoids (kaempferol and quercetin) and their glycosides, polysaccharides, organic acids, vitamin C, minerals – all of which are valuable for perfumery, cosmetics and pharmacy (Akram et al., 2020; Mileva et al., 2021). Vijayanchali (2017) reported that rose petal powder has high quality phytocompo-

nents and antioxidant activity, along with nutritional capacity of 70.4% carbohydrates, 0.5% protein, 0.2% fat, 1.2% crude fiber, 0.0002% vitamin C, 0.0037% iron and 0.12% calcium. After extraction of the essential oil, the phytocomponents remain in the waste flowers in the same quantities and can be used as a material for further processing or direct application (Schieber et al., 2005; Slavov et al., 2017a).

The wastes after distillation of the rose flowers can have various uses. Various approaches are proposed for the valorisation of rose waste in order to utilize the valuable biomass (Slavov et al., 2017b). They become a valuable organic fertilizer after their joint composting only with manure or with manure and straw (Onursal & Ekinci, 2015). There are attempts to use rose waste for biogas production by mixing it with manure at different proportions and temperatures. The results show that treatment with 20% fertilizer at 45°C gives a maximum cumulative gas production of 0.4268 m³/ (kg VS), containing 72% methane at the same time (Doaguei et al., 2011). Another study investigated the anaerobic degradation of residues from the rose oil industry using a fully mixed laboratory-scale reactor. The hydrolyzed rose residue produces slightly more methane than the untreated one (Tosun et al., 2004).

A study by Slavov et al. (2016) showed that chamomile, calendula and rose waste are a promising source of pectin. They can also be used as flour for inclusion in heat-treated foods to enrich them with dietary fibre. The content of protein and ash in the pectin extract of rose is the highest, 12.00 ± 1.3% and 1.09 ± 0.2%, respectively. Treatment of waste rose flowers with 50%, 70% and 95% ethanol yields extracts rich in polyphenols and could be used as antioxidant additives. The protein and ash content in them is from 1.65 ± 0.2 to 1.84 ± 0.6% and from 2.51 ± 0.5 to 2.67 ± 0.9%, respectively (Slavov et al., 2017a).

Waste materials from distilled rose flowers can be successfully used as a supplement to functional foods (Vasileva et al., 2019). They have an enormous potential since they have good nutritional value and a beneficial effect on animal health (Singh & Gaikwad, 2020).

According to Windisch et al. (2008) phytogetic feed supplements can increase total feed consumption, stimulate growth and improve the digestive enzyme activity and reduce intestinal pathogenic pressure in swine and poultry. This is explained by the antioxidant and antimicrobial actions of phytogetic compounds, which have a beneficial effect on animal health.

According to Sabino et al. (2018), the food supplement with essential oils can be used as a new strategy to improve animal health. Research on the use of natural sources of biologically active substances in farm animals has proven their

beneficial effect on their health status and productivity. Feeding lambs with dihydroquercetin from distilled roses increases the fat content of the carcass and the haemoglobin in their blood and at the same time lower blood sugar level has been registered (Stancheva et al., 2020).

In recent years, the scientific literature amounts in research related to the properties of *R. damascena* products and their huge potential for application in medicine, pharmacy and cosmetics (Mahboubi, 2016), but scientific information on the practical use of waste flowers is scarce.

It is known that the yield and composition of rose oil are strongly influenced by environmental conditions and the agricultural cultivation system (Mileva et al., 2021). Although there is some information on the nutritional value of rose waste, there are no data on the impact of conventional and organic production on the value of feed (such as fiber, crude protein, crude fat, extracts ash) and the release of gases from these products. All this provokes interest in our country to study the possibilities of using the residues of oil-bearing rose after distillation as a supplement to animal feed. The main questions we would like to find an answer to in the present study are: What is the relative feed value of the waste rose flowers? And is there a difference in the quality of marc obtained from rose flowers grown under conventional and organic production?

Material and Methods

The rose flowers were collected from 6 fields located in the Kazanlak valley, Southern Bulgaria in the period 2019-2020 (Figure 1). Three of the fields are with organic production and three are with conventional rose production. The waste flowers were obtained after microdistillation of the fresh raw materials, then dried and ground with a grinder.

The chemical composition of *R. damascena* waste flowers (g/kg dry matter (DM)) has been determined by the Weende method (AOAC, 2010) and includes the following indicators: crude protein – by Kjeldahl (ISO 5983-2:2009), crude fat – by Soxhlet (ISO 6492:1999), crude fibre (AOAC, 2007), ash (ISO 5984:2002) and nitrogen-free extracts (NFE) – calculated by the formula: 1000 – (crude protein + crude fat + crude fibre + ash).

The analysis of the neutral detergent fibre (NDF) and acid detergent fibre (ADF) has been carried out by Fiber Analyzer 2000, Ankom, USA. The analysis of digestibility has been carried out by Daisy Incubator, Ankom, USA. Neutral detergent fibre (NDF, %) has been determined by the method of Mertens et al. (2002). Acid detergent fibre (ADF, %) has been determined without specific sequence with respect to the neutral detergent fibre.

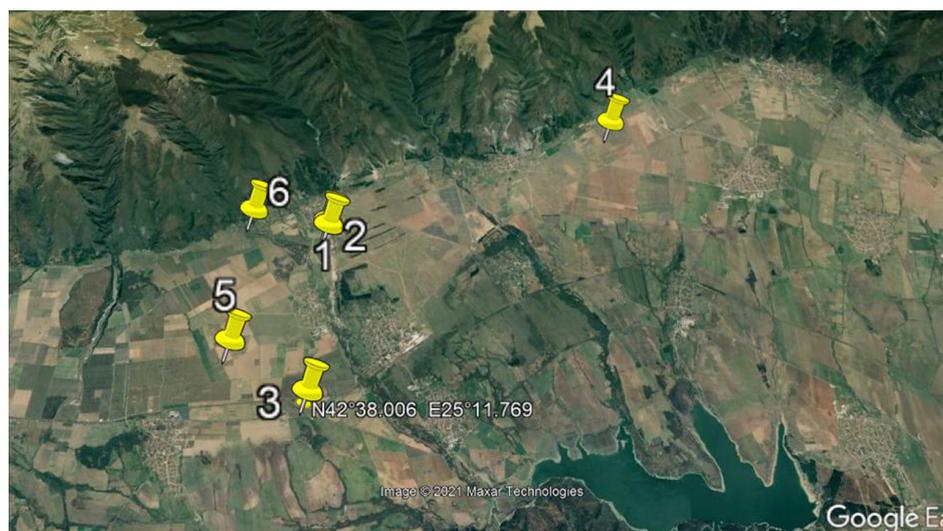


Figure 1. Distribution of fields with conventional and organic production: fields with conventional production: 1 – Damascena 1; 2 – Damascena 2; 3 – Koprinka; fields with organic production: 4 – Yasenovo; 5 – Asen; 6 – Skobelevo

Neutral detergent fibre digestibility (NDFD, %) has been determined by using („*in situ*“) analysis according to Spanghero et al. (2010), with incubation time 48 h. Each sample was weighed (0.45-0.50 g feed) in three replications by means of *Ankom F57 filter bags* (ANKOM Technology Corporation, Fairport, NY, USA). All samples have been incubated in 2 incubation times – 24 h and 48 h and analysed in Ankom 2000 (ANKOM Technology Corporation, Fairport, NY), according to Mertens et al. (2002).

The amount of the carbon emissions (CH₄ and CO₂) has been determined by a method for analysis with Gas Production System ANCOM RF/Ankom®, Tech. Co., Fairport, NY, USA.

The relative feed value (RFV) has been determined by Undersander & Moore (2002); Jeranyama & Garcia (2004); Stallings (2006); Boga et al. (2014) by the formula:

$$\text{RFV (relative feed value)} = (\% \text{ DDM} * \% \text{ DMI}) / 1.29.$$

$$\text{DDM} = \text{dry matter digestibility} = 88.9 - (0.779 * \% \text{ ADF}).$$

$$\text{DMI} = \text{dry matter intake (\% of live weight)} = 120 / (\% \text{ NDF}).$$

Results

Chemical composition of waste *R. damascena* flowers

In the dry matter of waste flowers from *R. damascena* Mill. the content of NFE is the highest (Table 1). In organic production, the established average values are 494.40 g/kg DM in the first year of the experiment and 474.06 g/kg DM in the following year. These results are higher than those obtained in conventional production – 469.58 g/kg DM and 443.65 g/kg DM, respectively. In both methods of production the values of NFE are higher in the first year.

The average values of crude protein are 155.65 g/kg DM in 2019 and 146.80 g/kg DM in 2020 for conventional and 150.44 g/kg DM and 135.53 g/kg DM for organic production, respectively (Table 1). In both methods of production, they were higher in the first year of the study. In conventional production, higher average values have been found in both years compared to organic production. The mean content of all variants was 147.11 g/kg DM.

Table 1. Chemical composition, g/kg DM; content of structural fiber components, %; gas production, dm, ml of waste flowers of *R. damascena*

Years	2019	2020	Average	2019	2020	Average	Average
Indicators	Conventional			Organic			
Crude protein, g/kg	155.65	146.80	151.23	150.44	135.53	142.98	147.11
Crude fat, g/kg	32.93	53.41	43.17	22.15	49.13	35.64	39.41
Crude fibre, g/kg	303.26	315.72	309.49	299.48	308.39	303.93	306.71
Ash, g/kg	38.58	40.41	39.49	33.53	32.89	33.21	36.35
NFE, g/kg	469.58	443.65	456.61	494.40	474.06	484.23	470.42
NDF, %	53.72	53.71	53.72	52.82	49.24	51.03	52.37
ADF, %	45.05	44.51	44.78	43.61	43.64	43.63	44.21
Gas Production, 24 dm, ml	105.7	161.53	133.62	105.84	131.47	118.66	126.14
Gas Production, 48 dm, ml	116.92	182.46	149.69	121.49	137.61	129.55	139.62

The mean crude fat content for both production methods was 39.41 g/kg DM (Table 1). The values were higher in the second year. Higher content was found in conventional production.

Crude fibre content in waste *R. damascena* flowers was high – 306.71 g/kg DM average for the study period (Table 1). The average values in conventional production (303.26 and 315.72 g/kg DM) were higher than those of the organic one (299.48 and 308.39 g/kg DM) for both experimental years. Slightly higher were the values in the second year in both types of production.

A similar tendency was observed for mineral substances (ash). The average values varied in slam interval – from 32.89 to 40.41 g/kg DM (Table 1). The mean content for all variants was 36.35 g/kg DM.

NDF, ADF, gas production

The analysis of fibre components showed that in waste *R. damascena* flowers the amount of neutral detergent fibre (NDF) was 52.37% on average (Table 1). The values in conventional production were higher, 53.72 % on average compared to the organic one – 51.03 %. In both ways of production the average values by years were similar. Acid detergent fibre (ADF) was 44.21% on average. The average values both by years and as a whole for the experiment were higher in conventional production by about 1.0%.

The amount of gas production after biomass incubation for 24 hours recorded by the *in vitro* gas production method was 126.14 dm, ml on average (Table 1). Greater amount of gas was formed during the second year in both types of production. In conventional production the amount was greater – 133.62 dm, ml, on average, while in the organic one it was 118.66 dm, ml.

The total amount of gas produced for 48 hours was 139.62 dm, ml on average. The greatest amount was produced in the second year in conventional production – 182.46 dm, ml on average. Greater were the gas production values in conventional production – 149.69 dm, ml on average against 129.55 dm, ml on organic one.

Digestibility, consumption and relative feed value

For waste *R. damascena* flowers mean total digestibility for all variants of 54.46% has been established, varying from 53.28% to 55.67% (Table 2). The greatest values obtained were in field 4, where ADF and NDF were the lowest. Gas production on hours 24 and 48 was also low. Greater digestibility has been found in organic production compared to conventional one, 54.92% on average. Field 1 from the conventional production has the lowest DM digestibility value – 53.28%. There the highest ADF, NDF, crude protein content was recorded, as well as the highest gas production values on hours 24 and 48.

Table 2. Dry matter digestibility, %, dry matter intake, g/kg and relative forage value of waste flowers of *R. damascena*

Indicators	1	2	3	Average conventional	4	5	6	Average organic	Average
DDM, %	53.28	53.86	54.90	54.01	55.67	54.94	54.13	54.92	54.46
DMI, g/kg	2.21	2.24	2.25	2.23	2.39	2.32	2.35	2.35	2.29
RFV	91.34	93.56	95.76	93.55	102.99	98.83	98.54	100.12	96.84

Table 3. Specific indices related to the approximate determination of gas production of waste flowers of *R. damascena*

Indicators	Conventional production				Organic production				Average
	1	2	3	Average conventional	4	5	6	Average organic	
NDF, %/ADF, %	1.19	1.19	1.22	1.20	1.18	1.19	1.15	1.17	1.18
GP 48 dm, ml/ 48 h	3.32	3.03	3.01	3.12	2.70	2.87	2.53	2.70	2.91
GP 48 dm, ml/ GP 24 dm, ml, %	113 %	115 %	109 %	112 %	109 %	110 %	109 %	109 %	111 %
GP 48 h dm, ml/ ADF %	3.48	3.23	3.31	3.34	3.04	3.15	2.72	2.97	3.16
GP 48 h dm, ml/ NDF %	2.94	2.71	2.71	2.79	2.58	2.66	2.38	2.54	2.66
GP 48 h dm, ml/ DDM, %	2.99	2.70	2.63	2.77	2.33	2.50	2.25	2.36	2.56
GP 48 h dm, ml/ RFV	1.74	1.55	1.51	1.60	1.26	1.39	1.23	1.29	1.44

The calculated waste *R. damascena* flowers consumption, g/kg live weight was 2.29 on average. It had higher values in organic production – 2.35 g/kg live weight on average, for field 4 it was the highest – 2.39 g/kg. In that variant the highest total digestibility was found.

The relative feed value of *R. damascena* was 96.84 on average. It was the lowest in field 1 – 91.34. It was higher in the organic production, 100.12 on average. That value calculated for the alfalfa biomass in flowering phase has been accepted to be 100 with 41 % ADF and 53% NDF content. The results obtained by us show that the relative feed value of waste *R. damascena* flowers in organic production is equal to that of alfalfa.

The NDF to ADF ratio was 1.18 on average (Table 3). That indicator has quite similar value in all fields studied.

Specific gas production in *R. damascena* was 2.91 dm, ml on average for 1 hour at 48-hour recording of the gas production incubation (Table 3). The gas production speed (the amount of gas produced ration on hour 48 to hour 24) was 111% on average. It was lower in organic production – 109% on average.

Gas production on hour 48 was 3.16 dm, ml/1% ADF; 2.66 dm, ml/1% NDF; 2.56 dm, ml/1% digestible dry matter and 1.44 dm, ml/unit if relative feed value.

The relations between chemical composition, gas production, ADF and NDF are presented through correlation dependencies (Table 4).

Discussion

The mean crude protein content in waste *R. damascena* flowers in the present study was 147.11 g/kg DM (Table 1). Iftikhar et al. (2009) found crude protein content of $0.21 \pm 0.05\%$ in waste *Rosa centifolia* biomass.

From data by Sood et al. (2006) *R. damascena* dry weight was significantly more than *R. bourboniana* till the beginning of opening the petals (beginning of flowering). That difference was

non-existent in full blossom. The percentage moisture content in *R. bourboniana* was higher than in *R. damascena* during all states of development with a maximum of 88.41% in full blossom. Protein content in *R. damascena* was almost permanent in all stages of flower development – between 120 and 180 mg gm^{-1} DW, while *R. bourboniana* had significantly higher protein content during the first stages before full opening of the petals. Considerably higher reducing sugar content has been found in *R. damascena* from opening the sepals to full blossom than in *R. bourboniana*. In both species starch content in petals was the highest in the middle stages of the blossom development being significantly higher in *R. bourboniana*.

When assessing the feed value, indicators related to the structure of the fibre components are used (content of acid and neutral detergent fibres, *in vitro* digestibility, amount of gas produced). These indicators allow the relative feed value to be calculated.

The comparison between the obtained data about fiber components, digestibility and metabolic energy of *R. damascena* (Table 4) and other grass feed shows that in terms of NDF and ADF content the waste from *R. damascena* is close to that of perennial cereal and legume grass feed (Todorov et al., 2007).

According to Naydenova et al. (2015) fiber components in cat grass mixtures (*Dactylis glomerata* L.) with legume forage grasses – bird's-foot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis* Adans.) and white clover (*Trifolium repens* L.), show values lower than those when cat grass is grown individually and higher than the ones in individually grown legume grasses. The results obtained by us about NDF and ADF in waste *R. damascena* flowers are the closest to those for cat grass. The best is the quality of feed in mixtures from cat grass with white clover. In new varieties of spring forage peas Naydenova & Todorova (2009) found high digestibility – 75.84 to 80.12%. The higher values found by us in the waste flowers in organic production of oil-bearing rose were from 54.13 to 55.67%.

Table 4. Correlation between chemical composition, gas production, ADF and NDF of waste flowers of *R. damascena*

	ADF, %	NDF, %	Gas 24 h, dm,ml	Gas 48 h, dm,ml	Crude protein, g/kg	Crude fat, g/kg	Crude fibre, g/kg	Ash, g/kg	NFE, g/kg
ADF, %	1.00								
NDF, %	0.50	1.00							
GP 24 h, dm,ml	0.11	0.01	1.00						
GP 48 h, dm,ml	0.14	0.15	0.95	1.00					
Crude protein, g/kg	0.56	0.74	-0.14	-0.08	1.00				
Crude fat, g/kg	0.03	-0.21	0.70	0.68	-0.32	1.00			
Crude fibre, g/kg	0.29	-0.01	0.31	0.37	-0.07	0.32	1.00		
Ash, g/kg	0.45	0.65	0.38	0.45	0.46	0.27	0.36	1.00	
NFE, g/kg	-0.52	-0.34	-0.53	-0.59	-0.36	-0.58	-0.74	-0.72	1.00

Close to our results were obtained by Yucel & Avci (2009) in vetch: ADF – 40.11%, NDF – 46.64%, digestibility – 57.58%, consumption – 2.58%. The values established by Ammar et al. (2010) of NDF, ADF and gas produced on hour 24 in oats, vetch, clover and mixtures with vetch and clover were lower than our results.

Georgieva et al. (2016) determined the characteristics for assessing the qualities of new varieties of wheat, triticale and rye. Crude protein content in the grain of some of the studied wheat varieties was 14%. The relative feed value and digestibility were highest for triticale grain, 118.35 and 68.42%, respectively. Waste rose flowers in our experiment are characterized by crude protein content as in wheat grain, but RFV and DDM are lower.

According to Dragoev et al. (2021), distilled *R. damascena* flowers show limited potential for use as food supplements in small ruminants and poultry, but have a positive effect in pigs.

Lower values for NDF, ADF and higher for digestibility was obtained by us for *R. damascena* compared to those of Kafilzadeh & Maleki (2012) for the straw from four varieties of chickpeas (*Cicer arietinum* L.).

The relative feed value of waste *R. damascena* flowers can be defined as good due to the values of the structural fibre components. In organic production, they are very close to those of alfalfa harvested in the flowering phase. This puts them in the group of good fodder.

For an approximate calculation of the amount of gas produced when feeding ruminants with bulky and concentrated feed, specific indices can be used, showing the quantitative characteristics of gas produced in relation to the composition of feed (NDF and ADF content, digestibility, energy value and relative feed value). This can lead to reduced gas emissions and environmental pollution.

The amount of gas produced at hour 48 is higher by 10.69% compared to hour 24. This confirms that waste *R. damascena* flowers have good nutritional value and are decomposed just like high quality feed. For roughage, this difference is high – over 40%, i.e. they degrade slowly at the beginning and rapidly at hour 48. The difficult initial decomposition of NDF and ADF is due to their higher content in those feeds.

The analysis of the relationship between chemical composition, gas production, ADF and NDF shows that there is a very good positive correlation between crude protein with NDF (Figure 2) and ADF; between crude fat with gas production at hours 24 (Figure 3) and 48; between ash with NDF; between NDF with ADF.

A negative correlation has been found between NFE and crude fiber (Figure 4), ash, crude fat, the amount of gas produced at hours 24 and 48 and ADF.

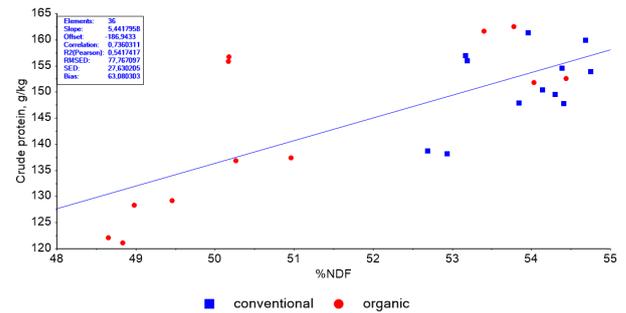


Figure 2. Positive correlation between crude protein and NDF

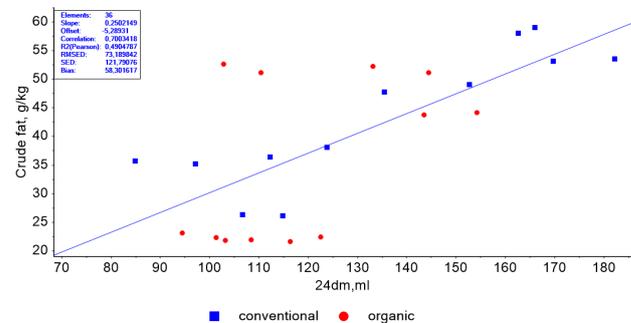


Figure 3. Positive correlation between crude fat and gas production at hours 24

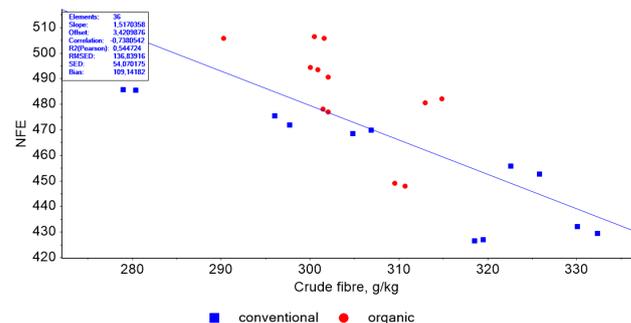


Figure 4. Negative correlation between NFE and crude fibre

Conclusions

In waste *R. damascena* flowers the average crude protein content is 147.11 g/kg DM, of crude fat – 39.41 g/kg DM, of crude fiber – 306.71 g/kg DM, of ash (mineral substances) – 36.35 g/kg DM and of NFE – 470.42 g/kg DM on average. In conventional production higher crude protein, crude fat, crude fiber and ash content have been found than in organic one.

Higher ADF, NDF and gas production at hours 24 and 48 values are typical for conventional production, and those for digestibility, consumption and relative feed value – for organic production.

The relative feed value of waste *R. damascena* flowers in organic production is 100.12 on average, i.e. 7% more than in the conventional one, being as close as possible to that of alfalfa in the flowering phase.

The high content of crude protein and the relative feed value of the waste rose flowers make them suitable for use as a feed supplement for ruminants.

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