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AROMA ACTIVE COMPOUNDS IN MILK FROM GOAT FED BASIL (*OCIMUM BASILICUM*)

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Abstract

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The study deals with the addition of basil (*Ocimum basilicum*) green tops into goat feeding ratio and with the effect of this addition on aroma active compounds in milk. The experiment comprised 8 goats crossbreed of White Goat and Brown Mountain Goat. These goats, which were on the second lactation, were divided into two groups – control (n = 4) and experimental (n = 4). The addition of 0.1 kg of basil green tops into goat feeding ration had no statistically significant effect on differences in total solid, fat or total nitrogen substances in milk during basil green tops feeding. However, the content of aroma active compounds (1-octanol, 1-undecanol, 2-nonanone), which were obtained by simultaneous distillation-extraction (SDE) and determined with usage GC-MS, reached statistically significant differences in days of basil green tops feeding. The amount of 1-octanol, 1-undecanol and 2-nonanone rose from 18.24 ± 0.019, 4.48 ± 0.019 and 5.37 ± 0.032 μl/100 ml of milk in days without basil green tops in feeding ration to 24.15 ± 10.398, 9.60 ± 0.118 and 23.29 ± 0.408 μl/100 ml of milk in the first day of basil green tops feeding when the increase was mostly the highest. In the next days of experiment the amount of 1-octanol, 1-undecanol and 2-nonanone was falling despite of readily goats' acceptation of basil green tops.

goat milk, simultaneous distillation-extraction (SDE), aroma active compounds, GC-MS, 1-octanol, 1-undecanol, 2-nonanone

Animal nutrition has a significant impact on the basic components of milk (Kováč *et al.*, 1989). Some authors confirm that the taste and flavor of the milk is directly affected by animal diet (Sehovic, 1988, 1991; Martin *et al.*, 1991; Ando *et al.*, 2001). The addition of herbs into feeding rations has a positive effect on fatty acids composition in milk (Collomb *et al.*, 2002) and positively affects rumen fermentation and digestibility (Ando *et al.*, 2003). Medicinal herbs in the feeding ration may even have a positive action against various diseases of animals, moreover, with no residual effect (Tipu *et al.*, 2006; Rochfort *et al.*, 2008; Gajula *et al.*, 2009). Some aroma active compounds could have a supportive impact for milk consumers such as 1-octanol which has a positive effect against essential tremor according to Bushara *et al.* (2004). A number of alcohols involved in taste and flavor of milk result from reduction of their aldehyde analogues (Burdock, 1997). Excluding alcohols also ketones create taste and flavor of milk (Wong, 1999; Berard *et al.*, 2007; Belitz *et al.*, 2009). From the perspective of species differences the qualitative representation of aroma active compounds in sheep, goat and cows´ milk is not different, however from a quantitative point of view the content of these constituents is higher in sheep and goat milk (Moio *et al.*, 1993; Friedrich and Acree, 1998; Park *et al.*, 2006). Sehovic (1988, 1991) mentions that grazing grassland rich in dicotyledonous plants, which occur mainly in the higher mountain areas, affecting flavor of cheese otherwise like grazing grasslands in lowland areas.

The aim of this study was to determine the effect of basil green tops addition into goats feeding ration on aroma active compounds in milk.

MATERIALS AND METHODS

Design of the feeding experiment

The experiment took a period of 15 days and comprised from goats (n = 8) crossbreed of White Goat and Brown Mountain Goat, which were on the second lactation. The groups were balanced in milk yield and lactation days. During the experiment no animal showed any disease, loss of appetite or selective choice of feeding.

Control group (n = 4) as well as experimental group (n = 4) were fed basic ration composed from 10 kg meadow grass, 0.5 kg oat meal and 0.03 kg NaCl. The experimental group has added 0.1 kg green tops of basil per head per day into basic ration during 4^{th} and 10^{th} days of experiment.

Both groups were housed in stable throughout the experiment.

Green tops of basil (*Ocimum basilicum*) were obtained from trade network of the Czech Republic. The dry matter of green tops of basil was determined according to Özcan *et al.* (2005). The samples of green tops of basil contained 15.01 ± 0.414 g/100 g of dry matter.

Sample analysis

The individual milk samples were collected. The milked samples from evening and morning milking were combined. Then the milk samples were homogenized and divided into two parts.

The first part of samples was lyophilized (–40 °C, 120 Pa, 48 hours; Alpha 1–4 LSC, Christ, Germany) for determination of total solid (ČSN ISO 6731), milk fat according to Soxhlet and nitrogen according Kjeldahl which was consequently converted to the total nitrogen substances according to Lynch *et al.* (2002).

The second part of milk samples was frozen (–20 °C) before aroma active compounds analysis. The samples were homogenized (35 °C, homogenizer IKA® RW 14 basic) prior to analysis. The aroma active compounds were extracted by simultaneous distillation-extraction (SDE) using Likens-Nickerson device involving a common condensation of volatile compounds from the studied material with solvent vapors (Chaintreau, 2001). Chaintreau (2001) recommended that as the most suitable organic solvent dichloromethane. Volatile substance soluble in organic solvent are continuously transferred from water vapor into the vapor of organic solvent and concentrated. The aroma active compounds were determined by GC-MS according to Mariaca and Bosset (1997). Alcohols (1-octanol, 1-undecanol) and ketone (2-nonanone) were found in samples of goat milk.

The samples of basil green tops were analyzed by the same method as milk samples and the results of aroma compounds in green tops of basil are shown in Table I.

Statistical methods

Results were analysed with Analysis of variance (ANOVA). The Paired test was performed according to Snedecor and Cochran (1967) using UNISTAT v. 5.5 software (Unistat, Ltd, London, Great Britain, 2003).

RESULTS AND DISCUSSION

The total solid of milk from the control group ranged 11.59 ± 0.109 g/100 g throughout the experiment. At the experimental group the total solid of milk was 11.85 ± 0.043 g/100 g during the first three days of experiment, then total solid of milk reached 11.78 ± 0.052 g/100 g in days of basil green tops feeding (from 4^{th} to 10^{th} day of experiment) and 12.64 ± 0.214 g/100 g from the 11^{th} day until the end of the experiment.

The concentration of fat in milk from control group ranged 2.94 ± 0.057 g/100 g throughout the experiment. Milk fat in milk from experimental group achieved 3.15 ± 0.059 g/100 g at the beginning of the experiment, slight increase 3.31 ± 0.048 g/100 g was observed in days of basil green tops feeding and in the end of the experiment milk fat rose to 3.79 ± 0.062 g/100 g.

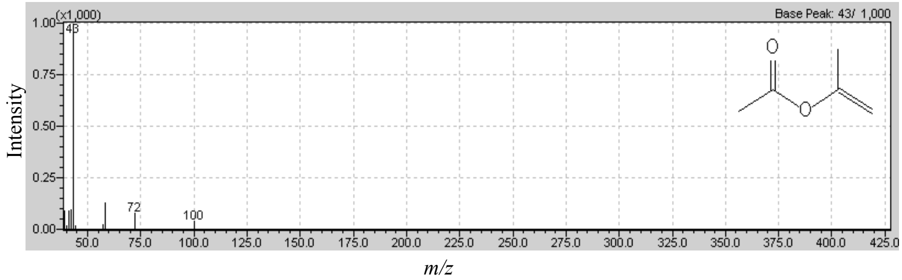
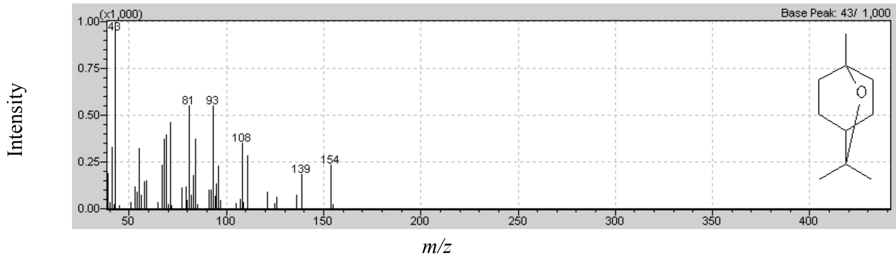
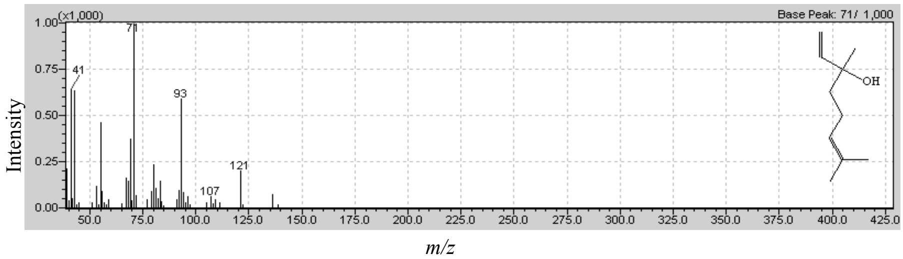
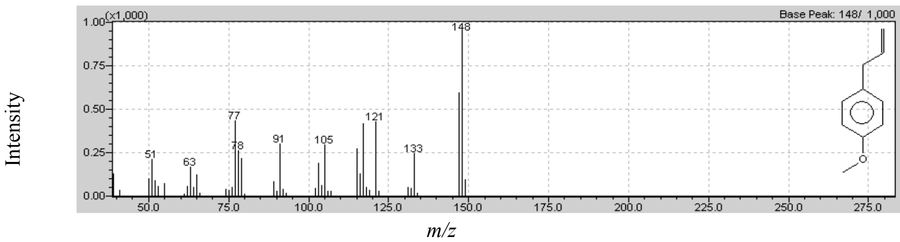
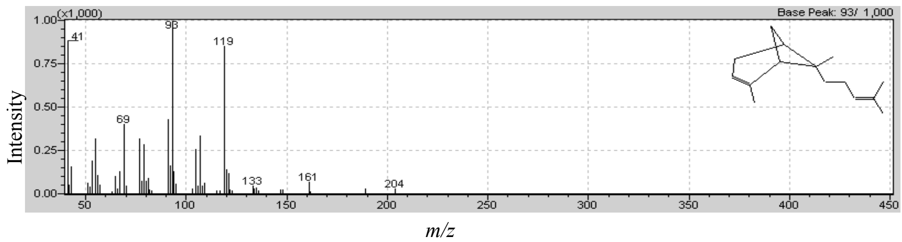
Milk from the control as well as experimental group contained the same amount of total nitrogen substances (2.82 ± 0.010 g/100g) throughout the experiment.

The addition of basil green tops had no statistically significant effect on differences in total milk solid, milk fat or total nitrogen substances in milk.

In green tops of basil were determined 1-propen-2-ol-acetate, eucalyptol (1, 8-cineole), linalool, estragol and α-bergamotene. The content, structure and mass spectrum of these compounds are described in Table I. Aroma active compounds that were observed in milk were not detected in basil green tops. This fact is related to the rumen metabolism (Broudiscou *et al.*, 2007). Linalool, the most contained aroma substance in basil green tops, is according to Malecky *et al.*, (2009) completely degraded in the rumen microbial ecosystem. According to the same study oxygenated monoterpenes as eucalyptol are much less degraded, but according to another study realized by Malecky and Broudiscou (2009) reducing of the culture redox potential by 50 mV contributes to degradation of eucalyptol and other monocyclic terpenes as well as linear terpenes.

Dynamic content changes of aroma active compounds in goat milk during the experiment are showed in Table II. In this Table we could see that milk from control group contained 18.24 ± 0.019 µl 1-octanol/100 ml of milk, 4.48 ± 0.019 µl 1-undecanol/100 ml of milk and 5.37 ± 0.032 µl 2-nonanone/100 ml of milk throughout the experiment (these values are graphically expressed on Fig. 1–3 where they are labeled by horizontal line which means 100% of content).

I: *Aroma active compounds determined in green tops of basil (Ocimum basilicum) (μl/100 g)*

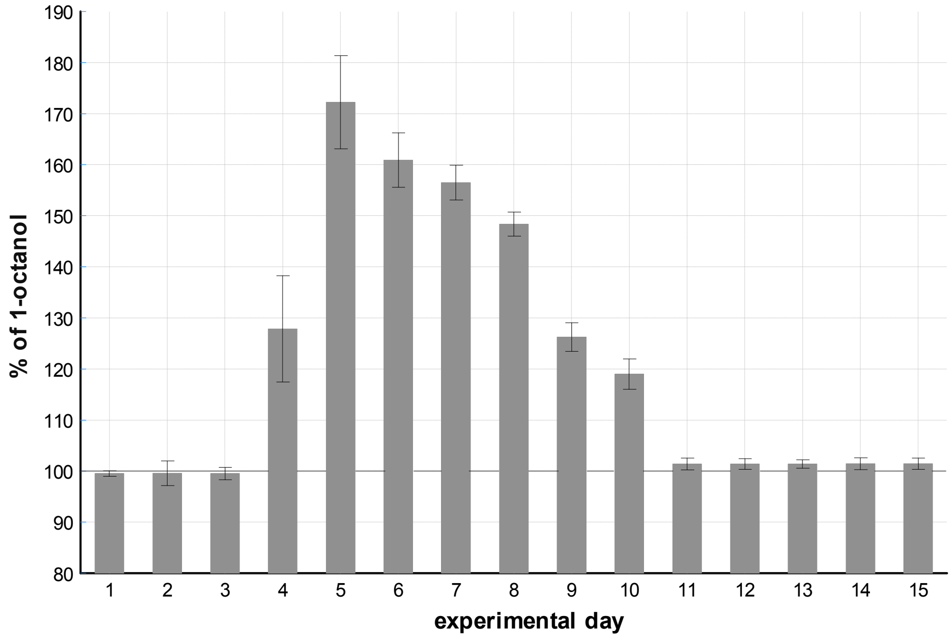
	amount mean ± S.E.	chemical structure and mass spectrum of the compound
1-propen-2-ol-acetate	163.15 ± 1.243	
eucalyptol (1, 8-cineole)	105.37 ± 1.459	
linalool	429.98 ± 1.999	
estragol	173.34 ± 1.453	
α-bergamotene	37.45 ± 1.280	

The changes in content of 1-octanol in milk from experimental group and their statistically significant differences are presented in Table II. If these values are expressed as a percentage increase compared with the control group (Fig. 1) then the comparison shows that the first day of the basil green tops feeding has amount of 1-octanol increased about 30% and the highest value of 1-octanol (about 70%)

II: *Dynamic content changes of aroma active compounds in goat milk by control (n = 4) and experimental (n = 4) group expressed in µl/100 ml of milk (mean ± S.E.)*

day	1-octanol		1-undecanol		2-nonanone	
	group					
	control	experimental	control	experimental	control	experimental
	mean ± S.E.	mean ± S.E.	mean ± S.E.	mean ± S.E.	mean ± S.E.	mean ± S.E.
1.						
2.		18.81 ± 0.136		5.33 ± 0.046		4.65 ± 0.071
3.						
4.		24.15 ± 10.398*		9.60 ± 0.118**		23.29 ± 0.408**
5.		32.53 ± 9.134**		9.02 ± 0.018**		19.53 ± 0.023**
6.		30.40 ± 5.334**		8.34 ± 0.256**		19.47 ± 0.718**
7.		29.56 ± 3.437**		7.50 ± 0.507**		15.05 ± 1.260**
8.	18.24±0.019	28.03 ± 2.386**	5.37 ± 0.032	7.18 ± 0.130*	4.48 ± 0.019	15.09 ± 0.081**
9.		23.85 ± 2.815*		7.05 ± 0.106*		13.73 ± 1.047**
10.		22.48 ± 2.982*		6.91 ± 0.100		12.79 ± 0.021**
11.						
12.						
13.		19.16 ± 0.067		5.53 ± 0.039		4.79 ± 0.277
14.						
15.						

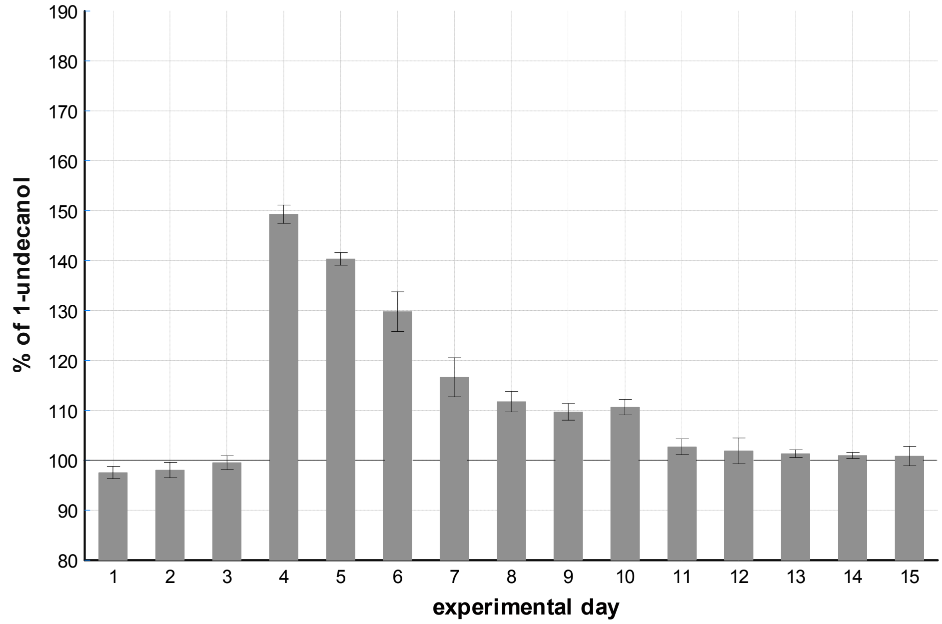
* … P ≤ 0.05
**… P ≤ 0.01



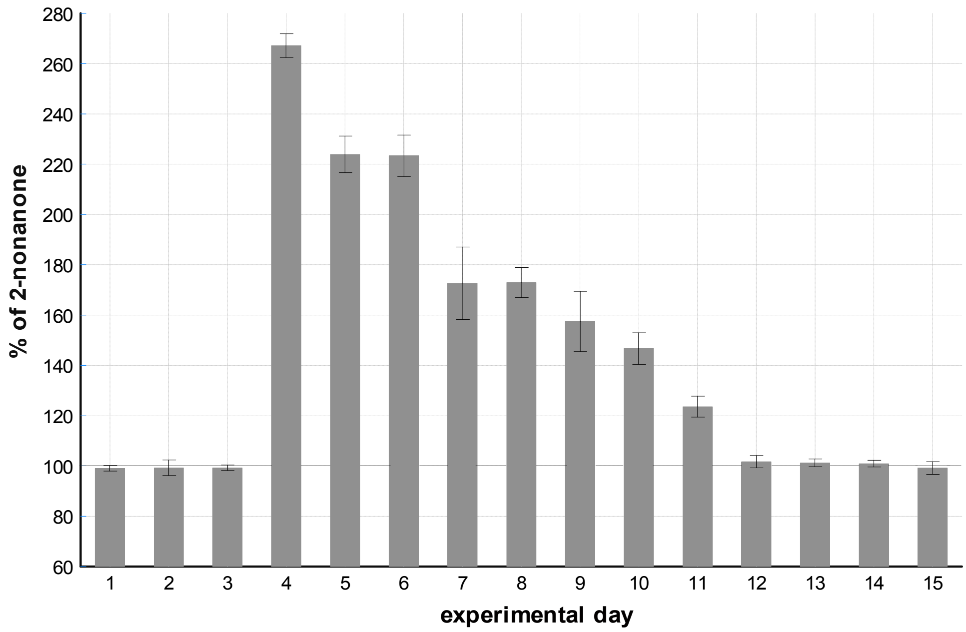
1: *Percentage increase of 1-octanol in goat milk of experimental group in comparison to control group (horizontal line on the value 100%)*

was reached on the second day of basil green tops feeding. From the 6th day of experiment the content of 1-octanol gradually decreased up to the 10th day when its value stabilized at current level.

The changes in content of 1-undecanol in milk from experimental group and their statistically significant differences presented in Table II. Its percentage increase against the control group is showed on Fig. 2. The highest amount of 1-undecanol was determined on the first day of basil green tops feeding (almost 50%), but the amount is already falling from the second day of basil green tops feeding.



2: *Percentage increase of 1-undecanol in goat milk of experimental group in comparison to control group (horizontal line on the value 100%)*



3: *Percentage increase of 2-nonanone in goat milk of experimental group in comparison to control group (horizontal line on the value 100%)*

The changes in content of 2-nonanone in milk from experimental group and their statistically significant differences presented in Table II. A high growth in the content of ketone 2-nonanone (Fig. 3) was observed by the first day of basil green tops feeding (almost 170%). The amount of 2-nonanone was gradually declined in the next days of experiment.

Initial concentrations of 1-octanol, 1-undecanol and 2-nonanone occured already on 13th day of experiment. The decrease of content of 1-octanol, 1-undecanol and 2-nonanone could be caused by gradual metabolic conversion of these compounds to acetic acid which serves like the major precursor of milk fat in the mammary gland (Kováč *et al.*, 1989; Kudrna *et al.*, 1998). Alternatively, they might be transformed into other volatile fatty acids constituting the fat in milk (Kováč *et al*., 1989; Kudrna *et al*., 1998). Increase in milk fat content of milk from the experimental group from the original value 3.15 ± 0.059 g/100 g to 3.31 ± 0.048 g/100 g by basil green tops feeding up to 3.79 ± 0.062 g/100 g in the end of experiment could also be related with this transformation of flavor compounds.

CONCLUSION

In conclusion, the addition of 0.1 kg of basil green tops had no statistically significant effect on differences in total solid, fat or total nitrogen substances in milk. The results of this study showed dynamic changes in concentration of 1-octanol, 1-undecanol and 2-nonanone. The statistically significant differences ($P \leq 0.05$, $P \leq 0.01$) were found from 4th to 12th days of experiment. Initial concentrations of 1-octanol, 1-undecanol and 2-nonanone occured already on 13th day of experiment.

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