

The Effects of Including Cinnamaldehyde or Carvacrol in Wethers' Diets on the Main Parameters of Rumen Metabolism

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Abstract

Essential oils are plants' secondary metabolites that have the potential to be valorized as feed additives. Literature background indicates that cinnamaldehyde (main component of the cinnamon oil) and carvacrol (present in oregano leaves) possess properties that might influence the rumen metabolism. In order to assess this potential, a 3x3 Latin square experimental design was organized using three wethers fed diets supplemented with cinnamaldehyde or carvacrol. The pH, ammonia concentrations and VFA concentrations (total and individual) of the rumen fluid were determined at the each of each experimental period. Supplementation with cinnamaldehyde and, in a lesser extent, with carvacrol significantly improved some of the parameters related to the rumen pH level. Slight decreases, not statistically significant, was observed in case of ammonia and total VFA concentrations, for both cinnamaldehyde- and carvacrol-supplemented diets. However, the cinnamaldehyde-supplemented diet significantly influenced the VFA profile, leading to a decrease of the butyric acid ($p=0.044$) and a decrease tendency for valeric acid ($p=0.067$). These changes confirmed the potential of the two essential oils to influence the rumen metabolism, especially in the case of cinnamaldehyde.

Keywords: carvacrol, cinnamaldehyde, essential oils, ruminal fluid

1. Introduction

Over the years, antibiotics were used in the livestock feeding due to their ability to improve animal performances and to reduce the risk of some animal's diseases developing [1]. Nowadays, the harmful effects of antibiotics use in animals feeding are well documented and it is known that, in certain conditions, their traces can be found in final animal products (eggs, meat or milk) thus contributing to the overall increase of the resistance of pathogens to antibiotics. As results, their use in animals feeding was banned in Europe [2]. In this context, the necessity of using alternative feed additives that can replace

antibiotics in animal diets and exhibits same activities is an important topic for animal products industry.

Essential oils are volatile secondary metabolites of plants, which can be obtained by steam distillation or by extraction with organic solvents and possess important properties such as antibacterial activity against a wide range of microorganisms. Therefore, they may be successfully used in animals' diets in order to improve their health and production performances. The inclusion of essential oils in ruminants' diet can improve some ruminal process such as inhibiting the protein degradation in rumen and may increase the supply of amino-acids in the small intestine [3, 4]. Also, the supplementation of ruminant's diet with essential oils can have beneficially effects on ruminal environment such as modulating the

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ruminal fermentations or improving the utilization of some nutrients [5].

Cinnamaldehyde is the main component of the cinnamon oil (accounting for 60 - 80% of its content) and possess antimicrobial activity that may have important implications in ruminal fermentation processes through the effects on rumen ecosystem. It was reported that it can reduce the concentration of total ammonia in the rumen and also can modulate the concentration of several volatile fatty acids, for example it can increase the propionate molar proportion in rumen [3, 4, 6].

Carvacrol is a phenolic monoterpene which possess antimicrobial activity, being one of the main components of the oregano leaves. It was reported that inclusion of carvacrol in combination with other active substances (i.e., thymol and eugenol) in ruminants' diet can improve the methane excreta and can modify the total bacterial population [7, 8-10].

However, the literature is still scarce in describing the effects of these two essentials oils, especially on the dynamics of rumen fermentations.

The aim of this study was to evaluate the effects of including high doses of cinnamaldehyde and carvacrol in ruminant diet on the main parameters that describe rumen metabolism.

2. Materials and methods

The feeding trial was performed in INCDBNA experimental farm, for 45 days, on three fistulated Merino wethers. In this experiment the effects of three diets were assessed: the control (C) diet, without essentials oils inclusion, the CIN diet, with an overall inclusion level of 4.5 g/kg dry matter (DM) cinnamaldehyde and CAR diet, with an overall inclusion level of 4.5 g/kg DM carvacrol.

The essential oils were included in the compound feeds, in order to ensure their 100% ingestion. The two essential oils were purchased from a local provider, which ensured 95% purity and 1.048 density for cinnamaldehyde and 99% purity and 0.975 density for carvacrol.

The effects of the three diets were assessed within a 3x3 Latin square experimental design. The diets were balanced and comparable from point of view of protein and energy supplies and consisted of a hay mixture (oat, vetch and alfalfa, 1:1:2) and a complementary compound feed based on corn, barley and sunflower meal. At the end of each of the three 15-days experimental periods, samples of rumen liquid were collected, two days consecutively, in dynamics, for a period of eight hours after the morning meal administration (at 0, 2, 4, 6 and 8 hours).

The pH of ruminal liquid was measured using a portable pH meter 330i WTW, priorly calibrated using two pH standard solutions: pH=4.01 and pH=7.00. Synthetic parameters describing the post-prandial ruminal pH evolution were estimated with a Visual Basic script in Excel, taking into account the following thresholds of biological significance in terms of ruminal acidosis pH=6.2, pH=6.0, pH=5.8, pH=5.5 [11]. The ammonia concentration in the ruminal liquid was measured using an ORION 710A+ ionometer, after a preliminary preparation that consisted in conditioning the electrode and obtaining a calibration curve with standard solutions (1700 ppm, 170 ppm, 17 ppm, 1.7 ppm ammonia). Determination of volatile fatty acids (VFA) concentrations in the ruminal liquid was performed using a Gas Chromatograph, set for 1.5 ml/min column flow, 30 ml/min. hydrogen flow and 300 ml/min. air flow. A 20.0 Hz acquisition frequency and 10 min. working time were used. Statistical analysis of the results was performed using Minitab® Statistical Software (version 16.0) by analysis of variance (ANOVA), followed by Tuckey post-hoc test. The differences between the three diets were considered significant for $P < 0.05$; for $0.05 < p < 0.1$ the differences were considered as trends.

3. Results and discussion

The intake of the main components of the three diets is presented Table 1.

Table 1. Consumption of diets (kg/head)

Parameter	C	CIN	CAR	SEM	P
Oat & vetch hay	0.633	0.626	0.627	0.005	0.671
Alfalfa hay	0.661	0.660	0.661	0.003	0.949
Compound feed	0.659 ^a	0.631 ^b	0.659 ^a	0.008	0.027

C-control diet, CIN-cinnamaldehyde diet, CAR - carvacrol diet, ^{a, b} Means within a row with no common superscript are significantly different (P<0.05).

The dry mater intake of the three diets (C, CIN and CAR) was 1676.51, 1638.58 and 1671.38 g/day, respectively.

This led to daily intake of essential oils of 0.75 g/head/day, approximately. The inclusion of essential oils had no noticeable effect on the total dry mater intake. Also, there were no significant changes in hays consumption. In case of compound feeds, the inclusion of cinnamaldehyde was associated with a significant decrease of the intake. This response of feed intake may be correlated with the high administration doses. In studies involving small ruminants it was reported that the administration of cinnamaldehyde at an inclusion level of 0.2 g/kg DM or 100-400 mg/kg DM [3, 12] did not influence the total dry mater intake. On the other hand, Yang et al., 2010

observed that supplementing the beef heifers' diets with increasing levels of cinnamaldehyde (400, 800 and 1600 mg/head/day) led to a response of DMI that was negatively corelated with the supplementing level [13].

The pH value of the ruminal liquid represents one of the most important rumen parameters, which can offer information about rumen fermentations' stability. A too low value of the ruminal pH impairs various rumen processes and may trigger rumen acidosis. The post-prandial evolution of rumen pH is presented in Figure 1. It can be observed that both experimental diets (CIN, CAR) led to higher pH values comparing to control but also that the pH curves were in the safe zone (above 6.2) from the point of view of rumen stability.

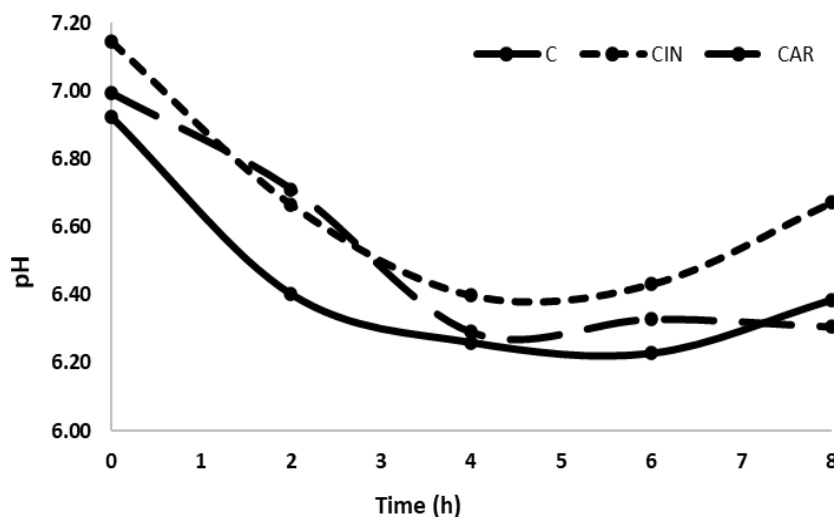


Figure 1. The pH values in C, CIN and CAR diets

This effect can be noticed also for the mean pH values (Table 2). Indeed, statistical analysis revealed a nearly significant influence of the essential oils inclusion on the average pH value

(p=0.053). The post-hoc test (Tuckey) revealed a significant difference between CIN and C diet (+0.223 pH units) but the other two-by-two differences were not statically significant.

Table 2. pH, ammonia and VFA obtained in CIN and CAR diets.

Parameter	C	CIN	CAR	SEM	P diet
pH	6.440 ^b	6.663 ^a	6.526 ^{ab}	0.064	0.053
NH3 (ppm)	358	328.4	341.8	25.35	0.712
Total VFA (mM/L)	148.3	135.3	143.3	4.869	0.171

C-control diet, CIN=cinnamaldehyde diet, CAR=carvacrol diet, a, b Means within a row with no common superscript differ (p<0.05).

It is known that average pH alone does not offer enough information from the point of view of rumen stability [14]; moreover, it can hinder relevant details. From Table 3 it can be observed

that although the pH averages corresponding to the three diets were close to each other, the minimum pH was significantly lower and below the thresholds of pH=6.2 and pH=6.0 in case of CAR diet comparing to CIN diet.

Table 3. Synthetic parameters of ruminal pH in CIN and CAR diets

Parameter	M	CIN	CAR	SEM	P diet
Min.	6.147 ^b	6.381 ^a	5.988 ^b	0.049	0.001
Max.	6.947	7.146	7.017	0.094	0.351
d<5.5	-	-	-	-	-
i<5.5	-	-	-	-	-
d<5.8	0.063	0.000	0.107	0.041	0.230
i<5.8	0.001 ^{ab}	0.000 ^b	0.005 ^a	0.001	0.029
d<6	0.910	0.028	0.318	0.283	0.125
i<6	0.086	0.000	0.048	0.040	0.350
d<6.2	2.355	0.552	1.468	0.555	0.116
i<6.2	0.403	0.061	0.171	0.092	0.062
a.u.c	51.09 ^b	52.81 ^a	51.96 ^{ab}	0.420	0.044

C-control diet, CIN=cinnamaldehyde diet, CAR - carvacrol diet; d=duration of pH decreases below the threshold (hours); i=intensity of pH decreases below the threshold (hours x pH units); a.u.c=total area under the pH curve representing the 8 hours post-prandial evolution of pH; ^{a, b}Means within a row with no common superscript differ (p<0.05).

Also, the duration and intensity of the pH decrease below these thresholds but also below pH=5.8 were more important in case of CAR comparing to CIN; however, statistical significance was detected in case of 5.8 threshold only.

The duration and intensity of pH decrease (parameters that synthesize the pH dynamics) were also more important in case of control group, comparing to both CIN and CAR diets, the most important differences being noticed in case of 6.0 or 6.2 thresholds.

These differences concerning the effects of the essential oils on pH dynamics can be important from the point of view of feeding conditions that can lead to ruminal acidosis and to the impairment of rumen processes such as cellulolytic activity, protein degradation and microbial protein synthesis.

Overall, the results showed a higher potential of

CIN diet, comparing with CAR and C diets, to counteract the acidogenic factors at rumen level. Such potential is less clear in case of CAR diet comparing to C diet.

The ammonia concentration in rumen (Table 2) was not significantly influenced by the inclusion of the studied essential oils (P=0.712). Therefore, the 9% and 5% decreases recorded in case of CIN and CAR diets, respectively, were not detected as being significant. However, changes of such magnitude may be important as the ammonia level in rumen is related to both protein degradability and microbial protein synthesis.

The effects of the experimental diets on the total VFA concentrations were also not significant (Table 2). The difference between CIN and C diets is noticeable (-9%), with potential implication on the diet energy supply as the VFA are the main source of dietary energy. Also, the level of VFA

can influence the milk and meat production [15]. However, this difference was not detected as being significant.

In literature there are only few studies that describe the effects of cinnamaldehyde and carvacrol on rumen fermentation process. In case of cinnamaldehyde it was reported that response of several ruminal parameters is related to the dose. It was observed that a CIN dose of 1g/head/day did not influence any ruminal parameters (such as pH level, ammonia concentration and total volatile fatty acids). In contrast, a higher dose of CIN (312 mg/L) led to a significant decrease of total VFA [5, 12, 13]. Still, this dose was lower than the level we have used in our study.

In case of carvacrol, it was reported that supplementation has increased the concentration of total VFA (and consequently reduced the pH level in ruminal liquid), suggesting that addition of carvacrol in ruminants' diet can lead to a higher rumen fermentability [12].

The concentrations of individual volatile fatty acids in ruminal fluid are important because their sum is correlated with the dietary energy supply, but also their relative proportions offer indications of rumen fermentation patterns and changes of the densities and activity of the rumen microbial populations.

As it is shown in the Table 4, there were no significant effects of the studied essential oils on acetate or propionate content.

Table 4. The main VFA (mM/L) profile of ruminal fluid in CIN and CAR diets

Parameter	C	CIN	CAR	SEM	P diet
Acetate	95.59	87.96	92.6	3.071	0.215
Propionate	28.11	25.19	26.48	3.071	0.243
Butyrate	18.57 ^a	16.35 ^b	18.13 ^{ab}	0.652	0.044
C2:C3	3.472	3.575	3.592	0.079	0.511

C-control diet, CIN=cinnamaldehyde diet, CAR=carvacrol diet, ^{a, b}Means within a row with no common superscript differ (p<0.05).

On the other hand, the concentration of butyrate was significant decreased in case of the CIN diet (-12%), comparing with the C diet (p=0.044). Other important parameter describing the ruminal fermentation is the ratio between acetate and propionate. The inclusion of CIN and CAR in wethers' diet had no significant influence on C2:C3 ratio. This ratio was high for all three diets, correlated with the high proportion of hays in diet, leading to high proportions of fibers and safe levels of pH.

An *in vitro* study using ruminal liquid collected from heifers reported that cinnamaldehyde inclusion led to higher concentrations of butyrate and propionate and lower concentration of acetate comparing to the control diet. On the other hand, it is obvious that effects on VFA profile are related to the dose of the essential oils. At a lower dose (31.2 mg/L) of CIN it was reported that proportions of propionate and acetate were increased. At a higher dose of CIN (312 mg/L) the supplementation also influenced the butyrate

concentration [6]. Also, there are other parameters that can interact with the effects of CIN on VFA profile, such as the pH level. In a study that used batch culture incubations it was reported that, at a neutral level of pH (7.00), the addition of CIN has influenced the acetate: propionate ratio, by increasing its value. At an acidic pH (5.5), the addition of CIN was associated, on the contrary, with a decrease of the acetate: propionate ratio [16].

In case of carvacrol, previous studies reported that its inclusion in ruminants' diets did not significantly changed the molar proportions of acetate, propionate and butyrate [12], which is consisted with data obtained in our study.

The effects on the minor VFAs concentrations in the rumen fluid are presented in Table 5. With exception of valerate concentration, which has expressed a decrease tendency in case of CIN supplementation (-0.395 units, p=0.067), the experimental diets did not change the profile of the minor VFAs.

Table 5. Minor VFA (mM/L) in C, CIN and CAR diets

Parameter	C	CIN	CAR	SEM	P diet
Isobutyrate	1.603	1.622	1.634	0.0726	0.953
Isovalerate	1.667	1.806	1.808	0.1118	0.593
Valerate	1.853	1.485	1.678	0.1101	0.067
Isocaproate	0.145	0.282	0.338	0.0948	0.338
Caproate	0.763	0.627	0.615	0.0727	0.286
Heptanoate	0.018	0.016	0.018	0.0108	0.992

C=control diet, CIN=cinnamaldehyde diet, CAR=carvacrol diet

It is well known that the concentration of valerate is an important parameter for ruminal fermentation processes, considering that valerate is used for synthesis of the long chain fatty acids and can be involved in protein synthesis process [17, 18]

There are very few studies in literature reporting effects of CIN and CAR on minor VFA's. Other authors found a general lack of effects, also in case of valerate, but they have used a much lower dose of cinnamaldehyde: 0.2 g/kg DM [12].

4. Conclusions

The supplementation of wethers' diets with cinnamaldehyde or carvacrol, at an overall dose of 4.5 g/kg DMI led to an improvement of the parameters describing the rumen pH level and dynamics, especially in the case of cinnamaldehyde. Although the pH was generally at a safe level, the improvement suggests that inclusion of these essential oils may induce changes that counteract the dietary acidogenic factors.

The supplementation led to slight decreases of ammonia and total VFA's concentrations which were not detected as being significant ($p=0.712$ and 0.171 , respectively). The two essential oils had no effects on acetate and propionate concentrations. However, it was associated with significant decrease of the butyrate content ($p=0.044$), especially in the case of cinnamaldehyde (-12%). The effects on the minor VFA's profile were not significant, with the exception of valerate, which tended to decrease.

Although the current study did not cover other rumen data that can substantiate the results, such as the changes of the rumen ecosystem, it can be concluded that cinnamaldehyde and carvacrol supplementation of wethers' diet didn't have noticeable adverse effects on ruminal metabolism,

but have expressed the potential to influence some rumen parameters that are relevant for applying feeding strategies based on the use of these essential oils as feed additives.

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