Effect of dietary supplementation with treated amaranth seeds on fermentation parameters in an artificial rumen

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ABSTRACT: The rumen simulating technique (RUSITEC) was used to evaluate the effect of dietary inclusion of amaranth seeds on the rumen fermentation and the concentration of fatty acids (FA) in fermentation fluid. Four fermentation vessels of the RUSITEC were used. The control diet (C) consisted of 70% meadow hay and 30% barley meal, whereas 10% of barley meal was replaced by milled (A_{mill}), mechanically ground (A_{gr}), and ground after heating in a microwave amaranth seeds ($A_{heat+gr}$) in other experimental vessels, respectively. All diets were isonitrogenous (11% of crude protein). With degradability of dry matter (DM) and acid-detergent fibre (ADF) not affected ($P > 0.05$), degradability of neutral detergent fibre (NDF) was significantly decreased by the inclusion of A_{mill} ($P < 0.001$) and $A_{heat+gr}$ ($P < 0.05$). Heating, compared to milling, decreased degradation of crude protein (CP) and addition of amaranth seeds generally increased production of microbial nitrogen. Production of fermentation gasses, methane, and total volatile fatty acids (VFA) was not affected by changes in composition of diets. Addition of amaranth seeds in a milled form (A_{mill}) caused a significant decrease in concentrations of acetate and propionate and growth in concentrations of butyrate, iso-valerate, and caproate. No effect ($P > 0.05$), compared to C, was found on the percentage of saturated or unsaturated FA, but changes in concentrations of some FA were observed. An absence of any detrimental effects on ruminal fermentation patterns indicated that grain amaranth seeds can partially substitute for the barley in ruminant nutrition.

Keywords: pseudocereals; fatty acids; *in vitro*; Rusitec

The modification of ruminant diets to improve health characteristics of meat and milk products for human consumption has received a considerable attention in animal nutrition. This includes a search for alternative additives and their influence on animal health.

In animals fed high-grain diets, the feeding of barley has been linked to an increased incidence of digestive disorders (Ørskov, 1986; Givens et al., 1993). It is assumed that the rapid rate of fermentation of barley grain starch, relatively to that in corn, predisposes the animal to these health problems. The optimal rate of starch fermentation by ruminal bacteria for maximal efficiency is influenced by the ruminal environment, including availability of nitrogen substrates necessary for microbial growth. Animals fed barley-based diets may compensate for the apparent inefficiency of ruminal starch degradation, through greater microbial protein synthesis. Boss and Bowman (1996) and Surber and Bowman (1998) reported a higher microbial synthesis when barley replaced corn in the diet.

Amaranth (*Amaranthus* sp.) is an old arable crop from Central America used as vegetable, grain, and for decorative purposes. The potential of both grain and vegetable amaranth as a food

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source for both humans and animals has been reviewed extensively by Haas and Kauffman (1984) and Saunders and Becker (1984). Grain amaranth (*A. hypochondriacus*) has been used as a dietary component for rabbits (Reddy and Reddy, 1993), rats (Fadel et al., 1996), and broiler chickens (Ravindran et al., 1996), green, ensiled and pelleted amaranth for cattle (Škultéty et al., 1991), and amaranth straw for lambs (Sanchez et al., 1988). The crude protein (CP) content of grain amaranth ranges from 12.5 to 17.6% on a dry matter (DM) basis, higher than found in most common grains. Grain amaranth protein contains around 5% lysine and 4.4% sulphur amino acids (AA), which are the limiting AA in other grains (Senft, 1980). In a comparision of the AA composition and biological value of its protein to egg protein, the essential AA index of popped and raw grain amaranth was found to be 84.5 and 90.4%, respectively (Písaříková et al., 2005). Grain amaranth also contains a relatively high proportion of fat (6–8%) (Carlsson, 1979), with a high level of unsaturation (about 75%). Linoleic acid (C18:2 n-6) was found to be the dominant (almost 50%) fatty acid (FA) in grain amaranth (Becker et al., 1981).

The objective of the present study was to determine the effect of dietary substitution of 10% of barley meal with amaranth seeds, processed by milling, grinding, and grinding with heat-treatment, on ruminal *in vitro* fermentation and FA profile.

MATERIAL AND METHODS

In vitro fermentation system

Incubation was performed by the rumen simulation technique (RUSITEC), with a unit consisting of four vessels with a nominal volume of 850 ml each (Czerkawski and Breckenridge, 1977). The control diet (C) included 70% of cutted meadow hay (1–1.2 cm long) and 30% of barley meal (ground through 1-mm screen sieve) on a DM basis. In other vessels, 10% of barley meal was replaced by treated amaranth (*Amaranthus hypochondriacus*, variety Koniz) seeds. So, experimental diets included 70% of cutted meadow hay, 20% of barley meal, and 10% of treated amaranth seeds on a DM basis. Seeds were milled (homogeneous mixture of seeds, ground through 1-mm screen sieve, A_{mill}), mechanically ground to all seed coat disruption (A_{gr}) or heated in a microwave (450 kW, 1 min) and after cooling mechanically ground ($A_{gr+heat}$). Each vessel had its own inflow of McDougall's solution of artificial saliva, supplemented with microelements (in mg/l: $ZnSO_4$ 1.92, $MnSO_4$ 1.02, $CoSO_4$ 0.06; pH 8.4) (McDougall, 1948). Defined amounts of urea were used to increase the CP contents of all diets to 11%.

Duration of the experimental period was 12 days, with the first 6 days used for equilibration and the last 6 days for sample collection. Ruminal fluid and inocula were obtained from 2 ruminally fistulated Slovak Merino sheep (mean body weight 42.1 ± 2.0 kg) fed on a diet consisting of 70% meadow hay and 30% barley meal. Fermentation inocula (solid and liquid) were collected through a ruminal cannula 1 h after the morning feeding and transferred to the laboratory under anaerobic conditions at 39°C. Each reaction vessel was filled with 450 ml of rumen fluid and 400 ml of artificial saliva. Compressed particulate rumen contents (100 g) were weighted into a nylon bag (pore size 100 μm), which was placed inside the feed container in each vessel together with a bag of test diet. After the first 24 h the bag with original solid inoculum was replaced with a bag containing the tested diet. Each bag with tested feed was incubated for a period of 48 h, resulting in two bags present in a vessel at all time periods. Dilution rates for solids and liquid were maintained at 0.03/h. Fermenters were purged with N_2 after manipulation to maintain anaerobic conditions and the temperature was maintained at 39°C. During sampling effluent collection flasks were cooled to 2°C in a refrigerated water bath to inhibit microbial growth and fermentation.

Sampling and laboratory analyses

Fluid was sampled on a daily basis from each vessel before replacement of bags and pH (Inolab Level 1, WTW, Weilheim, Germany) and members of microbes were determined immediately to control a quality of experimental media (values of pH on days 7–12 were statistically analyzed). Gas produced was collected daily in new rubber bags, and on days 7–12 analyzed for determination of total gas volume and methane (CH_4) concentration. The liquid effluent in flasks was also collected daily, and on days 7–12 analyzed for detection of volatile fatty acids (VFA), FA, and ammonia nitrogen (NH_3-N) production, respectively. The disappearance of DM after 48 h of incubation was calculated from the loss of weight after oven drying

at 55°C for 48 h, and residues were analyzed for NDF (Van Soest et al., 1991) (in the presence of sodium sulphite but without α-amylase treatment and is presented ash-free), ADF (AOAC 2005: method 973.18), CP (Kjeldahl method), ether extract (EE) and ash (AOAC, 2005). The VFA concentrations in effluent were determined by gas chromatography (Cottyn and Boucque, 1968) using Perkin-Elmer 8500 gas chromatograph (Perkin-Elmer, Waltham, USA), with crotonic acid as an internal standard. NH_3-N concentrations were measured by the microdiffusion method (Conway, 1962), gas volumes with a flow gasmeter, and CH_4 in a Perkin-Elmer 8500 gas chromatograph as described by Czerkawski and Clapperton (1968).

Lipid from diets and effluents was extracted and methylated according to the method of Folch et al. (1957). Fatty acid methyl ester profiles were determined by gas chromatography on a 6890N chromatograph with a G1315A autosampler (both Agilent Technologies, Inc., Santa Clara, USA). The 60 m × 0.25-mm × 0.25-μm i.d. fused silica capillary column (DB-23; Agilent Technologies, Inc., Santa Clara, USA) was used. Nitrogen was applied as carrier gas at 0.8 ml/min and as makeup gas at 30 ml/min. The temperatures of the injector and the flame ionization detector were 230 and 260°C, respectively. A timed-temperature program was used: (1) initial oven temperature 120°C with a hold for 6 min, (2) an increase by 15°C/min until reaching 170°C, (3) an increase by 3°C/min until reaching 210°C, followed by a hold for 13.5 min, (4) an increase by 40°C/min until reaching 230°C, with a final hold for 7 min. Total run time was 44 min. The split ratio was 1 : 1 for effluents and 1 : 40 for feeds, with 1 μl injected. Total run flow rates of air and hydrogen were 300 ml/min and 30 ml/min, respectively. Data were extracted with a GC ChemStation B.01.01 (Agilent Technologies, Inc., Santa Clara, USA).

The stoichiometric parameters of rumen fermentation (organic matter fermented (OMF) (Van Nevel and Demeyer, 1977), amount of microbial nitrogen (N_M), efficiency of microbial synthesis (EMS)) were calculated according to Alves de Oliveira et al. (1997).

Statistical analyses

Results were performed to One-Way Analyses of Variance (ANOVA) with type of diet as categorical variable. When differences among vessels were significant at the 0.05 level, Tukey's *post hoc* test was used to compare means among treatments. Normality of values was evaluated with the Shapiro-Wilk test (Statistical Analysis System, Version 8.1, 2000). Results in tables are presented as means and standard errors of the mean (SEM).

RESULTS AND DISCUSSION

The chemical composition of components and diets are introduced in Table 1. Amaranth seeds contained 190 g/kg (in DM basis) of CP, compared to 152 g/kg, included in a barley meal. Ether extract (EE) in amaranth seeds amounted to 658 g/kg (in DM basis), with palmitic acid (C16:0), oleic acid (cis-C18:1), and linoleic acid (C18:2 n-6) as the most abundant fatty acids (FA). With dietary CP contents ranging from 735 g/kg for the control diet (C) to 806 g/kg (in DM basis) for diets containing amaranth seeds (A-diets), 177.5 and 144.7 mg of urea per 1 l of McDougall's solution of artificial saliva were added to equalize the CP contents in the respective vessels to 11%. EE varied from 126 to 158 g/kg (in DM basis) among diets. The inclusion of amaranth seeds in diets lowered the amount of NDF from 626 to 569 g/kg in DM basis, whereas contents of ADF were constant at 318 (320) g/kg in DM basis, in all diets.

The effects of dietary inclusion of amaranth seeds on fermentation parameters are presented in Table 2. The finding that addition of 10% of amaranth seeds into diets did not ($P > 0.05$) influence degradability of DM is consistent with results obtained by Illg et al. (1994) with soybeans. The degradability of NDF was decreased by amaranth inclusion; A_{mill} ($P < 0.001$) and $A_{heat+gr}$ ($P < 0.05$) decreased degradability of NDF in comparison with C. Degradability of ADF was not influenced by addition of amaranth seeds. This is in contradiction with the finding of Jalč et al. (1999) that whole, untreated amaranth seeds substituted (5, 10, and 20%) for barley meal reduced ADF degradability. Morgan et al. (1991) and Yang et al. (2000) reported no differences in digestion of NDF and ADF in the rumen of cows when they fed processed barley (barley was steam-rolled to a coarse, medium, medium-flat or flat thickness). The degradability of CP was influenced by amaranth seeds ($P < 0.05$), but we did not determine differences between C and A-diets in the degradability of CP; only $A_{heat+gr}$ compared with A_{mill} ($P < 0.05$) decreased

Table 1. Chemical composition of components and diets

	Meadow hay	Barley meal	Amaranth seeds	Control diet	Experimental diet
DM (g/kg fresh weight)	931	882	920	909	906
NDF (g/kg DM)	624	434	512	626	569
ADF (g/kg DM)	398	270	84	320	318
CP (g/kg DM)	78	152	190	74	81
EE (g/kg DM)	–	22	66	13	16
Fatty acid composition (g/100 g FA)					
C14:0	–	–	0.2	0.6	0.5
C16:0	–	–	20.4	24.0	23.0
C18:0	–	–	3.9	2.4	3.0
Cis-C18:1	–	–	22.3	14.6	17.4
C18:2 n-6	–	–	48.0	44.4	45.4
C18:3 n-3	–	–	1.5	7.8	5.6
SFA	–	–	26.7	29.9	29.1
MUFA	–	–	23.8	17.0	19.2
PUFA	–	–	49.5	53.1	51.7

ADF = acidodetergent fibre, CP = crude protein, DM = dry matter, EE = ether extract, FA = fatty acid, MUFA = monounsaturated fatty acids, NDF = neutral detergent fibre, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids

the microbial degradation of CP. Heat treatment of feedstuffs decreased the degradation of DM and CP by blocking reactive sites for microbial proteolytic enzymes in the study of Broderick and Craig (1980) and increased the supply of dietary protein to the duodenum (Tagari et al., 1986). Amaranth seeds included into diets decreased the degradability of EE ($P < 0.001$), mainly the ground ones, compared with C (A_{gr} and $A_{heat+gr}$ $P < 0.001$) and A_{mill} (A_{gr} $P < 0.01$ and $A_{heat+gr}$ $P < 0.05$).

We observed the affection of metabolism of nitrogen by the addition of amaranth seeds into experimental diets (Table 2). In contrast to *in vitro* (Illg et al., 1994) and *in situ* (Stern et al., 1985) results obtained with soybeans, content of NH_3-N (mg/l) and N (mg/l) in effluent was not influenced by the addition of amaranth seeds into the diet. Several studies (McCarthy et al., 1989; Casper et al., 1990, 1999) reported lower ruminal ammonia nitrogen concentrations for cows fed barley-based diets than for animals fed corn-based diets. In contrast, Surber and Bowman (1998) reported higher ruminal NH_3-N concentrations for steers fed barley-based diet than for steers fed corn-based diet. These variable responses could be due to a number of factors, including grain variety, extent of processing of grain, and differences in quality of forage sources between diets for lactating cows and finishing steers. Although production of NH_3-N and N in the effluent was not influenced by dietary inclusion of amaranth seeds, production of N_M (mg/day) and EMS (mg/g) was increased in all vessels with added seeds, compared by C ($P < 0.001$). This is in contrast to *in vitro* results with the dietary inclusion of whole, untreated amaranth seeds obtained by Jalč et al. (1999), when the production of N_M was neither influenced by adding 20% of untreated amaranth seeds into the diet.

The mean pH of ruminal fluid (6.76) did not fluctuate to a noticeable extent during the course of the experiment, and was not influenced ($P > 0.05$) by dietary treatment. Furthermore, addition of amaranth to diets did not have an effect ($P > 0.05$) on production of the total gas (ml/day) or methan (CH_4). Reduction of the latter greenhouse gas has received considerable attention in animal nutrition due to its destruction of the stratospheric ozone layer. The addition of fat by feeding of canola or whole cottonseed did not reduce CH_4 production in dairy cattle (Johnson et al., 2002). However, reductions have been obtained by inclusion of coconut, canola, and cod liver oil (Dong et al., 1997), and C18:1, C18:2, and linolenic (C18:3) acids (Czerkawski et al., 1966) to ruminant diets.

Table 2. Effect of dietary amaranth seeds on fermentation parameters

	C	Amaranth seed treatments			SEM	*P*-value
		A_{mill}	A_{gr}	$A_{heat+gr}$		
pH	6.67	6.73	6.69	6.74	0.01	ns
Degradability (%)						
DM	56.8	54.5	53.0	51.8	0.9	ns
NDF	43.1^{b}	27.5^{a}	35.0^{ab}	33.7^{a}	1.6	< 0.01
ADF	29.2^{ab}	20.8^{a}	35.1^{a}	27.3^{ab}	1.6	< 0.01
CP	49.8^{ab}	55.2^{b}	52.2^{ab}	47.7^{a}	1.0	< 0.05
EE	34.9^{b}	27.7^{b}	13.2^{a}	14.5^{a}	2.3	< 0.001
Production of N in effluent						
NH_3-N (mg/l)	193.1	189.5	187.5	182.5	1.56	ns
N (mg/l)	221.3^{a}	255.0^{b}	236.7^{ab}	238.3^{ab}	4.4	< 0.05
N_M (mg/day)	51.6^{a}	80.9^{b}	82.9^{b}	97.5^{b}	4.4	< 0.001
EMS (mg/g)	16.1^{a}	26.6^{b}	27.4^{b}	32.5^{b}	1.6	< 0.001
Production of gasses						
Gas production (ml/day)	3578.3	3437.5	3384.2	3376.7	49.1	ns
CH_4 production (ml/day)	121.1	100.5	98.5	109.4	6.5	ns
Production of volatile fatty acids						
Total VFA (mmol/l)	55.3	50.2	52.9	49.8	0.9	ns
Acetate : propionate ratio	3.00^{b}	2.82^{a}	2.73^{a}	2.74^{a}	0.03	< 0.001
Acetate (mol %)	56.9^{b}	53.1^{a}	55.0^{ab}	55.2^{b}	0.4	< 0.001
Propionate (mol %)	19.1^{a}	18.9^{a}	20.1^{b}	20.1^{b}	0.2	< 0.01
Butyrate (mol %)	17.2^{a}	20.3^{b}	17.9^{a}	18.3^{a}	0.1	< 0.001

C = control diet, A_{mill} = milled amaranth seeds, A_{gr} = mechanically ground amaranth seeds, $A_{heat+gr}$ = amaranth seeds ground after heating in a microwave, ADF = acid detergent fibre, CH_4 = methane, CP = crude protein, DM = dry matter, EE = ether extract, EMS = efficiency of microbial synthesis, N = nitrogen, NDF = neutral detergent fibre, NH_3-N = ammonia nitrogen, N_M = microbial nitrogen, VFA = volatile fatty acids, ns = non significant ($P > 0.05$), SEM = standard error of the mean
$^{a, b}$values within a row with different superscript letters differ at $P < 0.05$

Amaranth seeds in the diet had no influence ($P > 0.05$) on the production of total volatile fatty acids (VFA, mmol/l) and decreased the acetate : propionate ratio ($P < 0.001$) (Table 2). This is in accordance with declining ratios obtained when soybeans (Michalet-Doreau et al., 1985) or soybean oil (Bateman and Jenkins, 1998) were added to ruminant diets. Moreover, addition of milled amaranth seeds (A_{mill}) decreased the concentrations of acetate (mol %) compared to C ($P < 0.001$) and $A_{heat+gr}$ ($P < 0.05$). The ground seeds (A_{gr} and $A_{heat+gr}$), compared to the milled ones, enhanced the molar percentage of propionate ($P < 0.05$). Yang et al. (2000) reported linear increase in total ruminal VFA concentrations with larger demand of hulls of barley used in the diets fed to cows. Feeding medium-flat rolled barley significantly reduced the molar percentage of acetate but increased the molar percentage of propionate, which resulted in a large decrease in the acetate : propionate ratio, compared to feeding coarsely or medium rolled barley. Furthermore, milling of amaranth (A_{mill}) increased the production of butyrate (mol %), compared to C ($P < 0.001$), A_{gr} ($P < 0.01$), and $A_{heat+gr}$ ($P < 0.01$), respectively. Also, Illg et al. (1994) and Abdelgadir et al. (1996) found lower concentrations of butyrate due to extrusion and roasting (heat treatment), respectively, of soybeans. Compared to other treatments, milling also increased ($P < 0.05$) the production of isovalerate and caproate (data were not mentioned in Table 2).

Table 3. Effect of dietary amaranth seeds on some fatty acids in the fermentation fluid

	C	Amaranth seed treatments			SEM	*P*-value
		A_{mill}	A_{gr}	$A_{heat+gr}$		
Fatty acids (g/100 g of total FA)						
C12:0	2.05[b]	0.51[a]	0.56[a]	0.58[a]	0.18	< 0.001
C14:0	6.8[b]	2.9[a]	3.6[a]	4.2[a]	0.4	< 0.001
C16:0	35.3	31.1	32.2	33.6	0.7	ns
C18:0	15.0	13.2	13.0	13.7	0.6	ns
C18:1-n9t	0.47	0.61	0.40	0.72	0.06	ns
C18:1-n11t	1.6	3.3	2.1	2.8	0.2	ns
C18:1-n9	17.4	20.2	21.4	18.0	0.9	ns
C18:2-n6	6.3	12.8	11.6	9.7	1.0	ns
C18:3-n3	0.81[a]	0.92[b]	1.46[b]	1.37[b]	0.14	< 0.001
C18:2 (c9,t11)	0.09[ab]	0.12[b]	0.06[a]	0.13[b]	0.01	< 0.001
C18:2 (t10,c12)	1.23	0.86	1.10	0.91	0.10	ns
SFA	66.7	55.8	56.4	60.3	1.7	ns
MUFA	22.6	28.1	27.9	25.9	0.8	ns
PUFA	10.7	16.1	15.7	13.8	1.0	ns
n-3/n-6	0.11[b]	0.07[a]	0.11[b]	0.12[b]	0.01	< 0.01

C = control diet, A_{mill} = milled amaranth seeds, A_{gr} = mechanically ground amaranth seeds, $A_{heat+gr}$ = amaranth seeds ground after heating in a microwave, FA = fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, ns = non significant ($P > 0.05$), SEM = standard error of the mean

[a, b]values within a row with different superscript letters differ at $P < 0.05$

Concentrations of individual fatty acids (g/100 g FA) are indicated in Table 3. The addition of 10% of amaranth seeds into the diet had no influence ($P > 0.05$) on the total amounts of saturated or unsaturated FA; only addition of milled seeds (A_{mill}, $P < 0.01$) lowered the ratio of n-3 and n-6 PUFA, in comparison to other treatments. Seeds of amaranth decreased amount of lauric (C12:0; $P < 0.01$) and miristic (C14:0; $P < 0.01$) acids in effluent, in comparison with C. Furthermore, they enhanced amount of linolenic acid (C18:3 n-3) in effluent (A_{mill}, $P < 0.05$; A_{gr} and $A_{heat+gr}$ $P < 0.001$, respectively, compared to C). A_{mill} and $A_{heat+gr}$ enhanced the production of c9,t11-CLA (conjugated linoleic acid; $P < 0.01$) and A_{gr} decreased the amount of this isomer in effluent ($P < 0.01$), compared to C.

CONCLUSION

The replacement (10%) of barley by amaranth seeds in ruminant diets had no effect on degradability of DM and ADF and lowered degradability of NDF. No changes were observed in values of pH and production of fermentation gasses and total volatile fatty acids. The milling of seeds resulted in a significant decrease in production of acetate and propionate and increase in production of butyrate, iso-valerate, and caproate. The addition of amaranth seeds into the diet enhanced the production of microbial protein in effluent. No changes were observed in concentrations of saturated or unsaturated FA. It can be concluded that the addition of 10% of treated amaranth seeds into ruminant diets caused no detrimental changes in *in vitro* fermentation parameters, and can be used as substitute for barley.

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