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Maca (*Lepidium meyenii*): In Vitro Evaluation of Rumen Fermentation and Oxidative Stress

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Abstract: The aim of this study was to investigate the chemical composition of three maca (*Lepidium meyenii* Walp.) ecotypes (yellow, black, and red) and their in vitro fermentation characteristics and antioxidant effects on cow rumen liquor. The three ecotypes were added to a total mixed ratio (TMR) in different doses (0, 150, and 300 mg/g) and incubated in vitro under anaerobic conditions for 120 h. Methane production was recorded after 24 h of incubation. Antioxidant status and degree of lipid peroxidation were also evaluated after 24 and 120 h of incubation with the fermentation liquor. An analysis of the chemical composition showed high concentrations of non-structural carbohydrates in all maca ecotypes, particularly in the yellow ecotypes. Moreover, despite an increase in gas production, it seems that the TMR supplemented with each maca ecotype, particularly at the highest dose, increases the amount of volatile fatty acids and reduces methane production. Finally, the addition of maca can induce an antioxidant effect. Our findings suggest that the three ecotypes of maca are rich in non-structural carbohydrates which affect the in vitro fermentation kinetics and reduce methane production.

Keywords: fermentability; methane production; volatile fatty acids; pro- and antioxidative status

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1. Introduction

In recent years, several studies have analysed the bioactive compounds of many plants traditionally considered beneficial for humans and animals [1–4]. In this regard, maca (*Lepidium meyenii* Walp.) is a plant belonging to the *Brassicaceae* family that is native to the Andes of Peru [5]. Maca root shows high nutritional value, including important fatty acids, and it is considered to be a medicinal plant due to the presence of several bioactive antioxidant compounds, such as polyphenols, macamides, macaridine, alkaloids, and glucosinolates [5,6], which are considered to be cholesterol-lowering, anti-inflammatory, and anti-cancer factors [3]. The three most important ecotypes of cultivated maca root differ depending on the colour of their hypocotyls: yellow, red, and black. These ecotypes show different biological activities depending on the type of cultivation and processing that they undergo and on the concentration of different bioactive metabolites. The yellow ecotype is the most widely used, and it is studied for its energetic properties, beneficial effects on reproductive function, and immunomodulatory properties [7–9]. Red maca is the sweetest variety and has the highest levels of phytochemicals among the various maca powders [10]. Black maca is the least common ecotype, accounting for about 15% of the annual harvest [10]. Scientific research has mainly used laboratory animals to investigate the antioxidant effects of maca, whereas few studies have been published in recent years on farm animals.

The research on laboratory animals includes work by Sahin et al. [11] who demonstrated that *Lepidium meyenii* root powder moderately improved feed intake and nutrient digestibility in rats. Considering livestock animals, Korkmaz et al. [12] observed that maca powder had neither positive nor adverse impacts on laying hens' performance, including egg quality, egg yolk cholesterol content, serum parameters, and hormones. Olgun et al. [13] studied the effects of different levels of maca powder added to the diet of Japanese growing quails and found that their body weight increased linearly with the addition of up to 1.0 g/kg of maca powder to the diet. Furthermore, the authors suggested that adding up to 2.0 g/kg of maca powder to growing quails' diets could improve performance, serum hormone concentrations, bone biomechanical traits, and ileum parameters. Similarly, the addition of maca to the basic diet of Japanese quail improved eggshell, ileum, and bone traits that deteriorate with age without affecting performance and reducing serum total cholesterol levels [14].

Furthermore, Staerfl et al. [15,16] studied the in vitro and in vivo effects of different extracts and plants, including maca hypocotyls. The authors observed in both studies a low and/or lack of effect of maca on methane production, probably due to the low dosage that was utilised. It was considered useful to investigate this aspect for the presence of compounds in the powder that can influence rumen fermentation, methane production, and oxidative stress. Oxidative stress is currently defined as "an imbalance between oxidants and antioxidants in favour of the oxidants, leading to the disruption of redox signalling and control and/or molecular damage" [16]. The assessment of the antioxidant barrier provides insight into the type of defenses the body adapts to achieve a balance between pro- and antioxidant species [17]. When pro-oxidant species have the upper hand, they can damage proteins, lipids, DNA, and carbohydrates, changing the organism's structure and functions [18,19].

The present study primarily aims to investigate the chemical and nutritional characteristics, in vitro rumen fermentation ability, and antioxidant activity of three different maca roots (yellow, black, and red) for possible use as a supplement in ruminant feeding plans. A secondary objective is to choose the most appropriate concentration to use. The hypothesis is that the three ecotypes influence the in vitro parameters differently.

2. Materials and Methods

2.1. Plant Collection

The yellow, red, and black ecotypes of maca roots (*Lepidium meyenii* Walp.) used in this research were harvested from the Junín district (Andean Highlands of Peru, 4100 m above sea level); Prof. Domenico Carotenuto conducted the taxonomic recognition of the plants. Maca roots were exposed to extreme temperature cycles, strong light conditions, and atmospheric pressures typical of a high-altitude environment (>3500 m) for two months, replicating traditional open field drying. After drying, hypocotyls were selected, washed, ground to obtain a flour with a particle size of 0.8 mm, and stored until used.

2.2. Chemical Composition

Samples of the three different ecotypes of maca roots were analysed to determine their chemical composition (dry matter, crude protein, ether extract, and ash) following the procedures reported by the AOAC [20] (ID number: 2001.12, 978.04, 920.39, and 930.05 for DM, CP, EE, and ash, respectively). Neutral detergent fibre, acid detergent fibre, and free ash acid detergent lignin were also determined as indicated by Van Soest et al. [21]. The non-structural carbohydrates (NSC) were calculated as follows:

$$NSC = 100 - (DM - CP - NDF - EE - ash)$$

where: DM is dry matter, CP is crude protein, NDF is neutral detergent fibre, and EE is ether extract.

2.3. In Vitro Fermentation

The three maca ecotypes were tested for in vitro fermentation ability according to the method proposed by Theodorou et al. [22]. For each variety, three levels of supplementation (none, low, and high) of maca powder were added to a total mixed ratio (TMR) commonly used for lactating ruminants. TMR contained brewers' grain, corn silage, corn mash feed, alfalfa hay–silage, complementary commercial feed, oat hay, and a vitamin and mineral supplement and had the following chemical composition (% on dry matter basis: CP 14.1, NDF 32.7, EE 4.57, ash 9.29). TMR with no maca added (CTR) and TMR supplemented with 150 and 300 mg/g of yellow, red, and black maca [23] were incubated (1.0059 ± 0.0026 g) in serum flasks with buffered buffalo rumen fluid at 39 °C under anaerobic conditions, replicated six times. The rumen liquors were collected at the slaughterhouse from three healthy animals according to EU legislation (EU Council, 2004). All procedures involving animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Protocol 2019/0013729 of 08/02/2019). After slaughter, the rumen fluids were placed inside pre-heated thermoses and transported within two hours to the Laboratory of Feed Evaluation (Department of Veterinary Medicine and Animal Production, University of Napoli Federico II). Here, the rumen fluids were pooled (200 mL/rumen fluid), mixed, strained through a cheese cloth, and added to the flask (10 mL). Furthermore, a buffered medium (buffer, macromineral, micromineral, and resazurin solutions) was added to the flasks (75 mL, 1:7.5 ratio). Subsequently, the reducing agent (4 mL) was added. During the 120 h of incubation, the gas produced was recorded 23 times (at intervals of 2–24 h) using a manual pressure transducer (Cole and Palmer Instrument Co., Vernon Hills, IL, USA). The cumulative volume of gas produced at 120 h of incubation was related to incubated organic matter (OMCV, mL/g). At the end of the incubation period, the pH of the fermentation liquor was analysed using a pH meter (ThermoOrion 720 A+, Fort Collins, CO, USA). The organic matter degradability (OMD, %) was determined by calculating the weight difference between the incubated organic matter and the undegraded matter filtered through crucibles (sintered glass crucibles; Schott Duran, Mainz, Germany, porosity #2) and burned in a muffle oven at 550 °C [24,25].

2.4. In Vitro End-Products

The fermentation liquor was cooled to 4 °C to determine the volatile fatty acids (VFA) content after 120 h of incubation. The samples were centrifuged at 12,000× *g* for 10 min at 4 °C (Universal 32R centrifuge, Hettich FurnTech Division DIY, Melle-Neuenkirchen, Germany). The supernatant (1.0 mL) was then mixed with 1.0 mL of 0.06 mol oxalic acid. The VFAs were measured using a gas chromatography machine (ThermoQuest 8000 top Italia SpA, Rodano, Milan, Italy) equipped with a fused silica capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness), using an external standard solution composed of acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric acids. The percentage of branched-chain fatty acids was calculated as (iso-butyric acid + iso-valeric acid)/(total VFA)/100 [24].

2.5. In Vitro Methane Production

Three flasks for each of the ecotypes (substrates) were stopped at 24 h to measure methane (CH_4) production [26]. The gas phase from each flask was sampled (3.0 mL) using an airtight syringe. Methane was determined by injecting the sampled gas into a gas chromatography machine (ThermoQuest 8000 top Italia SpA, Rodano, Milan, Italy) equipped with a loop TC detector and a column packed with HaySepQ SUPELCO (3/16 inch, 80/100 mesh). Helium at a flow rate of 30 mL min^{-1} was used as carrier. Oven and detector temperatures were 50 and 190 °C, respectively. Standard curves were obtained separately for CO_2, CH4, and N. Using the gas recorded in each flask, the amount of CH_4 produced was calculated as a percentage of the total gas area and then converted to mL. Methane production data were considered to include the mL of gas related to incubated organic matter (iCH_4) and the mL of gas related to 24 h degraded organic matter (dCH_4).

2.6. Measurement of Redox Status and Antioxidant Enzyme Activity Analysis

From each flask stopped at 24 and 120 h, the fermentation liquor was collected and immediately frozen at −30 °C for the measurement of redox status. In particular, the pro-oxidative status was assessed by the LP-CHOLOX test, which evaluates the levels of lipid peroxides, i.e., oxidized cholesterol, within samples [27]. The results, obtained following a photometric reading at 505 nm, were expressed in µEq/L. The antioxidant barrier was evaluated using the OXY-adsorbent test which measures the ability of the sample to counteract the oxidation induced by a solution of hypochlorous acid (HClO). Unreactive HClO radicals further react with the chromogen solution of N, N-diethyl-p-phenylenediamine and produce a coloured complex, which is measured at 505 nm. The results were expressed as µmol HClO/L [9]. All oxidative stress kits were purchased from Diacron International, Grosseto, Italy.

The analysis of antioxidant enzyme activity was carried out on the fermentation liquor collected from each flask stopped at 24 and 120 h. Superoxide dismutase (SOD) belongs to the antioxidant enzyme class and catalyzes the dismutation reaction of the superoxide anion to H_2O_2 [28]. Total superoxide dismutase (T-SOD) enzyme activity was determined in rumen fluid using an SOD assay kit (Elabscience, E-BC-K020, Houston, TX, USA). The reaction mixture, consisting of 20 µL of a sample, 20 µL of enzyme working solution, and 200 µL of substrate application solution, was mixed and incubated at 37 °C for 20 min. The absorbance was measured at a wavelength of 450 nm using a microplate reader (Thermo Scientific, Waltham, MA, USA) and the results were expressed as U/mg of protein.

2.7. Data Analysis

For each bottle stopped at 120 h, the gas production profiles were fitted to the sigmoidal model to estimate the fermentation parameters [29]:

$$G = A/(1 + \left(\frac{B}{t}\right)^{C})$$

where G is the total gas produced (mL per g of incubated OM) at time t (h), A is the asymptotic gas production (mL/g), B is the time at which one half of A is reached (h), and C is the curve switch. Maximum fermentation rate (R_{max}, mL/h) and the time at which it occurs (T_{max}, h) were calculated utilising the model parameters [30]:

$$R_{max} = \frac{\left(A \times C^{B}\right) \times B \times {T_{max}}^{(B-1)}((1 + CB) \times (T_{max} - B))^{2}}{\left(\left(1 + C^{B}\right) \times (T_{max} - B)\right)^{2}}$$

$$T_{max} = C \times \left(\frac{B-1}{B+1}\right)^{1/B}$$

The chemical composition data were examined to verify the differences in the three maca ecotypes. The in vitro data (i.e., parameters of rumen fermentation and antioxidant activity) were analysed using a two-way ANOVA considering the effect of variety and dose. The significance level was verified using HSD Tukey's test at $p < 0.05$. All statistical analyses were carried out using JMP®, Version 14 SW (SAS Institute Inc., Cary, NC, USA, 1989–2019).

3. Results

3.1. Chemical Composition

In Table 1, the chemical compositions of three maca ecotypes are reported. The yellow variety showed the highest level of non-structural carbohydrates and the lowest levels of ash, crude protein, structural carbohydrates, and lipids. The black maca showed the highest levels of ash, ADF, and ADL ($p < 0.05$), while it was the lowest in terms of NSC.

The red and black varieties showed the highest level of crude protein and NDF. However, the red maca was found to have the highest value of EE.

Table 1. Chemical composition of three maca varieties (% DM).

Ecotype	Ash	CP	NDF	ADF	ADL	EE	NSC
Yellow	1.00 ^{C}	3.60 ^{B}	1.16 ^{B}	0.87 ^{C}	ND	0.30 ^{C}	94.0 ^{A}
Black	7.06 ^{A}	13.5 ^{A}	13.6 ^{A}	9.52 ^{A}	1.19 ^{a}	1.50 ^{B}	63.9 ^{C}
Red	5.37 ^{B}	12.6 ^{A}	12.5 ^{A}	7.55 ^{B}	1.07 ^{b}	2.23 ^{A}	67.4 ^{B}
MSE	0.0003	0.05	0.28	0.02	0.002	0.0003	0.44

CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; EE: ether extract; NSC: non-structural carbohydrates. Along the column, different capital and lower letters indicate $p < 0.001$ and $p < 0.05$, respectively. MSE: mean square error.

3.2. In Vitro Fermentation Characteristics and Methane Production

In Table 2, the in vitro fermentation characteristics and methane production are reported. Considering the effects of the different varieties, yellow maca and red maca showed the greatest and least level of OMD, respectively ($p < 0.05$). Similarly, yellow maca showed the shortest time to reach the maximum fermentation rate (T_{max}) and the greatest fermentation rate (R_{max}) ($p < 0.001$). Regarding methane production, yellow maca produced the least amount of methane (dCH_4) ($p < 0.01$). Considering the effect of dose, the addition of maca at 300 mg/g resulted in the greatest OMCV and R_{max} levels ($p < 0.001$). Moreover, the diet supplemented with 300 mg/g of maca produced the least amount of methane (iCH_4 and dCH_4). For OMCV, R_{max}, and dCH_4, the Variety x Dose interaction was significant.

Table 2. In vitro fermentation parameters (120 h of incubation) and methane production (24 h of incubation) of three maca varieties and three different doses (average ± standard deviation).

	120 h				24 h	
	OMD	**OMCV**	**T_{max}**	**R_{max}**	**iCH_4**	**dCH_4**
	%	**mL/g**	**h**	**mL/g**	**mL/g iOM**	**mL/g dOM**
			Variety effect			
Yellow	72.4 ± 0.34	339 ± 32.3	2.03 ± 0.67	15.2 ± 0.28	10.8 ± 1.19	18.2 ± 5.53
Black	71.7 ± 0.40	328 ± 15.4	5.21 ± 0.82	11.5 ± 0.68	11.3 ± 0.95	21.8 ± 2.12
Red	71.0 ± 0.82	337 ± 14.6	5.42 ± 1.13	11.6 ± 0.41	11.5 ± 0.74	22.8 ± 1.62
p-Value	0.022	0.149	<0.001	<0.001	0.331	0.008
			Dose effect			
0	72.6 ± 0.91	262 ± 2.42	5.29 ± 0.30	7.89 ± 0.47	13.5 ± 0.69	27.8 ± 1.17
150	71.9 ± 0.41	318 ± 10.1	4.52 ± 0.81	11.8 ± 0.71	11.9 ± 0.48	23.2 ± 1.33
300	71.5 ± 0.98	352 ± 13.3	3.92 ± 0.93	13.7 ± 0.31	10.5 ± 0.70	18.7 ± 1.25
p-Value	0.139	<0.001	0.240	<0.001	0.006	0.001
			Variety × Dose			
p-Value	0.159	0.031	0.337	<0.001	0.804	0.013

Yellow: control diet with yellow maca; Black: control diet with black maca; Red: control diet with red maca. 0: control diet; 150: 150 mg/g of maca added to control diet; 300: 300 mg/g of maca added to control diet. OMVC: cumulative volume of gas related to the incubated organic matter; T_{max}: time at which R_{max} occurs; R_{max}: maximum fermentation rate; iCH_4: methane production related to incubated organic matter; dCH_4: methane production related to 24 h degraded organic matter.

3.3. In Vitro End-Products

As reported in Table 3, red maca showed the greatest levels of VFA and BCFA ($p < 0.05$), while yellow maca showed the least amount of VFA. Otherwise, black maca resulted in the lowest levels of BCFA, iso-valerate, and valerate. Moreover, black maca showed the greatest level of acetate and the highest A/P ratio, while yellow maca resulted in the greatest level of butyrate. The addition of 300 mg/g of maca to the control diet

resulted in the lowest levels of pH, BCFA, acetate, and iso-valerate. On the contrary, the diet with 300 mg/g of maca showed the greatest levels of VFA, butyrate, and valerate. The diet supplemented with 150 mg/g of maca showed the highest A/P ratio. Only for pH, BCFA, propionate, was the Variety x Dose interaction not significant.

Table 3. In vitro end-products of three maca varieties at different doses after 120 h of incubation (average ± standard deviation).

	pH	VFA	BCFA	Ace	Prop	Iso-But	But	Iso-Val	Val	A/P
		mmol/L				% VFA				
					Variety effect					
Yellow	6.44 ± 0.08	86.9 ± 10.6	2.17 ± 0.25	63.8 ± 4.22	19.0 ± 1.14	1.02 ± 0.11	13.0 ± 0.76	1.15 ± 0.15	1.94 ± 0.94	3.36 ± 0.38
Black	6.45 ± 0.03	89.4 ± 9.08	2.05 ± 0.25	69.1 ± 1.54	17.7 ± 0.83	0.97 ± 0.12	9.23 ± 0.45	1.08 ± 0.13	1.65 ± 0.54	3.86 ± 0.26
Red	6.42 ± 0.09	92.7 ± 7.66	2.39 ± 0.25	67.4 ± 0.72	19.0 ± 0.18	1.10 ± 0.09	9.12 ± 0.44	1.29 ± 0.17	2.11 ± 0.71	3.54 ± 0.07
p-Value	0.612	0.029	0.018	<0.001	0.093	0.074	<0.001	0.027	<0.001	0.005
					Dose effect					
0	6.60 ± 0.04	80.8 ± 1.32	3.42 ± 0.12	66.1 ± 0.76	19.0 ± 0.40	1.51 ± 0.37	9.62 ± 0.11	1.90 ± 0.08	1.89 ± 0.13	3.48 ± 0.11
150	6.49 ± 0.02	81.9 ± 4.31	2.29 ± 0.34	68.2 ± 1.77	18.3 ± 0.92	1.05 ± 0.14	9.46 ± 0.49	1.23 ± 0.20	1.71 ± 0.41	3.74 ± 0.28
300	6.39 ± 0.05	97.4 ± 1.71	2.12 ± 0.17	65.3 ± 3.99	19.0 ± 0.84	1.00 ± 0.08	11.4 ± 0.73	1.11 ± 0.10	2.09 ± 0.10	3.44 ± 0.31
p-Value	0.002	0.077	<0.001	<0.001	0.121	0.246	<0.001	0.048	<0.001	0.010
					Variety × Dose					
p-Value	0.307	0.009	0.353	<0.001	0.257	0.013	<0.001	0.015	<0.001	0.027

Yellow: control diet with yellow maca; Black: control diet with black maca; Red: control diet with red maca. 0: control diet; 150: 150 mg/g of Maca added to control diet; 300: 300 mg/g of maca added to control diet. VFA: volatile fatty acids; BCFA: branched-chain fatty acids; Ace: Acetate; Prop: Propionate; Iso-But: Iso-Butyrate; But: Butyrate; Iso-Val: Iso-Valerate; Val: Valerate; Ace/Prop: Acetate/Propionate ratio.

3.4. Oxidative Stress Analysis

The antioxidant activity and redox status after 24 and 120 h of incubation are shown in Table 4. Yellow maca showed the greatest level of T-SOD and LP cholox after 24 and 120 h of incubation. The diet with 150 mg/g of maca resulted in the greatest LP cholox level after 24 h of incubation. After 120 h of incubation, maca at 150 mg/g showed the greatest level of T-SOD whereas maca at 300 mg/g showed the lowest level of LP cholox. For the oxidative stress parameters, the Variety × Dose interaction was always not significant.

Table 4. Oxidative stress parameters of three maca varieties and three different doses after 24 and 120 h of incubation doses (average ± standard deviation).

	24 h			120 h		
	OXY	T-SOD	LP Cholox	OXY	T-SOD	LP Cholox
	mmol HClO/mL	U/mL	mEq/L	mmol HClO/mL	U/mL	mEq/L
			Variety effect			
Yellow	43.3 ± 16.1	20.4 ± 1.05	1045 ± 58.9	58.7 ± 17.5	23.3 ± 1.32	1656 ± 154
Black	32.1 ± 15.3	16.9 ± 1.35	772 ± 123	62.1 ± 20.0	22.8 ± 1.19	1580 ± 125
Red	57.7 ± 15.2	14.7 ± 1.84	904 ± 222	60.7 ± 21.7	20.5 ± 0.87	1266 ± 114
p-Value	0.182	0.004	0.001	0.997	0.002	<0.001
			Dose effect			
0	63.8 ± 2.58	20.5 ± 2.61	903 ± 46.7	163 ± 18.5	21.2 ± 0.35	1604 ± 9.90
150	52.0 ± 12.1	17.9 ± 2.16	1013 ± 118	66.6 ± 15.5	22.9 ± 2.03	1596 ± 216
300	36.8 ± 10.0	16.7 ± 3.38	801 ± 172	52.9 ± 19.0	21.6 ± 0.85	1406 ± 178
p-Value	0.174	0.232	<0.001	0.717	0.014	0.004
			Variety × Dose			
p-Value	0.115	0.401	0.209	0.997	0.085	0.589

Yellow: control diet with yellow maca; Black: control diet with black maca; Red: control diet with red maca. 0: control diet; 150: 150 mg/g of maca added to control diet; 300: 300 mg/g of maca added to control diet. Oxy-adsorbent: antioxidant capacity; T-SOD: superoxide dismutase; LP cholox: lipid peroxides.

4. Discussion

4.1. Chemical Composition

The chemical compositions of three ecotypes (yellow, black, and red) of maca root powder seem in line with data reported in the literature [31,32]. As reported by Rondán-Sanabria et al. [33], the roots contain up to 18% protein, 76% non-structural carbohydrates, and a small amount of lipids (2.2%). Similarly, our data showed that all the ecotypes had low levels of structural carbohydrates and lipids and were rich in non-structural carbohydrates. In particular, the yellow ecotype was mainly characterized by non-structural carbohydrates. Considering the agronomic and nutritional characteristics of the maca ecotypes, their chemical compositions are comparable to those of tubers such as potatoes and carrots which are normally rich in total sugars [33,34].

4.2. In Vitro Fermentation

Despite a decrease in pH with the inclusion of maca, the resultant pH levels in the rumen fluid were within the physiological range for rumen function [35]. The data obtained supported this, as the diets supplemented with maca did not affect OM fermentability. Moreover, the experimental diets supplemented with the maca varieties fermented faster, particularly those with the yellow ecotype. The addition of maca to the standard diet increased gas production and the fermentation rate (Figures 1 and 2). Probably the presence of sugar, particularly in the yellow ecotype, improved the diet's fermentability in terms of fermentation rate and extent of fermentation. Several in vitro studies have reported that the supplementation of sugar sources in a ruminant diet can improve fermentation by increasing kinetics [36,37]. Furthermore, NSC are the main energy source for ruminants. High performance ruminants are fed diets with a high concentration of NSC to promote short-chain fatty acid production in the rumen and, consequently, increase the energy supply [38]. In this regard, in our study, the addition of 300 mg/g of maca, particularly the red variety, to the control diet increased the VFA amount. Furthermore, the addition of black maca to a common TMR showed the highest values of acetate and the acetate/propionate ratio. Otherwise, the addition of 300 mg/g of yellow maca to the TMR resulted in a higher level of butyrate. Considering the obtained results, the inclusion of maca at 150 mg/g did not particularly affect volatile fatty acid production, except for black maca. On the contrary, the addition of maca (especially the yellow ecotype at 300 mg/g) affected VFA and butyrate production. In this regard, the addition of soluble carbohydrates to the diet can increase butyric acid concentration [39]. Malhi et al. [40] observed that in vivo injection of butyric acid into the rumen of goats increased the growth of rumen epithelium cells and the VFA absorption capacity.

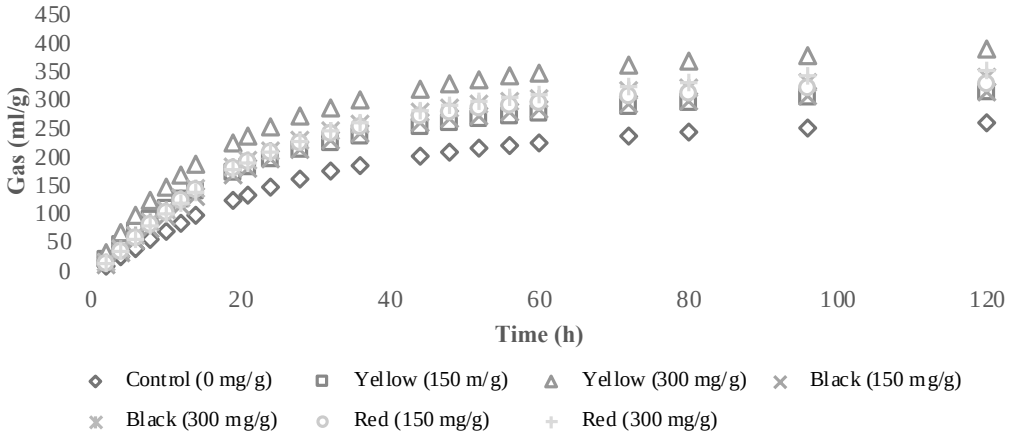

Figure 1. In vitro gas production profile of TMR incubated alone (control) and with the three maca varieties at different doses.

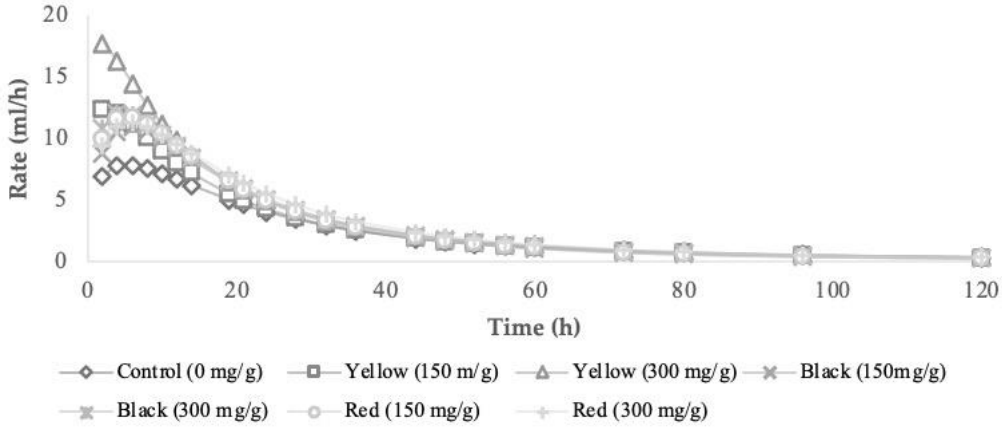

Figure 2. In vitro fermentation rate over time of TMR incubated alone (control) and with the three maca varieties at different doses.

4.3. Methane Production

The addition of maca to TMR decreased the in vitro methane production. The yellow maca, particularly at 300 mg/g, showed the lowest production of methane when related to incubated and degraded organic matter. Furthermore, all the diets supplemented with maca showed a lower amount of methane when related to degraded organic matter. Staerfl et al. [15] incubated different substrates of dietary dry matter, including maca at 75 and 150 g/kg, for 48 h in vitro. The authors observed that, among the tested feeds, maca hypocotyls were among the less promising substrates in terms of antimethanogenic properties. In our study, we tested maca hypocotyls at doses of 150 and 300 mg/g. The results obtained could be ascribed to the dosages used and the presence of some bioactive metabolites [5]. Maca contains several secondary compounds, such as polyphenols, macaridine, macaene, macamides, alkaloids, and glucosinolates. Other compounds include sterols such as beta-sitosterol, campesterol, and stigmasterol [41]. The presence of unsaturated fatty acids (e.g., macaene) could supress the methanogenic activity [26]. Moreover, glucosinolates and/or their breakdown products have long been known to have fungicidal, bactericidal, nematocidal, and allelopathic properties [42]. Therefore, it is possible to speculate that the presence of these secondary metabolites within maca may influence specific microbial populations, including methane-producing ones. Furthermore, the addition of polyphenolic sources into the diets of ruminants can modulate the diversity and activity of rumen microorganisms, the final fermentation products (i.e., pH, acetate, butyrate, propionate, etc.), and the degradability of nutrients, with an effect on methanogenesis [43].

4.4. Oxidative Stress Analysis

Despite the lack of bioactive compounds analysis, the obtained results could be due to the presence of various antioxidant compounds (i.e., phenols, glucosinolates, alkamides, polysaccharides, etc.) in the maca plant [2]. The increase in T-SOD values and the decrease in LP cholox values after 120 h of incubation could indicate a positive effect of maca on rumen fermentation. Moreover, Meissner et al. [44] demonstrated that the red maca ecotype has the highest concentration of glucosinolates when compared to black and yellow maca. The phytocomplex of the maca root varies according to the soil composition, maca ecotype, time of harvest, and drying process [45]. Clément et al. [46] observed that colour type largely influenced concentrations of macaene, macamides, β-sitosterol, campesterol, and glucosinolates and could have a biological effect. The environmental conditions and colour type must be considered when measuring the concentrations of distinct bioactive metabolites.

5. Conclusions

Based on its effect on in vitro rumen fermentation and antioxidant activity, maca could possibly be considered as a useful supplement in ruminant feeding plans. Our data confirmed the known chemical composition in all maca ecotypes and evidenced a high content of non-structural carbohydrates, especially in the yellow ecotype. These nutrients probably influenced some in vitro parameters, mainly improving the fermentation kinetics. Other differences in the ecotypes were also confirmed. Regarding the most appropriate concentration to use, it seems that the addition of 300 mg/g of maca to ruminants' standard diet could modulate the in vitro rumen fermentation in terms of gas and volatile fatty acid production. Furthermore, the supplementation of maca into the diet reduces the in vitro methane production, suggesting some antimicrobial activity on specific microbial populations and a possible ecological effect in terms of reduction of greenhouse gases. Moreover, the in vitro oxidative status analysis indicated that maca could have antioxidant activity after being ingested by the animal. These aspects linked to animal welfare, associated with those of environmental impact, could be of particular interest in intensive farms. The quantity and quality characterization of the bioactive components, probably mainly responsible for the in vitro effects, remains a key point to be investigated.

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