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ACTIVATED CHARCOAL DERIVATED FROM BIOCHAR AS A FEED ADDITIVE FOR GOATS: A RUMINAL ANALYSIS

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Abstract

Biochar, which is the product of biomass pyrolysis, has been suggested as a feed supplement to improve understanding of the rumen microbial ecosystem. The study aims to investigate the potential of activated charcoal to favorably modify rumen fermentation and reduce methane emission by measuring ruminal parameters, enzymes, and microbial communities. Thirty goats (weighing 14.35 ± 1.40 kg) were randomly put into three groups: a control diet group and groups with diet supplemented with activated charcoal at 50 mg/kg and 150 mg/kg. Each group comprised ten replicate pens. The basal diet contained 40% barley and 60% alfalfa hay, and diets were similar to isocaloric and isonitrogenous. The trial lasted 60 days following a 14-day adaptation. The biochar was obtained from pyrolysis of a river plant (Ceratophyllum demersum), impregnate with zinc chloride ($ZnCl_2$), and went through the carbonization process at 700 °C. Rumen fluids were sampled, filtered, and stored at -80 °C in liquid nitrogen to analyze eukaryotic diversity and enzyme activity; the rest was stored at -20 °C to measure fermentation parameters. The results showed that supplementation with activated biochar did not affect pH values and acetate/propionate molar ratio (P = 0.021). Ruminal ammonia (NH3-N), Total Volatile Fatty Acids, Acetate, and Butyrate (%) were significantly ($p < 0.05$) higher at 50 mg and 150 mg activated charcoal groups, compared with control group. Supplementation with activated biochar increased the activities of CMCase, Xylanase, Pectinase, and α-amylase in the rumen, but β-glucosidase and

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protease were not affected. These changes were associated with improved growth performance in the goats. Overall, the interplay of biochar's physical and chemical characteristics plays a major role in different living systems by influencing the different metabolic enzymes and processes, availability of different nutrients, and environmental conditions. Dietary supplementation with activated biochar increased total bacteria, *Fibrobacter succinogenes, Rumonococcus albus,* and *Ruminococcus flavefaciens*) and methanogenic, but did not affect protozoa. In conclusion, this study would further promote biochar applications in ruminal fermentation processes.

Key words: Goats, Biochar, Activated charcoal, Ceratophyllum Demersum, Microbial community, PCR

AKTIVNI UGALJ KAO ADITIV U HRANI ZA KOZE: ANALIZA SADRŽAJA RUMENA

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Kratak sadržaj

Aktivni ugalj, koji predstavlja proizvod pirolize biomase predložen je kao dodatak ishrani, u cilju boljeg razumevanja mikrobioma rumena. Cilj ovog rada je ispitivanje potencijala aktivnog uglja da povoljno utiče na fermentaciju u rumenu i tako dovede do smanjenja emisije metana, merenjem parametara rumena, enzima, i mikrobioma. Trideset koza (težine 14.35 ± 1.40 kg) nasumično je podeljeno u tri grupe: kontrolna grupa i grupe sa dodatkom aktivnog uglja 50 mg, i 150 mg/kg u obroku. Svaka grupa sastojala se iz 10 bokseva za ponavljanje. Bazalna ishrana saržala je 40% ječma i 60% lucerke, a ishrana je bila ujednačena po izokaloričnom i izonitrogenom sastavu. Ispitivanje je trajalo 60 dana, koje je usledilo nakon perioda adaptacije od 14 dana. Aktivni ugalj dobijen je priolizom iz rečne biljke Ceratophyllum demersum, impregniran je cink hloridon ($ZnCl_2$), a proces karbonizacije je izvršen na 700 °C. Tečnosti iz rumena su uzorkovane, filtrirane i

skladištene na -80 °C u tečnom azotu kako bi se analizirala raznovrsnost mikrobioma i aktivnost enzima; ostatak je čuvan na temperaturi od -20 °C kako bi se izmerili parametri fermentacije. Rezultati su pokazali da suplementacija aktivnim ugljem nije uticala na pH vrednosti i odnos molarne mase acetata i propionata (P = 0.021). Ruminalni amonijak (NH3-N), ukupne isparljive masne kiseline, acetat, i butirat (%) bili su značajno viši ($p < 0.05$) u grupama sa dodatkom od 50 mg i 150 mg aktivnog uglja u ishrani u poređenju sa kontrolnom grupom. Suplementacija aktivnim ugljem dovela je do povišenih nivoa aktivnosti CMCaze, ksilanaze, pektinaze, i α-amilaze kod rumena, dok na β-glukosidazu i proteazu nisu imali uticaja. Ove promene nisu povezane sa poboljšanim prirastom koza. Načelno, međudejstvo fizičkih i hemijskih karakteristika aktivnog uglja ima ključnu ulogu u različitim živim sistemima, utičući na različite metaboličke enzime i procese, dostupnost različitih nutrijenata i uslove sredine. Suplementacija aktivnim ugljem dovela je do povećanog ukupnog broja bakterija, *Fibrobacter succinogenes, Rumonococcus albus,* i *Ruminococcus flavefaciens* kao i metanogene, ali nije uticala na protozoe. Ovo istraživanje bi dodatno promovisalo primenu aktivnog uglja u procesima fermentacije u rumenu.

Ključne reči: koze, biougalj, aktivni ugalj, Ceratophyllum Demersum, mikrobna zajednica, PCR

INTRODUCTION

Dietary manipulation (feed additives) is critical for increasing the profitability of livestock industry through improving fermentation in the rumen and enhancing nutrient absorption efficiently, hence optimizing the health and production of ruminants. There is documented evidence that the use of biochar as a dietary supplement for both humans and animals for the treatment of digestive disorders (O'Toole et al., 2016). Biochar acts as a rumen modifier, particularly in reducing enteric CH_4 emissions. Schmidt et al., 2019 report that it may reduce methane emissions up to 20% in cow feed diet that contains 6 g biochar per kg dry matter (DM) (Leng et al., 2012). In addition, biochar's enhancing mechanisms, including immobilization effect (Yang et al., 2022), electron-mediating properties in biological redox reactions, good conductivity beneficial to electron transfer (Nguyen et al., 2021), biochar's alkalinity for improvement of digester buffering capability, adsorption effect (Wang et al., 2019), and contribution to microbial growth (Lü et al., 2019).

Biochar is a byproduct of biofuels industry, it is non-toxic and can be produced through pyrolysis or gasification processes under average temperatures

of 700 °C and low oxygen levels (Toth and Dou, 2016), and undergoes an activation process in order to increase surface of the substance to adsorption of a larger quantity of molecules (Soo et al., 2013) by a series of physical (CO_2, steam) or chemical (salt, organic and inorganic acid treatment) processes. The sources of biochar and pyrolysis conditions determine the characteristics of activated charcoal.

Ceratophyllum demersum (CD) is a submerged plant diffused in quiet streams and ponds whose increased growth might cause waterway clogging and acts as a secondary environmental pollutant (Yang, et al., 2022). Biochar production is an emerging strategy in waste biomass management, offering a new approach to enhance animal health and improve feed efficiency (Schmidt et al., 2017).

Activated charcoal has been used as a feed additive in ruminant diets due to promoting digestive health through removing toxins, supporting rumen ecology, and improving feed conversion efficiency (Wang et al. 2019). Biochar can inhibit butyric acid formation and enhance the quantity of lactic bacteria and provide a biofilm habitat for microbiota to proliferate (Pereira et al., 2014). In addition, biochar also possesses electron-mediating properties that support biological redox reactions in the rumen, enhancing energy conversion efficiency in livestock and helping to reduce greenhouse gas emission (Leng et al. 2012). Since most biochar is alkaline, it can act as a pH buffer in the rumen, resulting in improved livestock weight gains under high-energy diets (Schmidt et al. 2019). Several studies have been carried out to study the impact of biochar supplemented diet on ruminants (Al-Azzawi et al. 2021).

Despite its many potential benefits, further research is needed to fully understand the effectiveness of biochar in animal production, as current knowledge is limited and existing studies often report conflicting results. Therefore, the aim of this study was to deepen the understanding of microbial ecology and their functional roles in the rumen, while also exploring the potential of activated charcoal derivated from biochar as a feed additive for ruminant livestock.

MATERIAL AND METHODS

Ethical approval

The protocol used in this study was approved by the ethics committee on animal experimentation of the Faculty of Veterinary Medicine, University of Baghdad (no.1632/P.G.12 August. 2023).

Biochar Preparation

The biochar used in the experiment was obtained from pyrolysis of the river plant Ceratophyllum Demersum. The biomass was harvested from water surface of Tigris River, Iraq, from May, to July. The roots were cut off, discarded, and washed with distilled water. The samples were air-dried under sunlight for 48 hours by spreading them on sheets and turning them over multiple times to ensure even drying. The biomass was tightly packed into smaller barrels, which were then inverted into larger barrels. The space between the two barrels was filled with wood and ignited. The larger barrel was heated from below with a gas heat source until heating up to a peak temperature of either 350 or 550 °C for 3 h. The biomass was then impregnated with zinc chloride ($ZnCl_2$), and it went through the carbonization process at temperatures of 700 °C (Romanos et al., 2011).

Experimental procedure

This study was carried out from September up to November 2023, on Al-Zubaydiaa agricultural farms for goat breeding (private sector), Wasit, Iraq. Thirty male local-breed goats, aged between 8 and 11 months with an initial body weight of 14.35 ± 1.40 kg, were randomly assigned to three experimental diets using a completely randomized design (n = 10). Experimental diets contained the following:

1. Control (C), a basal diet of alfalfa hay and barley (60:40);
2. Activated charcoal (AC1), basal diet + 50 mg/kg activated charcoal (0.5%) and
3. Activated charcoal (AC2) basal diet + 150 mg/kg activated charcoal (1.5%), rations were an isocaloric and isonitrogenous (Table 1).

The experimental period lasted for 60 days. Goats were individually penned in adjacent pens with dirt floors, measuring 2.5 ×2.5 m, with free access to feed and water. Before the beginning of the experiment, the goats were fed experimental diets for 14 days as an adaptation period and weighed for three days at 8 am to fix the primary weight. The goats were fed 3% of their body weight twice a day (at 08:00 and 16:00). All goats were treated against ecto and endo parasites and vaccinated against clostridiosis before the beginning of the experiment.

Sampling schedule

Feed samples (50 g) were taken for each group in the morning. At the end of the experiment, feed samples representing 10% of the total weight were collected in a labeled polythene bag and stored at 10 °C until analyzed. Rumen fluids were sampled on days 0, 30, and 60 from each animal (after 2 hours of morning feeding) by using a stomach tube fitted to a vacuum pump to siphon fluid through a tube (length: 1 m, diameter: 1.5 cm). In order to prevent saliva interference, rumen pH was measured by using a pH electrode (Model PB-20, Sartorius, Germany). Ten and 5 mL of rumen fluids were collected from each goat, filtered and strained through four layers of compressed gauze, transferred to sterile tubes, and stored at -80 °C in liquid nitrogen for analysis (eukaryotic diversity and enzyme activity); the other was stored at -20 °C for measurement of fermentation parameters.

Chemical analysis

Feed samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) according to AOAC (2006), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the procedure by Van Soest, (1991). Using a Leco CHN1000 analyzer and coal reference material (Leco Corp., St. Joseph, MI, USA), the AC was analyzed by combustion. For further analysis, 0.1 g of AC was mixed with 10 ml of deionized water and shaken at 250 rpm for 24 hours, after which the supernatant was collected. The Tristar II 3020 (Micromeritics) was done to determine the surface area of the AC. Ammonia nitrogen (NH_3-N) and the total volatile fatty acids (TVFA) were evaluated according to AOAC (2006) and Martínez et al. (2010) respectively.

Rumen content samples were thawed at room temperature and placed in an ultrasonic beater at 4 °C, subjected to 4 cycles of 30 seconds each, with 30 second rest periods in between. The samples were then centrifuged at 20,000 × g for 15 minutes at 4 °C to separate cellular debris and enzymes (Martin and Michalet-Doreau, 1995). The supernatant was collected, and subsequent processing was done by the kit (R&D Systems, Minneapolis, MN, USA), to measure activities of carboxymethyl cellulase (CMCase), xylanase, pectinase and β-glucosidase. The activity of lipase and amylase was measured using commercially available reagent kits (NanJing JianCheng Bioengineering Institute, Nanjing, China).

Protease activity was measured using visual-light spectrophotometry at 680 nm. After reaction mixture preparation (1 mL of casein and 4 mL of protease enzyme), the mixture was incubated at 38 °C for 4 h. Then, the reac-

tion was stopped by adding trichloroacetic acid (10%), the sample centrifuged (3500×g for 15 min). The 1 mL of supernatant was mixed with 5mL 0.4M Na_2CO_3 with 1 mL Folin-Ciocalteu's reagent. The mixture was then incubated for 15 minutes before measuring.

Rumen microbial community analysis

The manufacturer provides the guidelines of the protocol. The DNA extraction using QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA), and Real-time PCR to determine the population of total bacteria, *Fibrobacter succinogenes, Rumonococcus albus, and Ruminococcus flavefaciens,* methanogens, and protozoa was used. The primers for detecting the ruminal bacteria 16S rRNA gene sequences were used through CFX 96 system to amplify 16S rDNA (Table 1).

Table 1. The PCR primer used for quantification of rumen microorganism.

Microbes	Primer	
	Forward	Reverse
General bacteria	5'-CGGCAACGAGCGCAACCC-3′	5'-CCATTGTAGCACGTGTGTAGCC-3'
Fibrobacter succinogenes	5'-GTTCGGAATTACTGGGCGTAAA-3'	5'-CGCCTGCCCCTGAACTATC-3'
Ruminococcus albus	5'-CCC TAA AAG CAG TCT TAG TTC G-3'	5'-CCT CCT TGC GGT TAG AAC A-3'
Ruminococcus flavefaciens	5'-CGAACGGAGATAATTTGAGTTTACTTAGG-3'	5'-CGGTCTCTGTATGTTATGAGGTATTACC-3'
Methanogenic archea	5'-TTCGGTGGATCDCARAGRGC-3'	5'-GBARGTCGWAWCCGTAGAATCC-3'
Protozoa	5'-GCTTTCGWTGGTAGTGTATT-3'	5'-CTTGCCCTCYAATCGTWCT-3'

The amplification reaction was carried out in a final volume of 25 µL, consisting of 12.5 µL of Maxima SYBR Green qPCR Master Mix, 8.5 µL of RNase-free distilled water, 2 µL of DNA elution, and 1 µL of species-specific forward and reverse primers. Amplification involved one cycle at 95 °C for 15 min for initial denaturation, then 39 cycles of 95°C for 30 s followed by 30 s at annealing temperature, and 20 s at 72°C for an extension.

Statistical analysis

All analyses were computed by SPSS software version 21.0 (Mean ± SE) and the obtained data were analyzed. Statistical comparisons were made using one-way ANOVA of the generalized linear model (GLM), using the following

model: yij = μ + Ti + eij, where yij is the dependent variable, μ is the overall mean, Ti is the treatment effect, and eij is the experimental error. Duncan's multiple range test to compare differences between groups was used. The ($p < 0.05$) level was adopted for statistical significance**.**

RESULTS

Chemical analysis of diets and activated biochar

The analysis of the experimental diets was similar (Table 2). The chemical composition of the activated charcoal is shown in Table 3.

Table 2. Ingredients and chemical composition of treated diets

Ingredient **g/kg, as-fed basis**	**Treatment**		
	C	**AC1**	**AC2**
Barley	40	39.75	39.25
Alfalfa hay	60	59.75	59.25
Activated charcoal	---	0.5	1.5
Chemical composition, % DM			
DM	89.3	89.1	89.7
CP	14.9	14.86	14.8
EE	5.46	5.3	5.1
NDF	35.6	34.9	34.7
ADF	27.2	26.5	26.1
Metabolizable energy (Mcal/kg DM)	2.37	2.31	2.3

Table 3. Physical properties and chemical composition of activated charcoal

Surface area (m^2/g)	516
Bulk density (kg/m^3)	387
Pores volume (cc/g)	9.21×10^{-2}
pH	8.91

Ruminal fermentation parameters

The effect of supplemented AC in diet on ruminal fermentation is presented in Table 3. The AC did not affect ruminal pH (P = 0.018) and sampling time and the highest value was obtained at 60 d (6.7) in the AC2 group, while the lowest levels were at 0 d (6.45) in control group.

The NH_3-N concentration in rumen was increased (P = 0.0048) in the AC1 and AC2 diets relative to the control diet. The sample time was an effect significantly ($p < 0.05$) which gradually increased over 30 and 60 days in all treated groups, while no change was observed between 0.5 and 1.5% AC.

The results show that the supplementation of AB significantly ($p < 0.05$) increased TVFA (mmol/dL) concentration during experimental feeding. Unlike this, TVFA levels tended to increase in AC1 and AC2 groups than control group. The acetate, propionic, and butyric acid concentration reveal significant ($p \leq 0.05$) increases with the higher level of AC inclusion, unlike acetic acid to propionic acid ratio (A/P) was not affected by the different levels of AC.

The activity of ruminal enzymes (CMCase, xylanase, pectinase, and α-amylase) was increased with high inclusion of AC. The activity of α-amylase in group AC1 was not significantly different from control ($p > 0.05$). However, the activities of these enzymes were significantly higher in AC2 group, whereas protease and β-glucosidase activity was not affected by supplement of AC (Table 4).

Table 4. Effect of activated charcoal on Rumen enzymes activity (mean ± SE) of goat

Parameter	Days	Treatment			p-value
		C	AC1	AC2	
CMCase (U/mL)	0	71.21±1.23	71.43±1.90	71.42±1.11	0.072
	20	72.64±1.97^{c}	75.18±4.32^{b}	77.12±3.55^{a}	0.018
	60	74.41±4.01^{c}	81.86±3.45^{b}	84.42±2.18^{a}	0.020
Xylanase (U/mL)	0	10.99±1.72	10.05±1.75	10.07±1.03	0.120
	30	13.31±2.12^{c}	15.22±1.43^{b}	18.20±1.37^{a}	0.004
	60	14.1±1.83^{c}	18.42±3.19^{b}	22.12±1.33^{a}	0.003
Pectinase (U/mL)	0	43.73±3.52	44.22±1.09	43.13±1.06	0.110
	30	42.48±4.52^{c}	50.25±3.31^{b}	52.76±2.66^{a}	0.004
	60	40.18±2.56^{c}	51.91±1.88^{b}	53.21±0.88^{a}	< 0.001

Parameter	Days	Treatment			p-value
		C	AC1	AC2	
β-glucosidase (U/ mL)	0	42.62±3.04	42.09±3.22	42.41±3.05	0.310
	30	44.61±4.32	44.87±4.77	45.38±3.62	0.250
	60	44.90±1.01	44.88±1.98	45.98±3.19	0.070
α-amylas (μg / min.mL^{-1})	0	20.95±0.75	20.92±0.32	20.89±0.34	0.051
	30	21.09±0.33^{b}	22.39±0.84^{b}	24.15±0.34^{a}	< 0.001
	60	21.45±0.53^{b}	21.55±0.43^{b}	24.99±0.54^{a}	0.003
Protease (μg / min.mL^{-1})	0	3.43±0.86	3.65±0.56	3.47±0.39	0.060
	30	3.51±0.69	3.63±0.22	3.42±0.73	0.140
	60	4.13±0.55	4.22±0.44	4.21±0.35	0.170

*The different lowercase letters refer to significant differences between different treated groups at ($p \leq 0.05$).

Table 5. presents the effect of supplement AC on the rumen microbial. Results show a significant ($p \leq 0.05$) increase in total bacteria count in the treated groups compared with the control group after 30 days of feeding. On the 60th day, group AC2 showed a significant ($p \leq 0.05$) increase in total bacteria count (P = 0.036) compared with other groups.

Table 5. Efect of activated charcoal on rumen microbial population (mean ± SE) of goat

Parameter (Log10 copy No/g)	Days	Treatment			p-value
		C	AC1	AC2	
Total bacteria	0	8.49 ± 0.39	9.06 ± 0.76	9.27 ± 0.61	0.063
	30	8.91±0.54^{b}	10.52±0.82^{a}	10.89±0.52^{a}	0.001
	60	9.01±0,71^{c}	10.04±0.65^{b}	11.6±0.46^{a}	0.036
Fibrobacter succinogenes	0	3.07±0.13	3.02±088	3.31±0.83	0.230
	30	3.20 ± 0.38^{b}	3.61 ± 0.16^{a}	3.8±0.63^{a}	0.026
	60	3.23±0.43^{b}	4.01±0.24^{a}	4.20 ± 0.11^{a}	0.001

Parameter (Log10 copy No/g)	Days	Treatment			p-value
		C	AC1	AC2	
Rumonococcus albus	0	7.61 ± 0.21	7.45 ± 0.18	7.27 ± 0.229	0.060
	30	7.82 ± 0.42	7.87±1.01	7.79±1.20	0.140
	60	7.81±0.37^{b}	7.90±0.75^{b}	8.38±0.24^{a}	0.040
Ruminococcus flavefaciens	0	4.42±0.18	4.57 ±0.07	4.66±0.62	0.120
	30	4.72± 0.22^{b}	4. 90±0.91^{b}	5.29±0.74^{a}	0.002
	60	5.03±1.10^{b}	5.1±0.65^{b}	5.32±0.31^{a}	<0.001
Methanogenic archea	0	3.71 ± 0.52	3.62±0.86	3.66±0.82	0.110
	30	4.2± 0.76^{b}	4.3±0.34^{ab}	4.72±0.57^{a}	0.041
	60	4.5± 0.85^{b}	4.62±0.74^{b}	5.13±0.66^{a}	0.037
Protozoa	0	3.63 ± 0.42	3.49 ± 0.22	3.51 ± 0.32	0.063
	30	3.57±0.16	3.50±1.32	3.52±0.89	0.071
	60	3.55±0.54	3.51±0.52	3.55±0.64	0.055

*The different lowercase letters refer to significant differences between different treated groups at ($P \leq 0.05$).

In addition, the AC supplement showed significantly ($p \leq 0.05$) increased population count of *Fibrobacter succinogenes, Rumonococcus albus, and Ruminococcus flavefaciens* in the AC2 group compared with other groups. Regarding the methanogenic archaea count, it increased significantly in the AC2 group compared to the other groups ($P = 0.041$). The inclusion of AC had no effect on the total protozoa count.

DISCUSSION

Rumen pH is a key indicator of rumen health, with a normal range of 5.50 to 7.50. Activated charcoal supplementation did not significantly affect pH levels but tended to result in slightly higher values compared to the control group. These results were consistent with Saleem et al. (2018) who found no significant link between the addition of pine activated charcoal and pH in RUSITEC system. Garillo et al. (1994) revealed that the ruminal pH in goats tended to increase slightly after 4 h of feeding on diets containing 0.3% and 0.6% of AC. Sun et al., (2021) report that activated charcoal, being alkaline in nature, has rich oxygen functional groups on the surface, which can produce a certain

amount of carboxyl, which can maintain a neutral environment. This result is in contrast with Wang et al. (2019) and Cabeza et al., (2018) who reported ruminal pH decreases after feeding activated charcoal.

The inclusion of AC increased ruminal NH_3-N concentration, which is consistent with Leng et al. (2012) who observed a significant increase in NH_3-N in yellow cattle after feeding them 0.6% activated charcoal. The increase in ruminal NH_3-N may be attributed to numerical increase in protozoal or an increase in protein digestion. This explanation is in line with Doreau and Ferlay, 1995. However, Garillo et al., (1995) revealed decreased NH_3-N in Suffolk ewe after feeding 0.3% AC. Pereira et al. (2014) in vitro and Cabeza et al., 2018 proved that there was no effect of NH_3-N when using different sources of activated charcoal.

Volatile fatty acids (VFA) are the end product of fermentation and indicate relative fermentability. Dietary AB supplementation tended to significantly ($p < 0.05$) increase total VFAs in the treated groups compared to the control group, which is consistent with increased AB levels. A similar result was reported by Pereira et al. (2014) in vitro when using activated charcoal from silage and hay. Garillo et al. (1994) observed an increase in ruminal total VFA when goat diet contained 0.6% biocarbon. In contrast, an increase in VFAs is consistent with an increase in the molar proportion of acetate, propionic, and butyric. In contrast, Pereira et al. (2014) found increased TVFA and acetate concentrations consistent with an increased level of activated charcoal, whereas the amount of propionate was decreased and butyrate was not affected. Terry et al. (2019) and Teoh et al. (2019) found that activated charcoal did not significantly affect rumen VFA. Cabeza et al. (2018) reported that the concentrations of TVFA and acetate were not affected by addition of 1.16 and 11.6 %. McFarlane et al (2017) reported that they did not observe any changes in VFA, acetate, propionate, and butyrate production.

The ruminal microbes play roles in digestive processes through their enzymes. The potential effects of AC on rumen enzymes could provide new information and deepen our understanding of the functions of the rumen microbiome, thereby improving management of the rumen microbiome. AC showed improved CMCase, xylanase, and pectinase activity compared with the control group ($p < 0.05$) throughout the experimental period. These results can be explained by an increase in the amount of surface area of activation activated charcoal, as well as enhanced growth of certain rumen microbial communities by forming micro-sites or formation of functional groups that can enhance microbial redox reactions (Schmidt et al. 2019). In addition, AC facilitates direct interspecies electron transfer (DIET) between microbial populations,

promoting more rapid microbial growth (Leng et al., 2012). A similar result was reported by Bagherpoor et al., (2023) in vitro when activated charcoal was given separately or in a mixture with probiotics. Activated charcoal may stimulate bacterial growth and enzyme activity by modulating biofilm formation and activity within the rumen (Lehmann and Joseph, 2015).

Our study revealed the activity of α-amylase increase ($p < 0.05$) in AC2 compared with other groups. These results may be attributed to the influence of AC on microorganisms based on their cell envelope structure — Gram-positive bacteria tend to be less effectively adsorbed by activated charcoal, whereas Gram-negative bacteria exhibit greater adsorption. Bagherpoor et al., (2023) observed that protease activity was not affected by the experimental treatments (probiotic and activated charcoal) in vitro. The effect of activated charcoal on extracellular enzyme activity is known to depend during interaction between the substrate and enzyme and could be affected by activated charcoal porosity or specific surface area (Lammirato et al., 2011). A previous study suggested that charcoal can form complexes with phenolic compounds, preventing hydrolysable tannins from inhibiting bacterial enzyme function and protein digestion, thereby potentially enhancing protein availability and protease activity (Van et al. 2006). The reason for these increases can the improvement of the cellulolytic bacteria population. Therefore, we speculate that the increase in relative abundance of *Fibrobacter* and *Ophryoscolex* was responsible for the increase in the activities of xylanase and pectinase and the decrease in activity of β-glucosidase.

The rumen provides a relatively stable environment for microorganisms, with their composition being primarily influenced by the animal's diet. Total bacteria appear to be affected by AC, particularly at higher supplement levels, results show that the number of bacteria significantly ($p < 0.05$) increased in treated groups compared with control, with the increase in the level of inclusion. These results differ from a study by Teoh et al. (2019) which found that activated charcoal may have shifted microbial communities but does not significantly influence the total bacteria in the rumen when 800 mg/per day is added. Qomariyah (2021) reported activated charcoal addition significantly decreased bacterial population in the rumen. Therefore, we speculate that the increased activities of xylanase and pectinase, along with the unchanged β-glucosidase activity, contributed to the relative abundance of *Fibrobacter* species, suggesting that the rumen bacterial community may be strongly oriented toward fiber degradation.

F.succinogenes, R. albus, and R. flavefaciens are predominant cellulolytic bacteria in goat rumen. In the present experiment, we found that cellulolytic

bacteria increased numerically in treated groups compared with control. Similar findings were reported by Sirjani et al. (2022), who observed a significant increase in cellulolytic bacterial populations ($p < 0.05$) following the separate inclusion of a lactobacilli mixture and activated charcoal in the diet. The increase in cellulolytic bacteria may be due to a form of different amino acids for protein synthesis and bacterial growth through incorporating ammonia into carbon skeleton by synthesizing enzymes (such as glutamate dehydrogenase, glutamine synthetase, and glutamate synthetase). This interpretation is in line with Antonopoulos et al., (2003). Another cause for increase is a shift of reduction methane mitigation to more propionic production. A similar report by Mitsumori et al., (2012) shows increased abundances of *Prevotella spp., Selenomonas spp*., and *F. succinogenes* as a result of decreased methane mitigation in ruminants, shifting towards a more propionic production. Activated charcoal has characteristics that improve the growth and preserve cellulolytic bacteria. This effect may be attributed to the anaerobic nature of cellulolytic bacteria, as activated charcoal helps reduce oxygen levels in the rumen environment. Barboza et al. (2009) also reported that neutral pH values promote the growth and activity of cellulolytic bacteria, supporting the findings of our study. During the activation process, carbon develops a larger surface area, enabling it to adsorb bacteria into its porous structure and potentially release them later, facilitating microbial colonization and activity (Villarreal et al., 2015).

Regarding methanogenic archea, the activated charcoal may increase the abundance of methanotrophs, inhibit methanogens, or absorb CH_4 produced by the rumen. This result is consistent with the findings of Leng et al. (2012) and Toth and Dou (2016), who proposed that activated charcoal reduces ruminal CH_4 production by modifying rumen microbial biofilms, decreasing methanogenic populations, and promoting the growth of methanotrophs.

The numbers of rumen protozoa increased numerically ($p > 0.05$) in treated groups, and this is due to the symbiotic relationship with methanogens. Similar observations were noted by Saleem et al. (2018) and Garillo et al. (1994) in goats. Machmüller et al., (2003) mention when protozoa are increased, the symbiotic methanogens also increases. The result is in disagreement with Terry et al. (2019) who found reduced protozoa counts in heifers fed pine activated charcoal at levels 0.5 and 1.0%.

CONCLUSION

Overall, this study demonstrates that adding activated charcoal to the goat diet is a simple and manageable intervention that can positively impact animal

health. It improves the rumen microbiota structure, alters fermentation processes, and enhances feed utilization efficiency, thereby potentially improving growth performance to some extent. The most important finding is that there was no adverse affected on ruminal ecology, total bacteria, and cellulolytic bacteria, while methanogenic archaea increased. In contrast, there was an increase in the activity of cellulose-degrading enzymes. However, further studies are needed to explore the impact of AB on the ruminal environment under different dietary conditions.

Author's Contribution:

A.M.M Mani designed the experiments. H.H Ghaza and A. Mani conducted the experiments. R.R. Al -Araji and A.MM Mani analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interest

The authors declare that there was no conflict of interest in carrying out this work.

REFERENCES

Al-Azzawi M., Bowtell l., Hancock K. Preston S. 2012.Addition of activated carbon into a cattle diet to mitigate GHG emissions and improve production. Sustainability.13(15): 8254. https://doi.org/10.3390/su13158254

Antonopoulos D.A., Aminov R.I., Duncan P.A., White B.A., Mackie R.I. 2003. Characterization of the gene encoding glutamate dehydrogenase (gdhA) from the ruminal bacterium R. flavefaciens FD-1. Archives of microbiology. 179 (3) :184–190. https://doi.org/10.1007/s00203-002-0513-4

AOAC. 2006. Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD.

Bagherpoor Z., Rezaei J., Rouzbehan Y. 2023. Potential of biochar in enhancing the effectiveness of probiotics Bacilli and Lactobacilli on in vitro microbial populations, hydrolytic enzymes, and ruminal fermentation in sheep.Animal Production Research, 12(3): 29-47. https://doi.org/10.22124/ar.2023.25526.1790

Barboza S.P., Parker K.L., Hume I.D. 2009. Integrative wildlife nutrition Berlin, Heidelberg, Germany: Springer-Verlag, 342. https:// doi.org/10.1007/978-3-540-87885-8

Cabeza I., Waterhouse T., Sohi S., Rookej A. 2018. Effect of biochar produced from different biomass sources and at different process temperatures on methane production and ammonia concentrations in vitro. Animal Feed Science and Technology, 237: 1-7. https://doi.org/10.1016/j.anifeedsci.2018.01.003

Doreau M., Ferlay A. 1995. Effect of dietary lipids on nitrogen metabolism in the rumen:a review. Livestock Production Science, 43(2): 97–110. https://doi.org/10.1016/0301-6226(95)00041-I

European Centre for Disease Prevention and Control (ECDC). Historical data by year - West Nile fever seasonal surveillance. Available at: https://ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical Accessed: 31.07.2019.

Garillo E.P., Pradhan R., Tobioko H. 1995.Effect of activated carbon on growth, ruminal characteristics, blood profiles and feed digestibility in sheep. Asian-Australasian Journal of Animal Sciences, 8(1): 43-50. https://doi.org/10.5713/ajas.1995.43

Garillo E.P., Pradhan R., Tobioko H. 1994. Effects of activated charcoal on ruminal characteristics and blood profiles in mature goats. The West Japan Journal of Animal Science, 37:85-89.

Lammirato C., Miltner A., Kaestner M. 2011. Effects of Wood Char and Activated Carbon on the Hydrolysis of Cellobiose by β-Glucosidase from Aspergillus Niger. Soil Biology and Biochemistry, 43(9): 1936–1942. https://doi.org/10.1016/j.soilbio.2011.05.021

Lehmann J., Joseph S. 2015. Biochar for Environmental Management: Science and Technology and Implementation. Routledge, London.

Leng R.A., Inthapanya S., Preston T.R. 2012. Biochar lowers net methane production from rumen fluid in vitro. Livestock Research for Rural Development, 24(6): 103.

Lü F., Yang L., Liming S., Pinjing H. 2019. Powdered biochar doubled microbial growth in anaerobic digestion of oil. Applied Energy, 247(2): 605-614. https://doi.org/10.1016/j.apenergy.2019.04.052

Machmüller A., Soliva C.R., Kreuzer M. 2003. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. The British journal of nutrition, 90(3):529–540. https://doi.org/10.1079/bjn2003932

Martin C., Michalet-Doreau B. 1995. Variations in mass and enzyme activity of rumen micro- organisms: Effect of barley and buffer supplements . The

Journal of the Science of Food and Agriculture, 67:407-413. https://doi.org/10.1002/jsfa.2740670319

Martínez M.E., Ranilla M.J., Tejido M.L., Ramos S., Carro M.D. 2010. Comparison of fermentation of diets of variable composition and microbial populations in the rumen of sheep and Rusitec fermenters. I. Digestibility, fermentation parameters, and microbial growth. Journal of dairy science, 93(8): 3684-3698. https://doi.org/10.3168/jds.2009-2933

McFarlane Z.D., Myer P.R., Cope E.R., Evans N.D., Bone T.C., Biss B.E., Mulliniks J.T. 2017. Effect of biochar type and size on in vitro rumen fermentation of orchard grass hay. Agricultural Sciences, 8(4):316–325. https://doi.org/10.4236/as.2017.84023

Mitsumori M., Shinkai T., Takenaka A. 2012. Responses in digestion, rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue. The British journal of nutrition, 108(3):482–491. https://doi.org/10.1017/S0007114511005794

Nguyen L.N., Vu M.T., Johir M.A., Pernice M., Ngo H.H, Zdarta J., Jesionowski T., Nghiem L. 2021. Promotion of direct interspecies electron transfer and potential impact of conductive materials in anaerobic digestion and its downstream processing-a critical review. Bioresource Technology, 341:125847. https://doi.org/10.1016/j.biortech.2021.125847

O'Toole A., Andersson D., Gerlach A., Glaser B., Kammann C., Kern J., Kuoppamäki K., Mumme J., Schmidt H.-P., Schulze M., Srocke F., Stenrød M. and Stenström J. 2016. Current and future applications for biochar. In *Biochar in European Soils and Agriculture: Science and Practice*, Eds. S. Shackley, G. Ruysschaert, K. Zwart, B. Glaser Routledge, London, UK , 253-280.

Pereira C., Muetzel R., Camps S., Arbestain M., Bishop P., Hina K., Hedley M. 2014. Assessment of the influence of biochar on rumen and silage fermentation: a laboratory-scale experiment. Animal Feed Science and Technology, 196:22–31. https://doi.org/10.1016/j.anifeedsci.2014.06.019

Qomariyah N., Retnani Y., Jayanegara A., Wina E., Permana IG. 2021. Influence of biochar and liquid smoke additives from cacao-pod husks on in vitroruminal fermentation characteristics. Advances in Animal and Veterinary Sciences, 9(4): 533-543. http://dx.doi.org/10.17582/journal.aavs/2021/9.4.533.543

Romanos, J., Beckner, M., Rash, T., Firlej, L., Kuchta, B., Yu, P. 2012. Nanospace Engineering of KOH Activated Carbon. Nanotechnology, 23(1): 15401. https://doi.org/10.1088/0957-4484/23/1/015401

Saleem A., Ribeiro G., Yang W., Ran T., Beauchemin K., McGeough E., Ominski K.H., Okine E.K., McAllister T.A. 2018. Effect of engi-neered biocarbon on rumen fermentation, microbial protein synthesis,and methane production in an artificial rumen (RUSITEC) fed a high for-age diet. Journal of Animal Science, 96(8):3121-3130. https://doi.org/10.1093/jas/sky204

Schmidt H.P., Hagemann N., Draper K., Kammann C. 2019. The use of biochar in animal feeding. Peer J, 7: 7373. https://doi.org/10.7717/peerj.7373

Schmidt H.P., Wilson K., Kammann C. 2017.Using biochar in animal farming to recycle nutrients and reduce greenhouse gas emissions. Geophysical Research Abstracts, 19: 5719.

Sirjani M.H., Rezaei J., Zahedifar M., Rouzbehan Y. 2022. Effect of adding biochar in diets containing probiotics on in vitro fermentation variables, health indicators, rectum bacteria, and blood enzymes of Holstein calves. Animal Production Research, 11(4): 11-19. https://doi.org/10.22124/ar.2023.23067.1727

Soo J., Malik B.A., Turner J.M., Persad R., Wine E., Siminoski K., Huynh H.Q. 2013. Use of exclusive enteral nutrition is just as effective as corticosteroids in newly diagnosed pediatric Crohn's disease. Digestive diseases and sciences, 58(12): 3584–3591. https://doi.org/10.1007/s10620-013-2855-y

Sun Y., Wu Q., Wang Y., Li C., Liang X. 2021. Protein-derived 3D amorphous carbon with N, O doping as high rate and long lifespan anode for potassium ion batteries. Journal of Power Sources, 512:230530. https://doi.org/10.1016/j.jpowsour.2021.230530

Teoh R., Caro E., Holman D.B., Joseph S., Meale S.J., Chaves A.V. 2019. Effects of hardwood biochar on methane production, fermentation characteristics, and the rumen microbiota using rumen simulation. Frontiers in Microbiology, 10: 1–13. https://doi.org/10.3389/fmicb.2019.01534

Terry S.A., Ribeiro G.O., Gruninger R.J., Chaves A.V., Beauchemin K.A., Okine E., McAllister T.A. 2019. A Pine Enhanced Biochar Does Not Decrease Enteric CH4 141.Emissions, but Alters the Rumen Microbiota. Frontiers in veterinary science, 6: 308. https://doi.org/10.3389/fvets.2019.00308

Toth J. D., Dou Z. 2016. Use and impact of biochar and charcoal in animal production systems. In *Agricultural and environmental applications of biochar: advances and barriers*. Eds. M. Guo, Z. He, S.M. Uchimiya, Soil Science Society of America. Inc, Madison: 199–224. https://doi.org/10.2136/sssaspecpub63.2014.0043.5

Van D.T.T., Mui N.T., Ledin I. 2006. Effect of method of processing foliage of Acacia mangium and inclusion of bamboo charcoal in the diet on performance of growing goats. Animal Feed Science and Technology, 130(3), 242–256. https://doi.org/10.1016/J.ANIFEEDSCI.2006.01.00.8

Van Soest P.J., Robertson J.B., Lewis B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science ,74(10):3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Villarreal J., Kahn C.A., Dunford J.V., Patel E., Clark R.F. 2015. A retrospective review of the prehospital use of activated charcoal. The American journal of emergency medicine, 33(1): 56-59. https://doi.org/10.1016/j.ajem.2014.10.019

Wang M., Wang J.J., Taft N.D., Hollier C.A., Myers G., Wang X. 2019. Effect of alkali-enhanced biochar on silicon uptake and suppression of gray leaf spot development in perennial ryegrass.Crop Protection, 119: 9–16. https://doi.org/10.1016/j.cropro.2019.01.013

Yang M., Zhan X., Wang K., Zhu S., Ye Z., Sheng K., Zhang G.X. 2022. Investigation of cascade valorization of Pistia stratiotes L. by hydrothermal treatment. Fuel. 324(1-3): 124473. https://doi.org/10.1016/j.fuel.2022.124473 OIE: Animal Diseases. Available at: http://www.oie.int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/. Accessed 07.08.2019.

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