atmosphere

MDPI

Article

Effects of Feeding a Commercial Starch Binding Agent during Heat Stress on Enteric Methane Emission, Rumen Volatile Fatty Acid Contents, and Diet Digestibility of Merino Lambs

Pragna Prathap [1,*], Surinder S. Chauhan [1], Brian J. Leury [1], Jeremy J. Cottrell [1], Aleena Joy [1], Minghao Zhang [1] and Frank R. Dunshea [1,2,*]

[1] School of Agriculture and Food, The University of Melbourne, Parkville, VIC 3010, Australia
[2] Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, UK
* Correspondence: pprathap@student.unimelb.edu.au (P.P.); fdunshea@unimelb.edu.au (F.R.D.)

Abstract: Twenty-four Merino lambs were allocated to three dietary treatment groups to determine the effects of a dietary starch and protein binding agent and heat stress on methane (CH_4) emissions and rumen parameters. The diets were a wheat-based diet (WD), a 2% Bioprotect™ (Bioprotect™, RealisticAgri, Rutland, UK) treated wheat-based diet (BD), and a maize-based diet (MD) for 3 periods of 1-week duration. During Period 1 (P1) the lambs were maintained under thermoneutral conditions and at a 1.7 × Maintenance (M) level. During P2 and P3, the lambs were maintained under cyclic heat stress conditions and fed at 1.7 × M and 2.0 × M, respectively. Total CH_4 production was lower for the BD diet than the WD diet, which in turn was lower than the MD diet ($p < 0.001$). Total CH_4 production was lower during P2 than P1 with P3 intermediate ($p = 0.04$). Rumen total volatile fatty acid (TVFA) concentrations were higher for the WD diet than the MD diet with the BD diet intermediate ($p = 0.01$). Rumen TVFA concentrations were lower during heat stress than under thermoneutral conditions ($p < 0.001$). Whole tract starch digestibility was higher for the BD and WD diets than the MD diet ($p < 0.001$). In conclusion, feeding Merino lambs the BD diet reduces CH_4 emissions without reducing starch digestibility.

Keywords: temperature; greenhouse gas; feed additives; sheep; wheat; maize

Citation: Prathap, P.; Chauhan, S.S.; Leury, B.J.; Cottrell, J.J.; Joy, A.; Zhang, M.; Dunshea, F.R. Effects of Feeding a Commercial Starch Binding Agent during Heat Stress on Enteric Methane Emission, Rumen Volatile Fatty Acid Contents, and Diet Digestibility of Merino Lambs. *Atmosphere* **2023**, *14*, 605. https://doi.org/10.3390/atmos14030605

Academic Editors: David Janke, Sabrina Hempel and Chaoyuan Wang

Received: 2 March 2023
Revised: 14 March 2023
Accepted: 20 March 2023
Published: 22 March 2023

1. Introduction

The link between climate change and sheep production is very complex and multidirectional, such that climatic stressors such as heat stress compromise animal production and animals emit greenhouse gases (GHGs) that contribute to global warming [1].

Heat stress can impair the nutritional status of ruminants by affecting the digestion and rumen fermentation characteristics [2,3]. There are conflicting results from studies investigating the effect of heat stress on digestion, with some studies finding an increase in dry matter (DM) [4], organic matter (OM) and starch, neutral detergent fibre (NDF) [5], and acid detergent fibre (ADF) digestibility [6] during heat stress, whereas others reported the opposite [7–9]. This increase in digestibility can result from a decrease in feed intake and an increase in the rate of passage of digesta [10,11]. While the lower digestibility is better explained by the variations in the rumen microbial diversity and motility [7,12], heat stress is also reported to play prominent effects on the rumen fermentation profile, as reflected by differences in the concentrations of individual and total volatile fatty acid (TVFA) profiles [13,14].

Ruminants' contribution to global warming is one of the most contentious topics of the 21st century and the emissions are mainly in the form of enteric methane (CH_4) [15]. The CH_4 alone accounts for 47% of the livestock sector's total GHG emissions and 17.3% of global GHG emissions [16,17]. Therefore, for countries to meet their Paris Agreement obligations they must reduce their CH_4 emissions since CH_4 is the major contributor to

ground-level ozone and global warming [18]. Several broad strategies are available to reduce enteric CH_4 emissions including feed additives and managemental approaches. However, the most common and most promising is the use of feed additives.

It is possible to reduce the effect of heat stress on the digestive and rumen functions and reduce enteric CH_4 by increasing the proportion of concentrate in the animals' diet and supplementing it with rumen bypass feeds [19,20]. Therefore, it is important to evaluate commercially available rumen starch and protein bypass agents for achieving heat stress amelioration and CH_4 mitigation. We, therefore, selected a commercial starch binding agent, Bioprotect (Bioprotect™, RealisticAgri, Rutland, UK) to promote post-ruminal digestion of starch and protein components in animal diets [21]. Previous in vitro and in vivo studies have demonstrated the positive effects of Bioprotect on heat stress amelioration and rumen degradation of grains [22–24].

The hypotheses to be tested were that heat stress may impact rumen fermentation and CH_4 production and that modifying the site of digestion of wheat through rumen protection may reduce CH_4 emissions. Therefore, the objectives of this study were to determine the impact of heat stress, level of feed intake during heat stress, and rumen starch fermentation on rumen volatile fatty acid (VFA) concentrations, CH_4 emissions, and whole tract digestibility.

2. Materials and Methods

The experiment was conducted at the Animal facility of Dookie Campus, The University of Melbourne. All the experimental procedures involving animal handling and usage were approved by the animal ethics committee of the Faculty of Veterinary and Animal Sciences, The University of Melbourne (Ethics ID: 1914950.1). A total of 24 1-year-old Merino lambs with an average body weight of 42.6 ± 3.6 kg were randomly allocated into 3 dietary treatment groups: wheat-based diet (WD), Bioprotect-based diet (BD), and maize-based diet (MD). The WD diet was composed of 50% cracked wheat grain, 25% lucerne, and 25% oaten chaff. The BD diet consisted of 50% cracked wheat grain sprayed with 2% Bioprotect, 25% Lucerne, and 25% oaten chaff. The MD diet constituted 50% cracked maize grain and 25% Lucerne and oaten chaff. The lambs were fed twice at 09:00 a.m. and 01:00 p.m. daily; the chemical composition and ingredients of the diets are listed in Table 1. The feed analysis was performed according to the Australian Fodder Industry Association Ltd. method with the help of DPI Laboratory services, NSW, Australia. All the animals were subjected to three consecutive weeks of variable environmental and feeding conditions. After acclimation, the lambs were maintained under thermoneutral (18–21 °C and 40–50% relative humidity; RH) conditions and fed at 1.7 × maintenance feeding requirements (M) for 1 week (P1). During the second week, the animals were shifted to a cyclic heat stress environment (28–40 °C and 30–50% RH) and fed at 1.7 × M (P2); the room temperature was maintained at 38–40 °C between 09:00 a.m. and 05:00 p.m. and at 28 °C overnight. During the third week, the lambs were fed at 2.0 × M and exposed to heat stress (P3). The temperature humidity index (THI) was calculated for each period, and the THI data can be found in a study by Prathap et al. [22]. Prior to the experiment, the animals were acclimatized for 1 week in group pens, followed by 15 days of feed and individual pen acclimatization and then metabolic cage adaptation for a further 3 days before commencing P1. For the 3 d prior to P1 and for the duration of P1, P2, and P3, the lambs were housed in individual metabolic cages in environmentally controlled rooms. Water was provided ad libitum in individual buckets attached to the metabolic cages. The maintenance feeding level was calculated using the reference [25].

Table 1. Chemical composition of the experimental diets.

	WD	BD	MD
Diet Composition, %			
Cracked wheat	50	0	0
Cracked wheat with 2% BP	0	50	0
Cracked maize	0	0	50
Lucerne chaff	25	25	25
Oaten chaff	25	25	25
Chemical Composition of the Diet			
Dry matter, %	89.0	89.0	88.2
Organic matter, %	95.0	95.0	95.5
Crude protein, %	14.7	14.1	13.1
Neutral detergent fibre, %	29	30	28.5
Acid detergent fibre, %	16.3	16.3	16.3
Metabolisable energy, MJ/kg DM	11.5	11.6	11.7
Total starch, %	38.0	39.5	40.5

WD—wheat diet, BD—Bioprotect diet, MD—maize diet.

2.1. Methane Estimation, Rumen Fluid and Faeces Collection Digestibility, and Volatile Fatty Acid Determination

Enteric CH_4 was measured using an indirect calorimetry facemask system (S128 NDIR Methane Analyzer, Qubit Systems Inc, Kingston, ON, Canada). To calibrate the gas analysers prior to CH_4 measurements, 2 reference gases, 0 ppm CH_4 and 5000 ppm CH_4 were used (BOC Gas & Gear, Victoria, Australia). Before the commencement of the study, the sheep were gradually trained to wear a facemask over a period of 15 days by gradually increasing the time from 1 min to 20 min, which allowed the quantitative collection of exhaled gases [26]. Padding was added to the face mask so it would fit comfortably on the animal's muzzle and face. Gas collection and CH_4 measurement were conducted on day 7 of each period 2 h after morning and afternoon feeding continuously for 20 min. Then, the daily CH_4 emission was calculated according to the method reported by Oss et al. [27].

Rumen fluid was collected on the final day of each period at 4 h after the morning feeding using a handheld stomach pump as described by Pragna et al. [13]. An initial 100 mL of rumen fluid was discarded to avoid possible saliva contamination and the subsequent 20 mL of the rumen fluid was strained using a muslin cloth. The pH of the strained rumen fluid was determined using a hand-held pH meter (Hanna Instruments, Australia). For the volatile fatty acid (VFA) analysis, 1 mL of strained rumen fluid was mixed with 4 mL of 25% metaphosphoric acid and the proteins were allowed to precipitate at room temperature for 30 min. After centrifugation at 4752 g for 20 min, the supernatant was stored at −20 °C for later analysis. The short chain VFA concentrations were measured using gas chromatography (7890B Agilent, Santa Clara, CA, USA) with 4-methyl-valeric acid as an internal standard.

The faecal samples were collected on days 4, 5, and 6 of each period before morning feeding at 08:30 a.m. Faecal pH was determined by homogeneously mixing 50 g of faeces sample with 50 mL of distilled water. An immediate pH measurement was performed using a portable pH meter (Hanna Instruments, Australia). Then, 300 g of the pooled faecal samples were stored at −20 °C for further analyses. Faeces samples were initially dried at 60 °C for 48 h to determine DM [4]. Later, laboratory DM was estimated by keeping powdered faecal samples at 105 °C for 3 h according to AFIA [28] protocol. The total tract apparent DM and OM digestibility were determined using the acid insoluble ash [29] technique developed by Van Keulen and Young [30] with slight modifications [31,32]. The NDF [5] and ADF were determined according to the method described by ANKOM technology using an ANKOM2000 Fiber Analyzer (ANKOM Technology, New York, NY, USA).

2.2. Statistical Analyses

The data collected from the live animal experiment were analysed using the restricted maximum likelihood analysis (REML) procedure in Genstat 16th edition (Version 16.1.0.10916, VSN International Ltd, Hemel Hempstead, UK). Diet and period were considered as fixed model effects whereas animal number and replication were included as random models. A p-value of $\leq$0.05 was considered significant, and a p-value between 0.05 and 0.1 was considered a trend.

3. Results

Enteric CH_4 production was lower for the BD diet than the WD diet, which in turn was lower than the MD diet (25.0 g/day, 19.3 g/day, and 32.4 g/day for WD, BD, and MD, $p < 0.001$) (Table 2). Methane production was lower during P2 than P1 with P3 intermediate (28.5 g/day, 23.0 g/day, and 25.2 g/day for P1, P2, and P3, $p = 0.04$). When expressed relative to dry matter intake (DMI) CH_4 production was lower for the BD diet than the WD diet, which in turn was lower than the MD diet (21.3 g/kg, 16.3 g/kg, and 26.0 g/kg DMI for WD, BD, and MD, $p < 0.001$). On this basis, CH_4 production was less during heat stress than under thermoneutral conditions, regardless of DMI (24.1 g/kg, 20.2 g/kg, and 19.3 g/kg DMI for P1, P2, and P3, $p = 0.01$). Qualitatively similar findings occurred if CH_4 production was expressed on a metabolic body weight basis.

Table 2. Effects of different experimental periods and diets on the methane variables [1].

Parameters	WD			BD			MD			SED	*p*-Values		
	P1	P2	P3	P1	P2	P3	P1	P2	P3		Diet	Period	Diet × Period
Total methane production (CH_4, g/day)	27.8	23.0	24.2	20.6	18.1	19.0	37.1	27.9	32.2	3.66	<0.001	0.04	0.77
Methane yield (CH_4, g/kg of DMI)	23.9	20.6	19.2	17.6	16.4	14.8	30.7	23.5	24	3.10	<0.001	0.01	0.73
Emission intensity (CH_4, g/kg of /BW)	0.67	0.54	0.57	0.49	0.43	0.44	0.87	0.64	0.73	0.09	<0.001	0.02	0.76

[1] P1—Thermoneutral conditions (18–21 °C and 40–50% RH) and lambs fed 1.7 × M for 1 week; P2—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 1.7 × M for 1 week; P3—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 2.0 × M for 1 week. CH_4—methane; DMI—dry matter intake; BW—body weight.

As anticipated, there was no effect of diet on DMI (1.19 kg, 1.19 kg, and 1.26 kg DMI/day for WD, BD, and MD, $p = 0.29$) (Table 3). However, there were differences in DMI between periods such that DMI decreased when heat stress conditions were initially applied (P2) and then increased during P3 when the allocated feed offered was increased from 1.7 to 2.0 × M (1.19 kg, 1.14 kg, and 1.32 kg DMI/day for P1, P2, and P3, $p < 0.001$). Qualitatively similar findings occurred when DMI was expressed on a per kg LWT basis. While there was no effect of either diet or period on rumen fluid pH. There were significant effects of period and diet on faecal pH. Faecal pH was lower in sheep fed the MD diet than either of the wheat-based diets (8.05, 8.16, and 7.42 for WD, BD, and MD, $p < 0.001$) and was lower in P3 than P1 with P2 intermediate (8.05, 7.88, and 7.71 for P1, P2, and P3, $p = 0.05$). Whole tract DM digestibility was higher for the BD diet than the WD diet, which in turn was higher than the MD diet (83.0%, 84.2%, and 81.4% for WD, BD, and MD, $p < 0.001$) and was higher during P2 than the other periods (82.7%, 83.4%, and 82.6% for P1, P2, and P3, $p < 0.001$). However, there was an interaction ($p < 0.001$) such that the increase in DM digestibility between P1 and P2 only occurred in those sheep fed the wheat-based diets, particularly those fed the BD diet (Table 3). Whole tract OM digestibility was higher for the BD diet than the MD diet with the WD diet intermediate (84.0%, 84.9%, and 82.0% for WD, BD, and MD, $p < 0.001$) and tended to be higher during P2 than the other periods (83.3%, 84.1%, and 83.5% for P1, P2, and P3, $p = 0.06$). However, there was an interaction ($p < 0.001$) such that the increase in OM digestibility between P1 and P2 only occurred in those sheep fed the wheat-based diets, particularly those fed the BD diet (Table 3). Whole tract starch digestibility was higher for the BD and WD diets than the MD diet (97.1%, 98.0%, and 90.4% for WD, BD, and MD, $p < 0.001$) and decreased from P1 to P3 (96.9%, 95.3%, and

93.3% for P1, P2, and P3, $p < 0.001$). However, there was an interaction ($p < 0.001$) such that the decrease in starch digestibility between P1 and P2 and P2 and P3 only occurred in those sheep fed the MD diet and not in those fed the wheat-based diets (Table 3). There was no effect of diet on NDF digestibility while NDF digestibility tended to be lower for the WD diet than the other diets, while period had an influence on NDF digestibility (70.2%, 72.2%, and 72.1% for P1, P2, and P3, $p = 0.06$). Whole-tract ADF digestibility was greater for the MD diet than the wheat-based diets (70.1%, 70.5%, and 73.7% for WD, BD, and MD, $p = 0.01$) and was higher during heat stress than under thermoneutral conditions (69.8%, 72.2%, and 72.3% for P1, P2, and P3, $p = 0.01$).

Table 3. Effects of different experimental periods and diet on the DMI, pH, and digestibility variables [1].

Parameters	WD			BD			MD			SED	*p*-Values		
	P1	P2	P3	P1	P2	P3	P1	P2	P3		Diet	Period	Diet × Period
DMI, kg/day	1.17	1.12	1.29	1.17	1.11	1.30	1.22	1.19	1.37	0.05	0.29	<0.001	0.84
DMI, g/kg/day	27.7	26.4	30.6	27.5	25.9	30.7	28.4	27.6	31.6	0.94	0.36	<0.001	0.89
Rumen fluid pH	6.60	6.73	6.60	6.59	6.58	6.73	6.68	6.84	6.73	0.17	0.61	0.58	0.72
Faecal pH	8.20	8.03	7.93	8.22	8.11	8.14	7.72	7.49	7.05	0.23	<0.001	0.05	0.51
DM digestibility, %	82.8	83.4	82.8	83.3	84.6	84.8	82.1	82.1	80.1	0.58	<0.001	<0.001	<0.001
OM digestibility, %	83.3	84.3	84.4	83.9	85.1	85.5	82.8	82.7	80.5	0.70	<0.001	0.06	<0.001
Starch digestibility, %	97.2	96.7	97.3	98.7	97.9	97.3	94.8	91.3	85.2	1.60	<0.001	<0.001	<0.001
NDF digestibility, %	68.5	71.4	72.0	69.3	73.0	73.0	72.8	72.2	71.4	1.62	0.38	0.06	0.13
ADF digestibility, %	68.1	70.7	71.6	67.6	72.1	71.6	73.6	73.8	73.6	1.71	0.01	0.01	0.28

[1] P1—Thermoneutral conditions (18–21 °C and 40–50% RH) and lambs fed 1.7 × M for 1 week; P2—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 1.7 × M for 1 week; P3—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 2.0 × M for 1 week. DMI—dry matter intake; DM—dry matter; OM—organic matter; NDF—neutral detergent fibre; ADF—acid detergent fibre.

There was no effect of either diet or period on the rumen fluid concentrations of acetate (Table 4). Rumen propionate concentrations were higher for the WD diet than the BD diet, which in turn was higher than the MD diet (22.1 mmol/L, 16.3 mmol/L, and 13.3 mmol/L for WD, BD, and MD, $p = 0.002$). Rumen propionate concentrations were lower during heat stress than under thermoneutral conditions (22.2 mmol/L, 14.4 mmol/L, and 15.2 mmol/L for P1, P2, and P3, $p < 0.001$). There was no effect of diet on the rumen fluid concentrations of iso-butyrate whereas the concentrations were higher during heat stress (0.46 mmol/L, 0.64 mmol/L, and 0.75 mmol/L for P1, P2, and P3, $p = 0.006$). There was no effect of diet on the rumen fluid concentrations of butyrate whereas the concentrations tended to decrease during heat stress (13.5 mmol/L, 11.0 mmol/L, and 9.89 mmol/L for P1, P2, and P3, $p = 0.06$). There was no effect of diet on the rumen fluid concentrations of iso-valerate whereas the concentrations tended to increase when DMI was increased (0.36 mmol/L, 0.38 mmol/L, and 0.51 mmol/L for P1, P2, and P3, $p = 0.10$). Rumen valerate concentrations were higher for the WD diet than the BD diet, which in turn was higher than the MD diet (1.22 mmol/L, 0.69 mmol/L, and 0.37 mmol/L for WD, BD, and MD, $p = 0.001$). Rumen valerate concentrations were lower during P3 than P1 with P2 intermediate (0.93 mmol/L, 0.74 mmol/L, and 0.60 mmol/L for P1, P2, and P3, $p = 0.02$). Rumen TVFA concentrations were higher for the WD diet than the MD diet with the BD diet intermediate (44.5 mmol/L, 39.7 mmol/L, and 35.4 mmol/L for WD, BD, and MD, $p = 0.01$). Rumen TVFA concentrations were lower during heat stress than under thermoneutral conditions (48.1 mmol/L, 36.5 mmol/L, and 35.0 mmol/L for P1, P2, and P3, $p < 0.001$).

Table 4. Dietary effect on the rumen volatile fatty acids (VFAs) of Merino lambs subjected to 3 different feeding period levels and environmental conditions [1].

	WD			BD			MD			SED	*p*-Values		
	P1	P2	P3	P1	P2	P3	P1	P2	P3		Diet	Period	Diet × Period
Acetate, mmol/L	11.7	9.20	7.88	9.47	9.98	7.42	10.7	9.03	8.82	1.97	0.79	0.12	0.83
Acetate, % total VFA	20.7	25.8	21.0	21.8	22.6	23.2	24.1	28.5	26.9	4.20	0.18	0.39	0.89
Propionate, mmol/L	29.7	15.6	21.1	20.7	15.6	12.7	16.3	11.9	11.8	3.18	0.002	<0.001	0.12
Propionate, % VFA	51.9	44.1	48.8	46.7	39.5	37.7	38.7	35.8	38.7	5.80	0.057	0.16	0.76
Iso-butyrate, mmol/L	0.40	0.57	0.78	0.39	0.80	0.81	0.60	0.55	0.67	0.14	0.29	0.006	0.21
Iso-butyrate, % VFA	0.72	1.57	1.92	0.96	2.02	2.54	1.61	1.81	2.19	0.34	0.078	<0.001	0.27
Butyrate, mmol/L	13.8	8.65	9.23	12.9	14.0	11.0	13.9	10.2	9.45	2.57	0.29	0.06	0.54
Butyrate, % VFA	23.4	24.0	23.9	27.1	33.3	33.3	33.3	31.9	29.7	4.34	0.017	0.77	0.56
Iso-valerate, mmol/L	0.21	0.35	0.59	0.52	0.45	0.51	0.36	0.33	0.42	0.15	0.57	0.10	0.30
Iso-valerate, % VFA	0.41	0.99	1.95	1.26	1.12	1.64	0.97	1.07	1.37	0.56	0.86	0.028	0.49
Valerate, mmol/L	1.46	1.28	0.92	0.90	0.62	0.54	0.44	0.33	0.35	0.23	0.001	0.02	0.45
Valerate, % VFA	2.54	3.51	2.40	2.22	1.46	1.61	1.15	1.02	1.13	0.53	<0.001	0.54	0.13
Total VFAs, mmol/L	57.4	35.6	40.5	44.6	41.5	32.9	42.3	32.4	31.5	5.02	0.01	<0.001	0.15

[1] P1—Thermoneutral conditions (18–21 °C and 40–50% RH) and lambs fed 1.7 × M for 1 week; P2—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 1.7 × M for 1 week; P3—heat stress conditions (28–40 °C and 30–50% RH) and lambs fed at 2.0 × M for 1 week. TVFAs—total volatile fatty acids.

4. Discussion

The major findings from the present study were that enteric CH_4 production by sheep decreased by substituting wheat for maize in a 50% concentrate diet and that there was an even greater reduction when the wheat was treated with a starch and protein binding agent such as Bioprotect. Thus, on a feed intake basis, there were 18% and 37% lower enteric CH_4 production rates in sheep consuming WD and BD compared with MD. Previously, Moate et al. [33] reported that for dairy cows consuming diets containing 45% grain, there was a 30% reduction in enteric CH_4 production rates in lactating dairy cows when wheat was substituted for maize. These authors attributed this reduction in CH_4 production rates to differences in starch digestion in the rumen and effects on either average rumen pH or the amount of time that the pH was below 6.0. Similarly, Moate et al. [34] reported a 43% lower reduction in enteric CH_4 production rates in lactating dairy cows when wheat was substituted for maize. However, in the present study there was no difference in rumen pH between the diets, albeit this was in a single sample taken 4 h post morning feeding. Moreover, the further effect of Bioprotect on enteric CH_4 production over wheat could not be explained by more rapid starch fermentation alone as one of the actions of Bioprotect is to reduce rumen starch fermentation by reversibly binding to hydroxyl groups of starches [22,23,35]. The rumen TVFA and propionate concentrations were higher for sheep fed the WD diet than the MD diet with the BD diet being intermediate which supports the relative differences in in vitro fermentation [36]. While higher starch fermentation and inhibition of cellulolytic fermentation may explain the reduction in enteric CH_4 production with the WD diet compared with MD, it does not explain the further decrease observed in sheep feed the BD diet since fermentation of BD is intermediate.

In addition to binding to starch, Bioprotect is also purported to react with the primary and secondary amino groups and form stable protein complexes at rumen pH, which dissociate at the more acidic conditions as they exist in the abomasum and duodenum. Thus, gas production from protein meals treated with Bioprotect is reduced indicating an increase in rumen undegradable digestible protein (RUDP) [36]. Therefore, it is possible that some of the decrease in enteric CH_4 emission from sheep consuming the BD diet may be due to an increase in RUDP. Wheat would have provided 40% of the dietary protein; therefore, Bioprotect-treated wheat could have been a not inconsequential source of RUDP in the BD diet. Lamba et al. [37] found that increasing RUDP was associated with decreased in vitro CH_4 production, which supports this concept and was consistent with their earlier work with conventional and unconventional protein sources [38]. Thus,

the lower rate of CH_4 emission from sheep fed the BD diet may be due to a reduction in rumen fermentation and digestion of both starch and protein and a shift in their site of digestion. Shipandeni et al. [23] confirmed this possible shift in digestion by showing a reduction in in vitro rumen starch degradation of maize and sorghum treated with Bioprotect. Another possible reason for the decrease in CH_4 from the WD and BD-fed animals could be attributed to the higher propionate concentration in the WD- and BD-fed animals compared with the MD-fed animals and the decreased concentration of butyrate in the BD-fed animals than the WD and MD-fed sheep. The increased propionate production and decreased butyrate production in the rumen of BD-fed sheep could have freely available hydrogen in the rumen that would otherwise be used by methanogens to reduce CO_2 to produce CH_4 [15].

In the current experiment, sheep exhibited higher CH_4 production (g/day) and CH_4 yield (g/kg of DMI) during P1 compared with P2 and P3. Even though the sheep had higher rumen propionate concentrations during P1, this increase in propionate did not translate into reduced CH_4 production. Similar to our results, Yadav et al. [39] observed an increase in the CH_4 production in Vrindavani crossbred cattle at 30 °C and 35 °C of heat stress exposure. However, they noted an increase in CH_4 production at 40 °C. In addition, an experiment conducted on dairy farms in India reported a decrease in CH_4 emissions from cows with an increase in the temperature humidity index [40]. However, the reason for this decrease in CH_4 emissions from the rumen during heat stress is unclear since the diversity of rumen bacteria was not assessed.

Differences in the DMI between periods are explained by the differences in the feeding level between the periods and the heat-stress-induced reduction in the feed intake by the sheep [22]. In general, digestibility variables and rumen fermentation characteristics are expected to vary substantially with the differences in the diet [41]. Diets with high starch content are generally associated with acidosis and lower rumen fluid pH in animals [42]. However, sheep in our experiment did not exhibit any changes in their rumen fluid pH either with diet or period. A positive effect of ration splitting could partially explain this non-significant effect on rumen fluid pH. Due to the sufficient interval between two feedings, the pH of the sheep's rumen fluid might have recovered after the feedings, reducing the impact on rumen microbes [43]. Among the dietary treatment groups, MD-fed sheep had very low faecal pH with the lowest values recorded during P3 (pH = 7.05), suggesting that these sheep had an increased amount of starch fermented in their hindgut [42]. Khorrami et al. [42] reported a decrease in hindgut fermentation during the daytime and this is associated with lower faecal pH. The lower pH during the heat stress periods are better explained by the possible imbalance in the production and use of acid in the rumen [8]. The decrease in faecal pH and possible low absorption rate of acid at the hindgut may explain the visual observation of undigested maize grains in our sheep faeces.

Both DM and OM digestibility showed a similar trend for the effect of diet and period. Lower digestibility values of starch during the periods of heat stress (P2 and P3) observed in this experiment agree with other studies conducted in ruminants [8,9]. These differences in the digestibility across the periods could be attributed to a number of factors such as variations in water intake, rumen motility, and the rumen microbiota [12]. Wheat treated with Bioprotect significantly improved its digestibility of DM, OM, and starch. Similar effects of Bioprotect supplementation on total tract digestibility were reported in in vitro experiments [21,44]. The results of this study support the hypothesis that Bioprotect treatment increases post-ruminal digestion of the grains fed to animals. In addition to our results, Philippeau et al. [45] observed higher digestion of DM, OM, and starch in steers fed wheat and wheat-based diets than those fed corn. According to Chandrashekar and Kirleis [46], starch particles in maize might have maintained constant structural integrity throughout digestion by remaining tightly embedded in the protein matrix, preventing microbes from accessing starch. These studies would indicate that the lower digestibility of the maize diet is linked to the structural integrity and particle size of the maize. Philippeau et al. [45] opined that the corn might require milling to reduce particle size for better performance.

Thus, the low starch digestibility of MD explains the low faecal pH and the remnants of the maize granules in the faeces of sheep. The NDF digestibility was unaffected either by diet or period. The reason for the decrease in the ADF digestibility in the BD and WD diets compared with the MD diet is not clear, but it may be related to the differences in the bacterial communities in the rumen. It should be noted that the NDF and ADF digestibility were relatively high although Garry et al. [47] found that NDF digestibility was higher than reported here and higher in sheep than lactating dairy cows, particularly in summer. Future studies to investigate the effects of WD, BD, and MD on the rumen microbiota of heat-stressed sheep could lead to a better understanding of the effects of different grains and treatment of wheat with Bioprotect on digestibility variables over a longer period of time.

The current study demonstrated a significant shift in the TVFA production of the sheep in response to the experimental periods and diets. Significantly higher TVFA concentration in P1 compared with P2 and P3 could be because of the increased water intake [22] and an associated increase in the rumen turnover rate and dilution of the rumen digesta [48,49]. This high turnover rate can sometimes culminate in the elimination of partially digested substrate from the rumen by not giving enough time for their fermentation [50]. A dramatically greater increase in TVFA production of WD-fed animals in P2 than in P1 is explained by the drastic increase in water intake (data published elsewhere; [22]) observed in WD animals during P2. The higher TVFA production in the WD-fed animals than those of the BD-fed animals indicate the higher fermentation in the rumen and this supports our hypothesis that Bioprotect-treated wheat can bypass the rumen to promote hindgut digestion. Regardless of the period and diet, acetate and iso-valerate did not show any significant variation. Contrary to our results, a difference in the concentrations of acetate and iso-valerate due to heat stress was observed in goats [13,51] and sheep [52]. Further, propionate production was lower during P2 and P3 compared with P1, which would have played a beneficial role in reducing the heat increment [49]. The lower concentration of propionate in the rumen fluid of sheep fed the MD diet could explain the higher CH_4 output from the MD group because propionate serves as a hydrogen sink [15]. Production of propionate consumes hydrogen atoms making less hydrogen available for the production of CH_4 [53]. The effect of dietary treatment was not significant for the concentration of iso-butyrate and butyrate. Our findings of decreased butyrate production during P2 and P3 are consistent with Nonaka et al. [14], who reported a reduction in butyrate production in Holstein heifers kept at 33 °C compared with the heifers at 20 °C or 28 °C. Higher butyrate production during P1 might account for the higher production of CH_4 during P1 [54]. The increase in the production of iso-butyrate during P3 might be due to the increased level of feeding and this indicates the high energy utilization in the rumen [55]. The WD-fed animals had higher valerate concentrations than their counterparts, and valerate concentrations decreased linearly across the period with the lowest values recorded in P3. Similar to our findings, Moate et al. [56] also found increased valerate concentrations in cows that were fed maize compared with cows fed wheat.

5. Conclusions

In conclusion, heat stress reduced rumen VFA concentrations and enteric CH_4 production with no decrease in whole tract digestibility except in lambs consuming the MD diet at high feed intakes. This was particularly for starch digestion. Lambs fed the MD diet produced more enteric CH_4 production than those consuming the WD diet. The treatment of the WD diet with a starch and protein binding agent further reduced enteric CH_4 production.

Author Contributions: Conceptualization, P.P. and F.R.D.; methodology, P.P.; software, P.P. and F.R.D.; validation, P.P., S.S.C., J.J.C., B.J.L. and F.R.D.; visualization, P.P.; formal analysis, P.P. and F.R.D.; investigation, P.P., S.S.C., A.J. and M.Z.; writing—original draft preparation, P.P.; writing—review and editing, S.S.C., J.J.C., B.J.L. and F.R.D.; supervision, S.S.C., J.J.C., B.J.L. and F.R.D.; funding acquisition, F.R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the guidelines from the Animal Ethics Committee (Ethics ID: 1914950.1) of the Faculty of Veterinary and Agricultural Sciences, The University of Melbourne.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available from corresponding author upon reasonable request.

Acknowledgments: Pragna Prathap received a Melbourne Research Scholarship for her Ph.D. at The University of Melbourne. The authors acknowledge the assistance provided by students and staff from the Faculty of Veterinary and Agricultural Sciences of The University of Melbourne. The authors gratefully acknowledge FeedWorks for supplying the grains and Bioprotect used in these experiments and Sumant Bhat for providing instrument training to Pragna.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Savsani, H.H.; Padodara, R.J.; Bhadaniya, A.R.; Kalariya, V.A.; Javia, B.B.; Ghodasara, S.N.; Ribadiya, N.K. Impact of climate on feeding, production and reproduction of animals-a review. *Agric. Rev.* **2015**, *36*, 26–36. [CrossRef]
2. Beede, D.; Collier, R. Potential nutritional strategies for intensively managed cattle during thermal stress. *J. Anim. Sci.* **1986**, *62*, 543–554. [CrossRef]
3. Gonzalez-Rivas, P.A.; Prathap, P.; DiGiacomo, K.; Cottrell, J.J.; Leury, B.J.; Chauhan, S.S.; Dunshea, F.R. Reducing rumen starch fermentation of wheat with 3% naoh does not reduce whole tract starch digestibility and increases energy utilization in wethers during heat stress. *Small Rumin. Res.* **2021**, *204*, 106523. [CrossRef]
4. Vanlierde, A.; Soyeurt, H.; Gengler, N.; Colinet, F.G.; Froidmont, E.; Kreuzer, M.; Grandl, F.; Bell, M.; Lund, P.; Olijhoek, D.W.; et al. Short communication: Development of an equation for estimating methane emissions of dairy cows from milk fourier transform mid-infrared spectra by using reference data obtained exclusively from respiration chambers. *J. Dairy Sci.* **2018**, *101*, 7618–7624. [CrossRef]
5. Silveira, S.R.; Terry, S.A.; Biffin, T.E.; Maurício, R.M.; Pereira, L.G.R.; Ferreira, A.L.; Ribeiro, R.S.; Sacramento, J.P.; Tomich, T.R.; Machado, F.S.; et al. Replacement of soybean meal with soybean cake reduces methane emissions in dairy cows and an assessment of a face-mask technique for methane measurement. *Front. Vet. Sci.* **2019**, *6*, 295. [CrossRef] [PubMed]
6. Christopherson, R.J.; Kennedy, P.M. Effect of the thermal environment on digestion in ruminants. *Can. J. Anim. Sci.* **1983**, *63*, 477–496. [CrossRef]
7. Bernabucci, U.; Lacetera, N.; Danieli, P.P.; Bani, P.; Nardone, A.; Ronchi, B. Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. *Int. J. Biometeorol.* **2009**, *53*, 387–395. [CrossRef] [PubMed]
8. Cai, L.; Yu, J.; Hartanto, R.; Zhang, J.; Yang, A.; Qi, D. Effects of heat challenge on growth performance, ruminal, blood and physiological parameters of chinese crossbred goats. *Small Rumin. Res.* **2019**, *174*, 125–130. [CrossRef]
9. Maloiy, G.M.O.; Kanui, T.I.; Towett, P.K.; Wambugu, S.N.; Miaron, J.O.; Wanyoike, M.M. Effects of dehydration and heat stress on food intake and dry matter digestibility in east african ruminants. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2008**, *151*, 185–190. [CrossRef]
10. West, J.W. Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.* **2003**, *86*, 2131–2144. [CrossRef] [PubMed]
11. Nejad, J.G.; Lohakare, J.D.; West, J.W.; Sung, K.I. Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in corriedale ewes. *Anim. Feed Sci. Technol.* **2014**, *193*, 1–8. [CrossRef]
12. Tajima, K.; Nonaka, I.; Higuchi, K.; Takusari, N.; Kurihara, M.; Takenaka, A.; Mitsumori, M.; Kajikawa, H.; Aminov, R.I. Influence of high temperature and humidity on rumen bacterial diversity in holstein heifers. *Anaerobe* **2007**, *13*, 57–64. [CrossRef]
13. Pragna, P.; Sejian, V.; Soren, N.; Bagath, M.; Krishnan, G.; Beena, V.; Devi, P.I.; Bhatta, R. Summer season induced rhythmic alterations in metabolic activities to adapt to heat stress in three indigenous (osmanabadi, malabari and salem black) goat breeds. *Biol. Rhythm Res.* **2018**, *49*, 551–565. [CrossRef]
14. Nonaka, I.; Takusari, N.; Tajima, K.; Suzuki, T.; Higuchi, K.; Kurihara, M. Effects of high environmental temperatures on physiological and nutritional status of prepubertal holstein heifers. *Livest. Sci.* **2008**, *113*, 14–23. [CrossRef]
15. Prathap, P.; Chauhan, S.S.; Leury, B.J.; Cottrell, J.J.; Dunshea, F.R. Towards sustainable livestock production: Estimation of methane emissions and dietary interventions for mitigation. *Sustainability* **2021**, *13*, 6081. [CrossRef]
16. Opio, C.; Gerber, P.; Mottet, A.; Falcucci, A.; Tempio, G.; MacLeod, M.; Vellinga, T.; Henderson, B.; Steinfeld, H. *Greenhouse Gas Emissions from Ruminant Supply Chains—A Global Life Cycle Assessment*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013.
17. Ritchie, H.; Roser, M.; Rosado, P. CO_2 and Greenhouse Gas Emissions. 2020. Available online: https://ourworldindata.org/ (accessed on 23 May 2022).

18. Chung, T. *Global Assessment: Urgent Steps Must Be Taken to Reduce Methane Emissions This Decade*; United Nations Climate Change: Bonn, Germany, 2021.
19. Chen, J.; Guo, K.; Song, X.; Lan, L.; Liu, S.; Hu, R.; Luo, J. The anti–heat stress effects of chinese herbal medicine prescriptions and rumen-protected γ-aminobutyric acid on growth performance, apparent nutrient digestibility, and health status in beef cattle. *Anim. Sci. J.* **2020**, *91*, e13361. [CrossRef] [PubMed]
20. Beauchemin, K.A.; Kreuzer, M.; O'Mara, F.; McAllister, T.A. Nutritional management for enteric methane abatement: A review. *Aust. J. Exp. Agric.* **2008**, *48*, 21–27. [CrossRef]
21. RealisticAgri. Bioprotect. Available online: https://www.realisticagri.com/nutrition/bioprotect/ (accessed on 22 April 2020).
22. Prathap, P.; Chauhan, S.S.; Leury, B.J.; Cottrell, J.J.; Joy, A.; Zhang, M.; Dunshea, F.R. Reducing the fermentability of wheat with a starch binding agent reduces some of the negative effects of heat stress in sheep. *Animals* **2022**, *12*, 1396. [CrossRef] [PubMed]
23. Shipandeni, M.N.T.; Cruywagen, C.W.; Raffrenato, E. Effects of a starch binding agent on in vitro rumen degradability of maize and sorghum starch. *S. Afr. J. Anim. Sci.* **2020**, *50*, 814–818. [CrossRef]
24. Gonzalez-Rivas, P.A.; Sullivan, M.; Cottrell, J.J.; Leury, B.J.; Gaughan, J.B.; Dunshea, F.R. Effect of feeding slowly fermentable grains on productive variables and amelioration of heat stress in lactating dairy cows in a sub-tropical summer. *Trop. Anim. Health Prod.* **2018**, *50*, 1763–1769. [CrossRef] [PubMed]
25. Liu, Z.; McMeniman, N. Effect of nutrition level and diets on creatinine excretion by sheep. *Small Rumin. Res.* **2006**, *63*, 265–273. [CrossRef]
26. Chauhan, S.S.; Celi, P.; Leury, B.; Liu, F.; Dunshea, F.R. Exhaled breath condensate hydrogen peroxide concentration, a novel biomarker for assessment of oxidative stress in sheep during heat stress. *Anim. Prod. Sci.* **2015**, *56*, 1105–1112. [CrossRef]
27. Oss, D.B.; Marcondes, M.I.; Machado, F.S.; Pereira, L.G.R.; Tomich, T.R.; Ribeiro, G.O.; Chizzotti, M.L.; Ferreira, A.L.; Campos, M.M.; Maurício, R.M.; et al. An evaluation of the face mask system based on short-term measurements compared with the sulfur hexafluoride (sf6) tracer, and respiration chamber techniques for measuring ch4 emissions. *Anim. Feed Sci. Technol.* **2016**, *216*, 49–57. [CrossRef]
28. AFIA. *AFIA—Laboratory Methods Manual*; Australian Fodder Industry Association Inc.: Ascot Vale, Australia, 2011; Volume 7.
29. Abeni, F.; Stefanini, L.; Calamari, L.; Maianti, M.G.; Cappa, V. Effects of heat stress on lactating dairy cows and feeding strategy to reduce its impact on milk yield and quality. *Ann. Della Fac. Agrar. Univ. Del Sacro Cuore Milano* **1993**, *33*, 151–170.
30. Van Keulen, J.; Young, B.A. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* **1977**, *44*, 282–287. [CrossRef]
31. Papadomichelakis, G.; Fegeros, K. Reliability of acid-insoluble ash as internal marker for the measurement of digestibility in rabbits. *World Rabbit Sci.* **2020**, *28*, 1–12. [CrossRef]
32. Gonzalez-Rivas, P. *Effect of Feeding Slowly Fermentable Grains as Protection against Heat Stress in Ruminants*; The University of Melbourne: Melbourne, Australia, 2017; p. 282.
33. Moate, P.J.; Williams, S.R.O.; Jacobs, J.L.; Hannah, M.C.; Beauchemin, K.A.; Eckard, R.J.; Wales, W.J. Wheat is more potent than corn or barley for dietary mitigation of enteric methane emissions from dairy cows. *J. Dairy Sci.* **2017**, *100*, 7139–7153. [CrossRef]
34. Moate, P.J.; Williams, S.R.O.; Deighton, M.H.; Hannah, M.C.; Ribaux, B.E.; Morris, G.L.; Jacobs, J.L.; Hill, J.; Wales, W.J. Effects of feeding wheat or corn and of rumen fistulation on milk production and methane emissions of dairy cows. *Anim. Prod. Sci.* **2018**, *59*, 891–905. [CrossRef]
35. Dunshea, F.R.; Russo, V.M.; Sawyer, I.; Leury, B.J. A starch-binding agent decreases the in vitro rate of fermentation of wheat. *J. Dairy Sci.* **2012**, *95*, 199.
36. Dunshea, F.; Pate, S.A.; Russo, V.M.; Leury, B.J. A starch binding agent decreases the in vitro rate of fermentation of wheat in a dose-dependent manner. In Proceedings of the 64th European Federation of Animal Science, Nantes, France, 26–30 August 2013.
37. Lamba, J.S.; Wadhwa, M.; Bakshi, M.P.S. Impact of level of rumen undegradable protein on in-vitro methane production and in-sacco degradability of concentrate mixtures. *Cellulose* **2019**, *8*, 15–70.
38. Lamba, J.S.; Hundal, J.S.; Wadhwa, M.; Bakshi, M.P.S. In-vitro methane production potential and in-sacco degradability of conventional and non-conventional protein supplements. *Indian J. Anim. Sci.* **2014**, *84*, 539–543.
39. Yadav, B.; Singh, G.; Wankar, A.; Dutta, N.; Chaturvedi, V.; Verma, M.R. Effect of simulated heat stress on digestibility, methane emission and metabolic adaptability in crossbred cattle. *Asian-Australas. J. Anim. Sci.* **2016**, *29*, 1585. [CrossRef]
40. Pinto, A.; Yin, T.; Reichenbach, M.; Bhatta, R.; Malik, P.K.; Schlecht, E.; König, S. Enteric methane emissions of dairy cattle considering breed composition, pasture management, housing conditions and feeding characteristics along a rural-urban gradient in a rising megacity. *Agriculture* **2020**, *10*, 628. [CrossRef]
41. Jefferson, A.; Costa-Campos, A.; Nascimento-Coutinho, D.; Soares-Freitas, C.A.; dos-Anjos, A.J.; Rocha-Bezerra, L. Effect of the diet on ruminal parameters and rumen microbiota: Review: Effect of the diet on rumen fermentation. *Rev. Colomb. De Cienc. Anim.* **2022**, *14*, e886.
42. Khorrami, B.; Kheirandish, P.; Zebeli, Q.; Castillo-Lopez, E. Variations in fecal ph and fecal particle size due to changes in dietary starch: Their potential as an on-farm tool for assessing the risk of ruminal acidosis in dairy cattle. *Res. Vet. Sci.* **2022**, *152*, 678–686. [CrossRef]
43. Metzler-Zebeli, B.U.; Schmitz-Esser, S.; Klevenhusen, F.; Podstatzky-Lichtenstein, L.; Wagner, M.; Zebeli, Q. Grain-rich diets differently alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats. *Anaerobe* **2013**, *20*, 65–73. [CrossRef] [PubMed]

44. Gonzalez, P.; Price, M.; Digiaconomo, K.; Henry, M.L.; Leury, B.; Russo, V.; Cakebread, P.; Dunshea, F. Rumen protection of wheat with a starch binding agent does not reduce whole tract digestibility in sheep. proc. *Aust. Soc. Anim. Prod.* **2014**, *30*, 107.
45. Philippeau, C.; Martin, C.; Michalet-Doreau, B. Influence of grain source on ruminal characteristics and rate, site, and extent of digestion in beef steers. *J. Anim. Sci.* **1999**, *77*, 1587–1596. [CrossRef] [PubMed]
46. Chandrashekar, A.; Kirleis, A.W. Influence of protein on starch gelatinization in sorghum. *Cereal Chem.* **1988**, *65*, 457–462.
47. Garry, B.; McGovern, F.M.; Kennedy, E.; Baumont, R.; Boland, T.M.; Wright, M.M.; O'Donovan, M.; Lewis, E. Comparison of sheep and dairy cows for in vivo digestibility of perennial ryegrass. *Animal* **2021**, *15*, 100258. [CrossRef]
48. Fraley, S.E.; Hall, M.B.; Nennich, T.D. Effect of variable water intake as mediated by dietary potassium carbonate supplementation on rumen dynamics in lactating dairy cows. *J. Dairy Sci.* **2015**, *98*, 3247–3256. [CrossRef] [PubMed]
49. Hou, Y.; Zhang, L.; Dong, R.Y.; Liang, M.Y.; Lu, Y.; Sun, X.Q.; Zhao, X. Comparing responses of dairy cows to short-term and long-term heat stress in climate-controlled chambers. *J. Dairy Sci.* **2021**, *104*, 2346–2356. [CrossRef] [PubMed]
50. West, J.W.; Coppock, C.E.; Milam, K.Z.; Nave, D.H.; Labore, J.M.; Rowe, L.D., Jr. Potassium carbonate as a potassium source and dietary buffer for lactating holstein cows during hot weather. *J. Dairy Sci.* **1987**, *70*, 309–320. [CrossRef]
51. Chaidanya, K.; Soren, N.M.; Sejian, V.; Bagath, M.; Manjunathareddy, G.B.; Kurien, K.E.; Varma, G.; Bhatta, R. Impact of heat stress, nutritional stress and combined (heat and Nutritional) stresses on rumen enzymes and fermentation metabolites in Osmanabadi bucks. In Proceedings of the Climate Change Adaptation and Biodiversity: Ecological Sustainability and Resource Management for Livelihood Security, Portblair, India, 8–10 December 2016; Andaman Science Association: Portblair, India, 2016.
52. Amini, A.; Pirmohammadi, R.; Khalilvandi-Behroozyar, H.; Mazaheri-Khameneh, R. Effects of heat stress on in vivo and in vitro ruminal metabolism in fat-tailed ewes. *Anim. Prod. Sci.* **2022**, *62*, 860–869. [CrossRef]
53. Wang, K.; Xiong, B.; Zhao, X. Could propionate formation be used to reduce enteric methane emission in ruminants? *Sci. Total Environ.* **2023**, *855*, 158867. [CrossRef] [PubMed]
54. Moss, A.R.; Jouany, J.-P.; Newbold, J. Methane production by ruminants: Its contribution to global warming. *Ann. De Zootech.* **2000**, *49*, 231–253. [CrossRef]
55. Cui, Z.; Wu, S.; Liu, S.; Sun, L.; Feng, Y.; Cao, Y.; Chai, S.; Zhang, G.; Yao, J. From maternal grazing to barn feeding during pre-weaning period: Altered gastrointestinal microbiota contributes to change the development and function of the rumen and intestine of yak calves. *Front. Microbiol.* **2020**, *11*, 485. [CrossRef]
56. Moate, P.J.; Jacobs, J.L.; Hannah, M.C.; Morris, G.L.; Beauchemin, K.A.; Hess, P.S.A.; Eckard, R.J.; Liu, Z.; Rochfort, S.; Wales, W.J. Adaptation responses in milk fat yield and methane emissions of dairy cows when wheat was included in their diet for 16 weeks. *J. Dairy Sci.* **2018**, *101*, 7117–7132. [CrossRef]