fermentation

MDPI

Article

Ruminal Degradation of Taurine and Its Effects on Rumen Fermentation *In Vitro*

Shuo Zhang †, Qinyue Liang †, Mengmeng Li and Guangyong Zhao *

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, 2 Yuan Ming Yuan West Road, Haidian District, Beijing 100193, China
* Correspondence: zhaogy@cau.edu.cn; Tel.: +86-10-62733379
† These authors contributed equally to this work.

Abstract: Taurine accounts for approximately 0.1% of an animal's body. It cannot be used for protein synthesis but plays a wide range of important roles in the animal body. Taurine does not exist in plants, while mammals can only synthesize 30–40% of the taurine they need. Supplementing taurine to beef cattle may be necessary to improve their nutrient utilization and health status. However, no data are available regarding the metabolism of taurine in the rumen. Two *in vitro* trials were conducted to investigate the ruminal degradability of taurine and its effects on rumen fermentation. In Trial 1, Tilley and Terry's *in vitro* rumen fermentation technique was used for incubation. As treatments, two levels of taurine, i.e., 0 and 10 mg, were added into plastic tubes containing 0.4000 g of feed mixture with a calibrated volume of 50 mL. Three adult cattle fitted with rumen cannulas were used as the donors for rumen fluid. The incubation was carried out at 39 °C for 48 h. The results showed that the taurine degradability increased with incubation time ($p < 0.001$) while its 2 h-degradability reached 99%. Taurine decreased the 48 h-dry matter degradability (DMD) ($p = 0.008$) and increased the 24 h- and 48 h-pH ($p = 0.005$; $p = 0.018$), respectively. In Trial 2, the Hohenheim gas test was used for incubation. Four levels of taurine, i.e., 0, 5, 10 and 20 mg, were added into glass syringes containing 0.2000 g feed mixture with a calibrated volume of 100 mL as treatments. The rumen fluid donors were the same as in Trial 1. The incubation was carried out at 39 °C for 48 h. The results showed that taurine increased the 48 h-pH ($p < 0.001$) linearly, decreased the cumulative gas production ($p < 0.001$) and the total volatile fatty acids (VFA) concentration ($p = 0.014$), and quadratically affected the ammonia–nitrogen ($p < 0.001$) and microbial crude protein (MCP) concentrations ($p < 0.001$). It was concluded that taurine was highly degradable in rumen fermentation. Taurine inhibits ruminal fermentation by decreasing DMD, VFA and gas production while improving MCP synthesis on a dose-dependent basis.

Keywords: degradability; *in vitro*; rumen; taurine

Citation: Zhang, S.; Liang, Q.; Li, M.; Zhao, G. Ruminal Degradation of Taurine and Its Effects on Rumen Fermentation *In Vitro*. *Fermentation* **2023**, *9*, 43. https://doi.org/10.3390/fermentation9010043

Academic Editor: Anusorn Cherdthong

Received: 14 November 2022
Revised: 10 December 2022
Accepted: 11 December 2022
Published: 3 January 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Taurine was first isolated as a component from *ox* (Bos taurus, from which its name is derived) bile in 1827 [1], and its chemical structure was determined in 1850. Since then, considerable efforts have been devoted to its chemical properties, functionalities, absorption and metabolism. Taurine is a sulfur-containing amino acid (AA) that accounts for approximately 0.1% of the animal body [2]. Taurine cannot be used for protein synthesis [3] but plays a wide range of pivotal roles in humans and animals, including functions in muscle growth [4], energy metabolism [5], bile acid conjugation [6], immune response, antioxidation [7], osmotic adjustment, regulation of blood pressure, management of the retinal and cardiac function, modulation of neuroendocrine activity [8], and therapy of fatty liver disease [9]. Taurine deficiency may cause weak energy metabolism and energy metabolism dysfunction [5]. Taurine has been widely used as a feed additive in aquatic and poultry animals to enhance feed efficiency, promote growth, and prevent diseases. Previous

studies showed that supplementing the diet with taurine at 2.5 or 5.0 g/kg improved the feed intake, egg production, and apparent digestibility of quails [10] and the addition of taurine at 12.3 g/kg and 16.7 g/kg improved the growth and health status of belugas [11]. It was also reported that adding taurine to the diet of chicken improved antioxidant activity and growth performance [12,13].

In mammals, taurine is mainly synthesized in the liver using methionine as the precursor [14]. However, the amount of taurine synthesized in the animal body can only meet 30–40% of their requirement [15]. Animal products, especially aquatic products, contain a high level of taurine, whereas plants almost do not contain taurine [16]. As herbivores, beef cattle cannot be fed with feeds of animal products. Therefore, adult beef cattle are quite possibly in a taurine deficiency status. Supplementing their diets with taurine could possibly improve the growth performance and feed utilization efficiency of beef cattle. To the best of our knowledge, however, no data are available regarding the ruminal degradation of taurine and its effects on rumen fermentation. Free amino acids (AA), such as methionine and lysine, are extensively degradable by ruminal microorganisms [17]. As a sulfur-containing AA, it could be hypothesized that taurine is also degradable and plays an important role in rumen fermentation. Therefore, the objectives of the present study were to investigate the effects of taurine on rumen fermentation and the ruminal degradation of taurine using *in vitro* rumen fermentation techniques to clarify the usefulness of taurine as a feed additive for beef cattle.

2. Materials and Methods

2.1. Trial 1—Ruminal Degradation of Taurine and Its Effect on DM Degradability

The animal experimental procedures and protocols were approved by the Animal Care and Use Committee of China Agricultural University, Beijing, China (No. 20130611–2).

2.1.1. *In Vitro* Rumen Fermentation and Experiment Design

The first step of Tilley and Terry's two-stage digestion method [18] was used for *in vitro* rumen fermentation. Three rumen-cannulated Angus steers (450 ± 50 kg live weight) were used as the donors for rumen fluid. The animals were fed a total mixed ration (TMR, Table 1), including 15 kg corn silage and 4 kg concentrate mixture. The daily ration was divided into two equal portions and supplied to the steers at 7:00 and 17:00, respectively. The steers had free access to drinking water, and no feed residues were left. About 500 mL of rumen fluid was taken from each steer before morning feeding. The rumen fluid samples from each steer were mixed and strained through four layers of cheesecloth into a prewarmed (39 °C) insulated bottle. The ruminal fluid and buffer were mixed at a ratio of 1:4 (*v*/*v*). Forty-five centrifuge tubes of a calibrated volume of 50 mL with a rubber plug and an air outlet slit were used as the incubators, of which 35 tubes were used as the treatment group, and 10 tubes were used as the control group. Each tube of the treatment group contained 0.4000 g of feed mixture, and 10 mg taurine (purity ≥ 99.0%, Aladdin Industries) was added, while each tube of the control group also contained 0.4000 g feed mixture but without taurine. A volume of 40 mL rumen fluid–buffer mixture was added into each tube and incubated in a biochemical incubator at 39 °C (SHP-205, Peiyin Instruments Co., Ltd., Shanghai, China). The tubes were shaken manually every 2–3 h during incubation. Five tubes of the treatment group were taken out at 2, 4, 8, 12, 24, 36 and 48 h of incubation time, and five tubes of the control group were taken out at 24 and 48 h of incubation time, respectively. The tubes were kept in ice water to stop the incubation. The pH of the 24 h- and 48 h-liquid samples of incubation was immediately measured using a pH meter (PHS-3C, Yueping Instrument Manufacturing Co., Ltd., Shanghai, China).

Table 1. Ingredients and chemical composition of the diet for the steers as rumen fluid donor (% DM).

Items	Contents
Ingredients	
Corn silage	30.00
Corn stover	15.00
Corn	31.90
Soybean meal	9.35
Chinese date powder	8.25
Premix	2.20
Sodium bicarbonate	1.10
Salt	1.10
Calcium hydrogen phosphate	1.10
Total	100.00
Nutrients	
CP	13.61
NDF	43.20
ADF	22.89
Calcium	0.47
Phosphorus	0.22

Notes: Composition of Premix: Fe 12 g/kg; Mn 1 g/kg; Cu 1 g/kg; Zn 11 g/kg; I 30 mg/kg; Se 30 mg/kg; Co 20 mg/kg; Vitamin A 450,000 IU/kg; Vitamin D 360,000 IU/kg; Vitamin E 2000 mg/kg; DM = dry matter; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

2.1.2. Chemical Analyses

The samples of TMR feeding the steers as the donors for rumen fluid were lyophilized for 72 h in a freeze dryer (LGJ-12; Songyuan Huaxing Technology Development Co., Ltd., Beijing, China) and then ground to pass through a 40-mesh sieve. The dry matter (DM) and ash contents of feeds were determined according to AOAC (2005) using methods No. 930.15 and 942.05 [19], respectively. The organic matter (OM) content was calculated by subtracting ash from the DM. The nitrogen (N) contents of feeds were analyzed by the Kjeldahl method, and crude protein (CP) content was calculated as N $\times$ 6.25, according to AOAC (2005), using method No.981.10 [19]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed on a fiber analyzer (Ankom Technology Corp., Macedon, NY, USA) using the methods of Van Soest et al. [20] with sodium sulfite and heat-stable α-amylase for analyzing NDF.

The liquid samples were centrifuged at 20,000$\times$ g for 15 min at 4 °C. The sediments of the 24 h- and 48 h-samples were for determining the DM degradability. The supernatants of all samples of the treatment group were for taurine analysis. The taurine of the supernatant samples was analyzed according to the method described by Yan et al. [21] on a liquid chromatograph (LC98-1, Wenfen Analytical Instrument Technology Development Co., Ltd., Beijing, China) using acetonitrile sodium dihydrogen phosphate solution (pH = 6.5) as the flow phase with the flow rate of 1.0 mL/min, the column temperature at 35 °C and the wavelength at 360 nm. Briefly, taurine was derivatized with 0.5 % 2,4-dinitro-fluorophenyl acetonitrile in a water bath at 60 °C for 10 min, then centrifuged at 20,000$\times$ g for 5 min at 4 °C. The supernatant was used for taurine analysis after filtering through a 0.45 µm filter and injected into a liquid chromatograph (LC98-1, Wenfen Analytical Instrument Technology Development Co., Ltd.). The sediments in the tubes were dried at 105 °C to a constant weight for calculating the DM degradability.

2.2. Trial 2—Effects of Taurine on In Vitro Rumen Fermentation Parameters

2.2.1. Experimental Design

Four levels of taurine (purity $\geq$ 99.0%, Aladdin Industries, Shanghai, China), i.e., 0, 5, 10 and 20 mg, were added to 0.2000 g air-dried feed mixture as substrate (Table 2) as treatments.

Table 2. Ingredients and nutritional composition of experimental ration (% DM).

Items	Contents
Ingredients	
Corn silage	50.0
Corn grain	26.5
Soybean meal	13.0
Wheat bran	9.5
Sodium bicarbonate	0.5
Salt	0.5
Total	100.00
Nutrients	
OM	94.01
CP	12.67
NDF	44.60
ADF	23.75

Notes: DM = dry matter; OM = organic matter; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

2.2.2. *In Vitro* Rumen Fermentation

The analysis of DM, ASH, total N, NDF, and ADF and the calculations of OM and CP contents of the feed mixture for incubation were the same as described in Trial 1. The *in vitro* rumen fermentation technique of Menke et al. [22] was used for the trial. The feed mixture and the steers as the donors for rumen fluid were the same as in Trial 1. The preparations of buffers and rumen fluid-buffer mixture were according to Menke et al. [22]. Rumen fluid and buffer mixture were mixed in a ratio of 1:2 (v/v) under continuous gassing with CO_2 in a bottle kept in a water bath at 39 °C. Glass syringes with a calibrated volume of 100 mL were used as incubation vessels. Each syringe contained 0.200 g feed mixture (Table 1) and 30 mL rumen fluid-buffer mixture. Six syringes were used as replicates for each treatment. Three syringes without feed mixture were used as the blank. The syringes were incubated in a biochemical incubator (SHP-205, Peiyin Instruments Co., Ltd.) at 39 °C for 48 h. During the incubation, the cumulative gas production (*GP*) was recorded at 0, 2, 4, 8, 12, 24, 36 and 48 h, respectively.

2.2.3. Sampling and Analyses

At 48 h of incubation, the syringes were placed into ice water to terminate the incubation. The gas from each syringe was collected. The pH of the incubation liquid was immediately measured using a pH meter (PHS-3C, Yueping Instrument Manufacturing Co., Ltd.). The liquid samples were frozen at −80 °C.

The CO_2, CH_4 and H_2 were analyzed by gas chromatography (GC-8600, Beijing Beifen Tianpu Instrument Technology Co., Ltd., Beijing, China) equipped with a thermal conductivity detector and a glass column (1 m × 3 mm × 2 mm). The volatile fatty acids (VFA) of incubation liquids were analyzed according to the procedures described by Zhao et al. [23] by gas chromatography (GC-8600, Beijing Beifen Tianpu Instrument Technology Co., Ltd.). The ammonia–nitrogen (NH_3-N) concentration of the incubation liquid was determined using a UV–Vis spectrophotometer (UV-1801; Beijing Beifen Ruili Analytical Instrument Co., Ltd., Beijing, China) with the phenol-hypochlorite method as described by Broderick et al. [24]. The microbial crude protein (MCP) of the incubation liquid samples was determined according to the method of Makkar et al. [25].

2.3. Calculation and Statistical Analysis

The cumulative gas production was calculated as:

$$GP = (V - V_0 - GP_0)/M \quad (1)$$

where *GP* refers to the cumulative instantaneous gas production at time *t*, mL/g DM; *V*, the syringe reading the at time *t*, mL; V_0, the syringe reading at time 0, mL; GP_0, the average gas production of the blank at time *t*, mL; *M*, the feed DM, g.

The gas production values at different incubation time points were fitted to the model of Ørskov and McDonald [26]:

$$dp = a + b(1 - e^{-ct}) \quad (2)$$

where *dp* is gas production (mL) at time *t*; *a*, the gas production of the fast degradable feed fraction, mL/g DM; *b*, the gas production of the slowly degradable feed fraction, mL/g DM; *c*, the gas production rate of the slowly degradable feed fraction, %/h.

$$\text{Taurine degradability (\%)} = (A - B)/A \times 100 \quad (3)$$

where *A* is the amount of taurine added to each tube, mg; *B*, the amount of taurine left in incubation liquid, mg.

The data of Trial 1 and the data on taurine degradability of Trial 2 among different treatments (Trial 1) and different time points (Trial 2) were statistically analyzed using the One-way ANOVA procedures of SPSS (version 6.1.3). The data were reported as the least squares means and standard errors, and the statistical differences were determined by Duncan's test. The data on DM degradability in Trial 2 were analyzed using the *t*-test. Differences were declared to be significant at $p < 0.05$ and a tendency at $0.05 < p < 0.10$.

3. Results and Discussion

Dietary carbohydrates can be fermented to produce VFA, and CP can be degraded into peptides, AA and NH_3 to some extent by rumen microorganisms. Amino acids such as methionine and lysine are easily hydrolyzed to NH_3 and CO_2 by rumen microorganisms [27]. Many *in vitro* studies showed that supplementing N sources (urea, casein or ovalbumin) or sulfur-containing substances (sodium sulfate) increased the concentration of NH_3-N [28,29]. The results in Table 3 showed that the ruminal degradability of taurine increased linearly with incubation time, and the 2 h *in vitro* degradability of taurine was found to be as high as 99 %, indicating that taurine was quickly and highly degradable for *in vitro* rumen fermentation. The results in Table 4 show that taurine significantly increased the 48 h-pH value ($p = 0.018$) and decreased the 48 h-DMD ($p = 0.008$). Ruminal microorganisms should have played important roles in hydrolyzing taurine. However, the actual reactions of the hydrolyzing process are unclear and need to be investigated in further research.

Table 3. Taurine degradability for *in vitro* rumen fermentation (Trial 1).

Items	Incubation Time, h							SEM	*p*-Value		
	2	4	8	12	24	36	48		T	L	Q
Taurine degradability, %	99.08	99.09	99.13	99.45	99.54	99.67	99.89	0.001	<0.001	<0.001	0.035

Notes: SEM = standard error of the means; $p < 0.05$ represents significant difference.

Table 4. Effects of taurine on *in vitro* rumen pH and DM degradability (Trial 1).

Items	Taurine Addition, mg		SEM	*p*-Value
	0	10		
Incubated for 24 h				
pH	6.76	6.85	0.019	0.005
DM degradability, %	45.51	32.93	0.006	0.200
Incubated for 48 h				
pH	6.74	6.79	0.010	0.018
DM degradability, %	51.49	34.80	0.012	0.008

Notes: SEM = standard error of the means; $p < 0.05$ represents significant difference; DM = dry matter.

The results in Table 5 showed that taurine increased the NH_3–N concentration of incubation liquid in a linear manner ($p < 0.001$), which was in accordance with taurine degradability. Previous *in vitro* rumen fermentation studies showed that adding methionine increased the ruminal MCP concentration [30] and confirmed that, in cattle, supplementing with methionine or sodium sulfate improved the ruminal MCP synthesis [31]. As a semi-essential AA, taurine contains N and sulfur, which are essential nutritional elements for MCP synthesis. The results in Table 5 showed that taurine quadratically affected the ruminal MCP synthesis ($p < 0.001$) and that low levels of taurine improved the ruminal MCP concentration, whereas a high level of taurine decreased the ruminal MCP concentration, suggesting that the effect of taurine on improving MCP synthesis was on a dose-dependent basis. The beneficial impact of low taurine on increasing ruminal MCP synthesis could possibly be resulted from the increased NH_3–N concentration and sulfur from taurine degradation. However, the effect should not be attributed to the direct use of taurine for MCP synthesis because taurine cannot be incorporated into protein [3]. The decreasing impact of high taurine on the ruminal MCP concentration could be a result of the inhibitive effect of a high level of NH_3–N [32,33]. The normal concentration of rumen NH_3–N ranges from 6.3 to 27.5 mg/100 mL, and the optimum ruminal concentration of NH_3-N required to support the maximum synthesis of MCP is 12.8 mg/100 mL [34]. The MCP synthesis can be inhibited when the rumen NH_3-N reaches or exceeds 27.5 mg/100 mL, as indicated in the present study (Table 5).

Table 5. Effect of taurine on *in vitro* rumen fermentation (Trial 2).

Items	Taurine Addition, mg				SEM	*p*-Value		
	0	5	10	20		T	L	Q
pH	6.48	6.54	6.54	6.60	0.010	<0.001	<0.001	0.695
NH_3-N, mg/100 mL	17.59	19.65	21.04	27.60	0.811	<0.001	<0.001	<0.001
MCP, µg/mL	37.98	38.57	43.18	34.84	0.701	<0.001	0.139	<0.001
Total VFA, mmol/L	86.26	88.84	87.24	80.33	5.438	0.008	0.014	0.008
Molar proportions, %								
Acetate	52.30	52.17	53.18	52.65	0.004	0.804	0.567	0.799
Propionate	19.71	19.89	19.87	20.19	0.002	0.780	0.352	0.836
Butyrate	18.29	18.63	17.59	17.97	0.002	0.277	0.252	0.969
Iso-butyrate	2.26	2.24	2.12	2.03	0.003	0.637	0.215	0.817
Valerate	2.29	2.25	2.37	2.30	0.001	0.744	0.658	0.925
Iso-valerate	5.15	4.82	4.87	4.87	0.001	0.458	0.281	0.309

Notes: MCP = microbial crude protein; VFA = volatile fatty acids; SEM = standard error of the means; $p < 0.05$ represents significant difference; T = treatment; L = linear; Q = quadratic.

Dietary carbohydrates can be extensively degraded and fermented by rumen microorganisms to produce VFA. The results in Table 5 also showed that taurine decreased the total VFA concentration ($p = 0.014$), but it did not affect the molar proportions of individual VFA ($p > 0.10$) of the incubation liquid. The results were in accordance with the decreased 48 h total gas production ($p < 0.001$), the potential gas production ($a + b$) (Table 6) ($p < 0.001$) and the 48 h-DM degradability (Table 4) ($p = 0.008$). The results suggested that taurine inhibited the ruminal fermentation of feed carbohydrates. Since taurine decreased the gas production of the slowly degradable fraction of feeds (b) ($p < 0.001$), which is mainly cellulose and hemicellulose, taurine should have an inhibitive impact on the activity of ruminal cellulolytic microorganisms. However, the hypothesis needs to be clarified by investigating the effects of taurine on ruminal microbiota in the future. The results in Table 6 showed that taurine quadratically affected the CH_4 ($p = 0.026$) and CO_2 ($p = 0.019$) production, suggesting that a suitable level of taurine had the potential to decrease ruminal CH_4 production.

Table 6. Effects of taurine on *in vitro* rumen gas production (Trial 2).

Items	Taurine Addition, mg				SEM	*p*-Value		
	0	5	10	20		T	L	Q
Gas production, mL/g DM								
2 h	28.55	30.81	36.70	30.81	0.935	0.006	0.070	0.012
4 h	67.06	61.62	57.54	56.18	1.796	0.130	0.072	0.550
8 h	111.46	98.32	94.24	91.52	2.736	0.037	0.017	0.287
12 h	150.43	129.13	125.96	124.60	3.306	0.008	0.005	0.076
24 h	269.14	229.26	220.20	211.14	5.367	<0.001	<0.001	<0.001
36 h	307.20	271.86	241.05	222.92	7.023	<0.001	<0.001	0.081
48 h	306.29	269.59	241.95	227.45	6.546	<0.001	<0.001	0.013
Gas production coefficients								
a, mL/g DM	−5.42	−3.66	−2.32	−4.48	0.342	0.004	0.094	0.001
b, mL/g DM	85.63	77.89	69.29	66.94	1.663	<0.001	<0.001	0.056
c, %/h	0.06	0.05	0.06	0.07	0.002	0.079	0.044	0.115
a + *b*, mL/g DM	80.21	74.23	66.97	62.46	1.578	<0.001	<0.001	0.626
Gas components, mL/g DM								
CO_2	177.10	160.49	131.34	133.76	4.338	<0.001	<0.001	0.019
CH_4	45.06	50.41	47.26	35.08	2.082	0.042	0.057	0.026
H_2	0.04	0.07	0.05	0.01	0.008	0.023	0.817	0.212

Notes: *a* = gas production of the fast degradable fraction, mL/g DM; *b* = gas production of the slowly degradable fraction, mL/g DM; *a* + *b* = potential gas production, mL/g DM; *c* = rate of gas production of the slowly degradable fraction, %/h; DM = dry matter; SEM = standard error of the means; $p < 0.05$ represents significant difference; T = treatment; L = linear; Q = quadratic.

Ruminal pH is mainly affected by the NH_3–N from feed CP degradation and the VFA from ruminal fermentation of feed carbohydrates [35,36]. The results in Tables 4 and 5 showed that taurine increased the ruminal pH. The results could be attributed to the increased NH_3–N and the decreased total VFA concentrations.

4. Conclusions

In summary, taurine was rapidly and highly hydrolyzable with *in vitro* rumen fermentation. Supplementation with taurine at 5, 10, and 20 mg to 0.20 g feed mixture inhibited *in vitro* rumen fermentation by linearly decreasing total gas production, VFA production, and DM degradability. The addition of 5 and 10 mg taurine improved whereas 20 mg taurine inhibited *in vitro* ruminal MCP synthesis, suggesting taurine increased MCP synthesis on a dose-dependant basis. The ruminal hydrolyzing processes of taurine and the impact mechanisms of taurine on rumen fermentation need to be studied in the future.

Author Contributions: S.Z.: conducted the research, data organization and statistics, and writing the original manuscript. Q.L.: conducted the research, data organization and statistics. M.L.: Statistics & editing. G.Z.: Conceptualization, methodology, supervision, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: The experiment was supported by the National Natural Science Foundation of China (grant number 32172748).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the content of this paper.

References

1. Seidel, U.; Huebbe, P.; Rimbach, G. Taurine: A regulator of cellular redox homeostasis and skeletal muscle function. *Mol. Food Res.* **2019**, *63*, 1800569. [CrossRef] [PubMed]
2. Kim, Y.C.; Kwon, D.Y.; Kim, J.H. Alterations in the metabolomics of sulfur-containing substances in rat kidney by betaine. *Amino Acids* **2014**, *46*, 963–968. [CrossRef] [PubMed]
3. Richard, N.; Colen, R.; Aragao, C. Supplementing taurine to plant-based diets improves lipid digestive capacity and amino acid retention of *Senegalese sole* (*Solea senegalensis*) juveniles. *Aquaculture* **2017**, *468*, 94–101. [CrossRef]
4. Takahashi, Y.; Tamura, Y.; Matsunaga, Y.; Kitoaka, Y.; Terada, S.; Hatta, H. Effects of taurine administration on carbohydrate metabolism in skeletal muscle during the post-exercise phase. *J. Nutr. Sci. Vitaminol.* **2016**, *62*, 257–264. [CrossRef] [PubMed]
5. Wen, C.; Li, F.; Zhang, L.; Duan, Y.; Guo, Q.; Wang, W.; He, S.; Li, J.; Yin, Y. Taurine is involved in energy metabolism in muscles, adipose tissue, and the liver. *Mol. Food Res.* **2019**, *63*, 1800536. [CrossRef]
6. Chen, W.; Guo, J.X.; Chang, P. The effect of taurine on cholesterol metabolism. *Mol. Food Res.* **2012**, *56*, 681–690. [CrossRef]
7. Maia, A.R.; Batista, T.M.; Victorio, J.A.; Clerici, S.P.; Delbin, M.A.; Carneiro, E.M.; Davel, A.P. Taurine supplementation reduces blood pressure and prevents endothelial dysfunction and oxidative stress in post-weaning protein-restricted rats. *PLoS ONE* **2014**, *9*, e105851. [CrossRef]
8. Idrissi, A.E. Taurine regulation of neuroendocrine function. In *Taurine 11*; Springer: Berlin/Heidelberg, Germany, 2019; pp. 977–985.
9. Ito, T.; Yoshikawa, N.; Ito, H.; Schaffer, S.W. Impact of taurine depletion on glucose control and insulin secretion in mice. *J. Pharmacol. Sci.* **2015**, *129*, 59–64. [CrossRef]
10. Orhan, C.; Kucuk, O.; Sahin, N.; Tuzcu, M.; Sahin, K. Effects of taurine supplementation on productive performance, nutrient digestibility and gene expression of nutrient transporters in quails reared under heat stress. *J. Therm. Biol.* **2020**, *92*, 102668. [CrossRef]
11. Slami, M.; Bahrekazemi, M.; Bahram, S.; Javadian, S.R. The positive effects of taurine on growth performance, immunohaematological parameters and stress response of farmed beluga (*Huso huso*) in both fresh water and brackish water. *Aquacult. Nutr.* **2021**, *27*, 2279–2293. [CrossRef]
12. Lu, Z.; He, X.; Ma, B.; Zhang, L.; Li, J.; Jiang, Y.; Zhou, G.; Gao, F. Dietary taurine supplementation improves breast meat quality in chronic heat-stressed broilers via activating the Nrf2 pathway and protecting mitochondria from oxidative attack. *J. Sci. Food Agric.* **2019**, *99*, 1066–1072. [CrossRef] [PubMed]
13. Lv, Q.; Sun, L.; Cui, Y.; Yang, J.; Yang, Q.; Yu, X.; Hu, J. Effects of replacement of methionine in diets with taurine on growth performance and blood index in broilers. In *Taurine 10*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 989–1000.
14. Jung, J.H.; Kim, S.J. Anxiolytic action of taurine via intranasal administration in mice. *Biomol. Ther.* **2019**, *27*, 450. [CrossRef] [PubMed]
15. O'Flaherty, L.; Stapleton, P.P.; Redmond, H.P.; Bouchier-Hayes, D.J. Intestinal taurine transport: A review. *Eur. J. Clin. Investig.* **1997**, *27*, 873–880. [CrossRef]
16. Spitze, A.; Wong, D.; Rogers, Q.; Fascetti, A.J. Taurine concentrations in animal feed ingredients; cooking influences taurine content. *J. Anim. Physiol. Anim. Nutr.* **2003**, *87*, 251–262. [CrossRef]
17. Mariz, L.; Amaral, P.; Valadares Filho, S.; Santos, S.; Detmann, E.; Marcondes, M.; Faciola, A. Dietary protein reduction on microbial protein, amino acid digestibility, and body retention in beef cattle: 2. Amino acid intestinal absorption and their efficiency for whole-body deposition. *J. Anim. Sci.* **2018**, *96*, 670–683. [CrossRef] [PubMed]
18. Tilley, J.; Terry, D.R. A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.* **1963**, *18*, 104–111. [CrossRef]
19. AOAC. *Official Methods of Analysis of AOAC International*, 18th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2005.
20. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
21. Yan, H.; Qiao, F.; Tian, M.; Row, K.H. Application of 2,4-dinitrofluorobenzene pre-column derivatization to quantitative determination of taurine and its intermediate in beverages and milk samples. *J. Liq. Chromatogr. Relat. Technol.* **2012**, *36*, 35–43. [CrossRef]
22. Menke, K.H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55. [CrossRef]
23. Zhao, Y.; Rahman, M.S.; Zhao, G.; Bao, Y.; Zhou, K. Dietary supplementation of rumen-protected methionine decreases the nitrous oxide emissions of urine of beef cattle through decreasing urinary excretions of nitrogen and urea. *J. Sci. Food. Agric.* **2020**, *100*, 1797–1805. [CrossRef]
24. Broderick, G.; Kang, J. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci.* **1980**, *63*, 64–75. [CrossRef] [PubMed]
25. Makkar, H.; Sharma, O.; Dawra, R.; Negi, S. Simple determination of microbial protein in rumen liquor. *J. Dairy Sci.* **1982**, *65*, 2170–2173. [CrossRef] [PubMed]
26. Ørskov, E.R.; McDonald, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* **1979**, *92*, 499–503. [CrossRef]

27. Gilbreath, K.R.; Bazer, F.W.; Satterfield, M.C.; Wu, G. Amino acid nutrition and reproductive performance in ruminants. In *Amino Acids in Nutrition and Health*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 43–61.
28. Zhang, Y.; Zhao, X.; Chen, W.; Zhou, Z.; Meng, Q.; Wu, H. Effects of adding various silage additives to whole corn crops at ensiling on performance, rumen fermentation, and serum physiological characteristics of growing-finishing cattle. *Animals* **2019**, *9*, 695. [CrossRef]
29. Wu, H.; Li, Y.; Meng, Q.; Zhou, Z. Effect of high sulfur diet on rumen fermentation, microflora, and epithelial barrier function in steers. *Animals* **2021**, *11*, 2545. [CrossRef] [PubMed]
30. Hassan, F.; Guo, Y.; Li, M.; Tang, Z.; Peng, L.; Liang, X.; Yang, C. Effect of methionine supplementation on rumen microbiota, fermentation, and amino acid metabolism in *in vitro* cultures containing nitrate. *Microorganisms* **2021**, *9*, 1717. [CrossRef]
31. Zhao, Y.; Xie, B.; Gao, J.; Xiao, M.; Zhao, G. Balancing the dietary ratio of nitrogen to sulfur by adding inorganic sulfur improves nitrogen retention and consequently decreases urine nitrous oxide emissions in steers. *Anim. Feed Sci. Technol.* **2021**, *274*, 114711. [CrossRef]
32. Zhou, Z.; Meng, Q.; Li, S.; Jiang, L.; Wu, H. Effect of urea-supplemented diets on the ruminal bacterial and archaeal community composition of finishing bulls. *Appl. Microbiol. Biot.* **2017**, *101*, 6205–6216. [CrossRef]
33. Wang, P.; Zhao, S.; Nan, X.; Jin, D.; Wang, J. Influence of hydrolysis rate of urea on ruminal bacterial diversity level and cellulolytic bacteria abundance *in vitro*. *PeerJ.* **2018**, *6*, e5475. [CrossRef]
34. OrtegaM, E.; Stern, M.D. The effect of rumen ammonia concentration on dry-matter disappearance *in situ*. *J. Dairy Sci.* **1979**, *62*, 76.
35. Shen, J.; Zheng, L.; Chen, X.; Han, X.; Cao, Y.; Yao, J. Metagenomic analyses of microbial and carbohydrate-active enzymes in the rumen of dairy goats fed different rumen degradable starch. *Front. Microbiol.* **2020**, *11*, 1003. [CrossRef] [PubMed]
36. Wedwitschka, H.; Gallegos Ibanez, D.; Schäfer, F.; Jenson, E.; Nelles, M. Material characterization and substrate suitability assessment of chicken manure for dry batch anaerobic digestion processes. *Bioengineering* **2020**, *7*, 106. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.