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ADDING GREEN AND BLACK TEAS IN A RICE STRAW-BASED SHEEP DIET IMPROVES *IN VITRO* DRY MATTER DEGRADABILITY WITHOUT AFFECTING GAS PRODUCTION AND PH

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Abstract
Rice straw (RS) is widely available in South East Asia and it can be used as a source of fibres for ruminant animals. However, RS has poor palatability and nutritional values. Thus, the use of concentrate (CON) as a dietary supplement is required to increase the utilisation of RS. Green (GT) and black (BT) teas can act as natural dietary additives in a CON. This study evaluated nutrient compositions of the diet ingredients and the impact of supplementing GT and BT in a rice straw-based sheep diet on *in vitro* dry matter degradability (IVDMD), total gas production (TGP) and pH during more than 24 hours of incubations. The CON, GT, and BT had higher crude protein, organic matter, and metabolizable energy but lower fibre fractions than RS. The GT and BT had greater total tannins than CON and RS confirming that they could potentially be used as natural dietary additives. In addition, GT supplementation improved IVDMD without affecting TGP and pH values. Conversely, BT supplementation did not improved IVDMD. Thus, BT is less preferable as a ruminant dietary supplement than GT.
Keywords: black tea, green tea, degradability, total gas production.

PENAMBAHAN TEH HIJAU DAN TEH HITAM PADA PAKAN DOMBA BERBAHAN DASAR JERAMI PADI DAPAT MENINGKATKAN DEGRADASI BAHAN KERING SECARA IN VITRO TANPA MEMPENGARUHI PRODUKSI GAS DAN PH.

Abstrak
Jerami padi (RS) banyak tersedia di Kawasan Asia Tenggara dan dapat digunakan sebagai sumber serat bagi ternak ruminansia. Tetapi, RS memiliki palatabilitas dan nilai nutrisi rendah. Sehingga, penggunaan Konsentrat (CON) sebagai pakan suplemen diperlukan untuk meningkatkan pemanfaatan RS. Teh hijau (GT), dan teh hitam (BT) mempunyai potensi sebagai pakan aditif alami di dalam CON. Penelitian dilakukan untuk mengevaluasi kandungan nutriens pada bahan pakan penyusun ransum dan pengaruh suplementasi GT dan BT dalam ransum domba berbasis RS terhadap kecernaan bahan kering in vitro (IVDMD), produksi gas total (TGP), dan pH dengan masa inkubasi lebih dari 24 jam. CON, GT, dan BT mempunyai kandungan protein kasar, bahan organik, dan energi metabolis yang lebih tinggi, tetapi memiliki kandungan fraksi serat yang lebih rendah dibandingkan RS. GT dan BT memiliki kandungan total tanin yang lebih tinggi dibandingkan CON dan RS mengkonfirmasi bahwa GT dan BT dapat digunakan sebagai pakan aditif alami. Sebagai tambahan, suplementasi GT dapat memperbaiki IVDMD tanpa mempengaruhi nilai TGP dan pH. Sebaliknya, suplementasi BT tidak meningkatkan IVDMD. Untuk itu, BT kurang direkomendasikan untuk menjadi pakan suplemen ruminansia dibandingkan GT.
Kata kunci: teh hitam, teh hijau, degradabilitas, produksi gas total.

INTRODUCTION

In West Java, sheep are mostly fed with grasses in a traditional cut and carry system. Unfortunately, high quality grass lands are now limited since many areas have been converted to housing, industrial, and cropping lands. Rice straws (RS) is a waste product of rice plants being the solely hugely available roughages. RS has poor tastiness and the nutritive values being low in organic matter (OM) and crude protein (CP), and high in fibre, lignin, and silica **(**Eun *et al.*, 2006; Khan and Chaudhry, 2010; Ramdani et al., 2020**)**. Increasing the value of cereal straws in ruminant animal diets using biological, chemical, or physical processing has been done (Chaudhry, 1998; Van Soest, 2006) but these are sometimes not preferable at small-traditional farming situation (Khan and Chaudhry, 2010). A simple alternative method is required to increase quality use of RS by offering suitable concentrate (CON) containing complementary additive supplementations.

Both green (GT) and black (BT) tea leaves could be used as potential natural dietary additives since they are rich in protein, fibres, minerals, and plant bioactive compounds (Ramdani et al., 2013; Ramdani et al., 2018). Ramdani et al. (2020) concluded that adding GT rich in phenolic tannins could increase the body weight gain of fattening lambs without affecting the dry matter and protein digestibility of feeds in those lambs. In addition, Ramdani et al. (2022) found that tea leaf supplementations in a sheep diet did not improve *in vitro* dry matter degradability (IVDMD) and total gas production (TGP). Unfortunately, their *in vitro* experiments were done for 24 hours incubation only. Longer *in vitro* incubation time is required in particular to observe *in vitro* dry matter (IVDMD) degradability. Hence, the objective of this study was to observe the impact of supplementing GT and BT into a rice straw-based sheep diet on IVDMD, TGP, and pH when incubated for more than 24 hours. The TGP is one of the fermentation outputs during an *in vitro* incubation and its measurement is required to observe the rate of fermentation and to predict the quality of different substrates.

MATERIAL AND METHODS

This study consisted of 2 different *in vitro* experiments:

1. A 7 x 5 factorial design was used to observe the impacts of 7 various tea supplementations in a sheep diet (Table 1) on IVDMD during 5 (0h, 6h, 24h, 48h, and 72h) various incubation times using 6 replicates.
2. A randomized experimental design was utilized to observe the impacts of 5 various tea leaf supplementations in a sheep diet (Table 1) on rumen *in vitro* TGP and pH up to 48h incubations.

Feed Ingredients

Each feed ingredient was ground to pass a 1 mm sieve with the help of a sample mill (Cyclotec 1093, Tecator, Sweden). Detailed GT and BT were similar to those described in Ramdani et al. (2018).

Meanwhile, CON consisted of (%) sugar beet pulps (26), barley and wheat mixtures (26), soybean meal (22), maize distillers' grain (15), molasses (8), and mineral mix (3). The CON was made in a feedmill at Cockle Park farm, Newcastle University in the spring season while dried RS was IR50 variety and obtained from Prof. M. Mehedi Khan from Bangladesh. Nutrient composition analyses of feed ingredients had the same procedure with those described in Ramdani et al. (2022). Table 2 shows chemical compositions for GT, BT, CON and RS.

Table 1. The proportions (%) of tea leaves, RS, and CON in a sheep diet that were tested in the 1st and 2nd *in vitro* experiments.

Diets	GT	BT	CON	RS
1st experiment				
T0	0 %	0 %	70 %	30 %
GT5	5 %	0 %	70 %	25 %
GT10	10 %	0 %	70 %	20 %
GT20	20 %	0 %	70 %	10 %
BT5	0 %	5 %	70 %	25 %
BT10	0 %	10 %	70 %	20 %
BT20	0 %	20 %	70 %	10 %
2nd experiment				
T0	0 %	0 %	70 %	30 %
GT5	5 %	0 %	70 %	25 %
GT10	10 %	0 %	70 %	20 %
BT5	0 %	5 %	70 %	25 %
BT10	0 %	10 %	70 %	20 %

Note: *BT, black tea; GT, green tea; CON, mixed concentrate; RS, rice straws*

Table 2. Nutrient compositions of the feed ingredients (%, except MJ/kg for ME).

Feeds	DM	OM	Ash	EE	CP	NDFom	ADFom	ME	TT
GT	93.7	93.8	6.18	2.08	24.0	25.4	21.1	7.08	20.4
BT	94.2	93.9	6.14	1.26	24.2	32.3	30.9	6.40	13.3
RS	94.5	81.8	18.2	9.90	6.04	78.7	68.4	4.01	0.11
CONC	86.4	92.1	7.89	5.66	17.6	27.1	14.4	10.1	0.16

Note: *ADFom, acid detergent fibre exclude ash; BT, black tea; CON, concentrate; CP, crude protein; DM, dry matter; EE, ether extract; GT, green tea; ME, metabolizable energy; NDFom, neutral detergent fibre exclude ash; OM, organic matter; RS, rice straws; TT, total tannins.*

Rumen Liquor Collection and Buffer Solution

Rumen liquor (RL) sources were obtained from a local abattoir (Linden Foods, Ltd.) at Buradon, Newcastle upon Tyne UK. The RL of experiment 1 was obtained from 3 freshly slaughtered lambs (Mule Suffolk). They were fed fresh grass-based diet with a mixture of bread, beans, and red clover silage for the last 3 weeks before slaughtering. The RL of experiment 2 was obtained from 2 freshly slaughtered lambs (Texel cross) that were fed fresh grass-based diet. The procedures of RL collection and buffer solution preparation were similar to Ramdani et al. (2022). Each experiment had the same method for preparing buffer solution using the synthetic saliva procedure of McDougall (1948).

Buffered Inoculum, Incubation, and Total Gas Production

Each buffered inoculum was immediately prepared after returning from the abattoir using a similar procedure to Ramdani et al. (2022).

In experiment 1, approximately 0.4 g each of substrate diet was placed into each 50-ml polypropylene tube which also received 40 ml of the buffered inoculum. Each tube contents were purged with CO_2, sealed with rubber stoppers fitted with gas pressure release valves, and incubated in a temperature-controlled waterbath (39^{o}C). Each tube was gently hand mixed for a few seconds, three times a day during incubation. The tubes were then taken out at 0h, 6h, 24h, 48h, and 72h from the waterbath and stored in an ice box to terminate fermentation. Next, the separation of liquids and residues were done by centrifuging each tube at 2500 rpm for 10 min. After centrifugation, the supernatant was collected and the residues were dried for IVDMD measurement which was done using a similar procedure to Khan and Chaudhry (2010). In prediction, the residues from buffered inoculum were degraded along with the substrate samples during incubation. However, IVDMD values at 0h and 6h were corrected for the average DM weight of the residue from 3 replicated buffered inoculum only blank representatives.

In experiment 2, approximately 200 ± 3 mg each of substrate diet was placed in a 50 ml glass syringe (SAMCO, UK), lubricated with Vaseline, and fitted with a 4 way-male-slip stopcock (Cole Palmer Instrument, UK) before adding about 20 ml buffered inoculum. The syringes were stored in a shaking water bath at 39^{o}C. In each syringe, TGP was measured every two hours up to 48h while pH of the inoculum was determined at the end of 48h incubation employing a calibrated pH meter (Hanna Instrument, Portugal).

Statistical Analysis

Two-way ANOVA of GLM procedures in Minitab 16 software was utilized to assess the statistical impacts of various tea leaf supplementations in a diet and incubation times alongside their interaction on IVDMD. Meanwhile, One-way ANOVA was used to examine the statistical impacts of various tea leaf supplementations in a diet on TGP at either 24 or 48h of incubation along with their pH. Differences were predicted to be significant if $P<0.05$.

RESULTS AND DISCUSSION

IVDMD

Table 3 shows the impacts of GT and BT supplementations into a diet at 0, 5, 10, and 20% on IVDMD at 0h, 6h, 24h, 48h, and 72h of incubation times. Various supplementations and incubation times gave significant impacts on IVDMD but not their interaction. Across incubation times, all GT supplementations improved IVDMD significantly but no differences among the GT5, GT10, and GT20 supplementations on IVDMD. On the other hand, all BT supplementations resulted in no significant impacts on IVDMD. In addition, the IVDMD of substrate diets were adjusted significantly by their incubation times. The longer the incubation times were the greater IVDMD were in all samples of substrate diet.

Total Gas Production and pH

Table 4 reports the impacts of various GT and BT supplementations in a sheep diet at doses of 0, 5, and 10% on TGP for up to 48h of incubation time. According to the incubation times, the highest rise in TGP (L/kg OM) was within 24h, especially from 6h (24.4 - 28.2) to 20h (131 - 137). Furthermore, TGP was likely to have a slow increase reaching between 185 and 192 at 48h. All GT or BT supplementations did not affect pH significantly after 48h incubation time.

Based on the current study, the GT supplementations at 5, 10, or 20% in an RS-based sheep diet increased IVDMD significantly when compared with the control diet (T0) but it was not observed for all BT supplementations. The primary cause for increased IVDMD due to GT supplementations because GT had lower fiber fractions than RS but GT contained greater nutritive values such as CP, OM, and EE. According to several studies (Eun et al., 2006; Khan and Chaudhry, 2010; Van Soest, 2006), RS is classified as a low nutritive value of roughage with low CP and OM but high fiber, lignin, and silica concentrations. Surprisingly, BT also contained lower fiber and greater OM and CP than RS; yet, BT inclusions did not improve IVDMD. This could be caused by the "maillard browning" reaction, which occurs during the BT production process resulting in the formation of more insoluble organic components and polyphenols. According to Ramdani et al. (2018), the majority of phenolic catechins in fresh tea leaves undergo conversion into theaflavins during BTL processing. These theaflavins exhibit longer retention times on chromatograms during HPLC analysis than catechins, indicating their altered polarity and subsequently poorer solubility.

Table 3. Impact of GT or BT supplementations at 0, 5, 10, and 20% of the substrate diets on IVDMD (%) at various incubation times.

Treatment	0 h	6 h	24 h	48 h	72 h	Means	SEM
T0	55.4	113	316	422	472	276^b	4.87
GT5	65.7	146	391	433	506	308^a	5.15
GT10	60.5	141	386	451	521	312^a	5.12
GT20	77.1	157	418	471	511	327^a	5.36
BT5	35.9	110	353	413	479	278^b	5.12
BT10	49.3	125	341	407	479	280^b	5.15
BT20	37.1	121	364	407	459	277^b	5.17
Means	54.4^E	130^D	367^C	429^B	489^A		P<0.001
SEM	4.60	4.44	4.24	4.22	4.26	P<0.001	

Note: *Means with different letters in the same column for the inclusions (small letters) or row for the incubation times (capital letters) are significantly different; BT and GT, black and green teas; SEM, standard error of mean*

Table 4. Impact of GT or BT supplementations at 0, 5, and 10% of the diets on TGP (L/kg OM) at various incubation times and the pH at 48h.

Treatment	0h	2h	4h	6h	20h	22h	24h	26h	28h	30h	44h	46h	48h	pH
T0	0	2.79	17.5	24.4	131	141	**145**	152	155	159	181	184	**185**	**6.74**
GT5	0	2.74	16.5	26.5	134	136	**148**	154	156	161	181	185	**187**	**6.75**
GT10	0	4.55	16.4	26.4	134	137	**154**	159	164	168	187	190	**192**	**6.73**
BT5	0	3.63	16.4	28.2	137	138	**153**	157	160	164	184	187	**188**	**6.74**
BT10	0	3.66	16.4	25.6	132	136	**149**	156	162	165	183	186	**187**	**6.75**
SEM		0.67	2.08	2.02	3.60	3.38	**3.73**	3.81	3.96	3.82	4.14	3.91	**4.15**	**0.07**
P value		P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	**P>0.05**	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	**P>0.05**	**P>0.05**

Note: *Means with letters in the same column are not significantly different (P>0.05); BT and GT, black and green teas; SEM, standard error of mean.*

The differing effect of GT and BT on degradability suggests that the processing methods of tea leaf fabrication can significantly influence their nutritional properties. Research by Li et al. (2015) and Czernicka et al. (2017) highlights that the GT processing retains more original nutrients present in fresh tea leaves compared to the BTL processing, which involves fermentation and oxidation processes that may alter the composition and bioavailability of nutrients. Additionally, Li and colleagues (2015) found that theaflavins, which are abundant in BT due to oxidation during processing, exhibited lower solubility compared to the catechins found in GT, potentially impacting their degradability in the rumen. Studies by Li et al. (2021) have demonstrated that certain polyphenols, including theaflavins, could form complexes with dietary proteins and carbohydrates, leading to the reduced enzymatic hydrolysis and impaired nutrient absorption in the gastrointestinal tract. This mechanism could contribute to the limited improvement in the *in vitro* digestibility (Correddu et al., 2020) observed with the BT inclusion in the current study.

Furthermore, the role of fibre composition in tea leaves, particularly the differences in lignin content between GT and BT, may also influence their effects on digestibility. Previous research by Baurhoo et al. (2008) and Khan and Ahring (2019) suggest that lignin, as a complex and indigestible component of plant cell walls, can impede the access of digestive enzymes to other nutrients, thereby reducing overall digestibility. Given that BT typically undergoes more extensive oxidation and fermentation processes compared to GT, it is plausible that the resulting alterations in lignin composition did contribute to the observed differences in digestibility between the two tea leaf types.

The findings of this study underscore the importance of considering not only the nutritional composition of feed ingredients but also the impact of processing methods on nutrient availability and digestibility. While the GT inclusion led to significant improvements in digestibility parameters, the BT inclusion did not yield similar outcomes despite its favourable nutritional profile. This suggests that factors beyond nutrient composition, such as processing-induced alterations, play a significant role in predicting the efficacy of feed ingredients. Next research is suggested to elucidate the mechanisms underlying the differential impacts of GT and BT inclusions on ruminant diet digestibility, with particular emphasis on understanding the implications of processing techniques on nutrient bioavailability and utilization. Such insights can inform the development of more effective dietary strategies to optimize ruminant nutrition and performance of production animals.

CONCLUSION

Green tea inclusions into a sheep diet can improve *in vitro* feed degradability without affecting total gas production and pH. There is no improvement in *in vitro* feed degradability due to black tea supplementations showing that black tea is less preferable as a ruminant dietary additive than the green tea.

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