Feeding frequency effects on consumption and nutrient digestibility on thin-tailed sheep infected with *Haemonchus contortus*

W Setyono¹, Kustantinah¹*, R W N Cahyo²

¹Department of Animal Nutrition, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia
²Department of Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, Indonesia

Corresponding author: kustantinah@ugm.ac.id

Abstract. In Indonesia, most sheep are reared by farmers on extensive rearing system. This system creates arising problem that nutrient deficiency and susceptibility to *Haemonchus contortus* are more prevalent. This study is a laboratory model – demonstrating the actual condition – by increasing the feeding frequency to evaluate the effect of nutrient utilization on sheep infected by *H. contortus*. The study used the sheep as livestock model, with age ±1 year and average body weight ±17 kg. 16 thin-tail sheep were randomly grouped into 4. Treatment on this study were different feeding frequency: A (2 times/day), B (3 times/day), C (4 times/day), and D (5 times/day). All groups were fed with field grass-based feed with no other feed supplementation. Worm infection process were carried out once a week as many as 200 *H. contortus* infective stage per head per infection. The egg number of *H. contortus* per gram of fecal egg number was observed and examined weekly for 10 weeks. According to this study, higher feeding frequency increases the consumption and digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) (P <0.05) and increases the percentage of fecal egg number (FEN).

1. Introduction
Sheep as grazing-type animal, has a better grazing ability compared to goat [1]. Most sheep in Indonesia are kept by farmers in an open space/field for continuous period of time. For a certain period of time when the feed is supplied from pasture land, nutrient deficiency might happen due to pasture land-forages commonly have low nutrient quality. Moreover, parasite infection might also occur among the animal. Sheep graze by bowing their head that might enable the parasite larvae on an infective stage was eaten up as well. Grass belong to one of places of reproductive cycle of *Haemonchus contortus* – one of nematode that commonly found infecting ruminant’s abomasum (up to 80%) [2–4].

In Indonesia, the most nematode infecting sheep and another small ruminant is *H. contortus*. The grass on the pasture land might be infected with the worm’s egg that eventually hatch into infective larvae [5]. Thus, more frequency of infected-grass feeding will widen the possibility of infection. Infective larvae will enter the gut tracts mediated by the grass consumed. The ingested larvae grow into either male or female worm in abomasum within 3 weeks [2].
This study is a laboratory model, demonstrating the actual condition. Infection model is performed by artificially and intentionally infecting the sheep with the parasite. Infection progress is evaluated weekly by grab sampling and counting the number of H. contortus egg. This study provides information about parasite infecting the small ruminants and compromising their health condition.

2. Material and method

2.1. Material
This study used 16 female thin-tailed sheep were used on this study (aged ±1 year, weighed ±17 kg). All animals were kept in individual stall, fed with field grass-based feed, provided with ad libitum drinking water, and on the adaptation phase of 3 weeks. Sheep were grouped into 4 groups based on the feeding frequency treatments: A (2 times/day, 08:00 and 15:00), B (3 times/day, 06:00, 12:00, and 18:00), C (4 times/day, 06:00; 10:00; 14:00; and 18:00), and D (5 times/day, 06:00; 09:00; 12:00; 15:00; and 18:00). Each treatment contains 4 sheep as replication.

2.2. Methods
Adaptation stage was conducted for 14 days. After adaptation phase, all animals were then infected with infective larva orally (weekly for 6 weeks). The number of eggs per gram (EPG) was evaluated every 2 weeks for 14 weeks period of time, which was performed by using McMaster method [5].

Sampling on feed, residual feed, and feces were performed daily during the collection phase (14 days) to evaluate the nutrient consumption and digestibility. Feces were collected daily, weighed, and sampled. At the end of collection phase, all samples were composited and sampled for chemical analysis to measure the content of dry matter (DM), organic matter (OM), crude fiber (CF), crude protein (CP), and extract ether (EE). Sampling was also performed on daily feed given and their residual feed – unconsumed by each animal. Both feed and feces sample were subjected to proximate analysis. Sample for analysis were dried in the 55˚C, ground into mash form (Willy Mills grinder), then randomly sampled to undergo proximate analysis (DM, OM, CF, EE, and CP) [6].

2.3. Statistical analysis
Variables observed on this study include nutrient consumption, nutrient digestibility, and fecal egg number. Nutrient consumption and nutrient digestibility data were statistically evaluated by one-way variance analysis using SPSS 16 computer program. Different among treatments were then subjected to further Duncan Multiple Range Test (DMRT). Data on haemonchosis rates were analyzed descriptively.

3. Results and discussion
Nutrient consumption of thin-tailed sheep on this study is presented in Table 1. The data shows that along with increased feeding frequency, daily nutrient consumption increased (gram DM). The D group (received 5 times feeding per day) showed significant differences on the consumption of DM, OM, CF, and CP compared to other groups (P<0.05). However, B and C groups also showed significant effects on the increasing feed intake. Feeding 3 or 4 times per day on ruminant increased cell proliferation of the ruminal epithelium. This condition might lead to stable ruminal fermentation, efficient feed intake, stable ruminal pH, and digestibility as well [7,8].

Feed frequency also increased nutrient digestibility values (%) DM, OM, EE, CP, NFE and TDN (Table 2). Feeding five times per day had significant effect (P<0.05) on digestibility. Increasing the frequency of feeding can increase the ingestion rate the rumen and have greater chance of nutrient absorption. Digestion of OM is an important factor that determine the value of feed. The higher the OM consumed, the higher the value of OM digestibility [7].
Table 1. Average of nutrient consumption (g/kg of body weight/day)

| Nutrient consumption          | Feeding frequency |
|-------------------------------|-------------------|
|                               | 2 x               | 3 x               | 4 x               | 5 x               |
| Dry matter (DM)               | 45.48±21.61<sup>a</sup> | 47.52±17.69<sup>ab</sup> | 48.67±20.45<sup>ab</sup> | 51.08±17.42<sup>b</sup> |
| Organic matter (OM)           | 39.28±12.47<sup>a</sup> | 40.31±13.93<sup>ab</sup> | 41.06±15.95<sup>ab</sup> | 43.67±17.23<sup>b</sup> |
| Extract ether (EE)            | 1.77±0.30         | 1.80±0.34          | 1.80±0.38          | 1.78±0.32          |
| Crude fiber (CF)              | 14.14±5.03<sup>a</sup> | 14.60±5.67<sup>a</sup> | 14.96±6.45<sup>a</sup> | 16.84±5.48<sup>b</sup> |
| Crude protein (CP)            | 2.01±1.80<sup>a</sup> | 2.17±2.03<sup>a</sup> | 2.44±2.30<sup>ab</sup> | 2.88±1.96<sup>b</sup> |
| Nitrogen free extract (NFE)   | 21.73±5.81        | 22.34±5.81         | 22.38±6.60         | 22.49±5.62         |
| Total digestible nutrient (TDN)| 41.51±12.84      | 42.62±14.47        | 43.31±16.43        | 44.25±14.00        |

<sup>a,b</sup> superscripts on the same rows indicate significant difference (P <0.05)

Table 2. Average nutrient digestibility (%)

| Nutrient              | Feeding frequency |
|-----------------------|-------------------|
|                       | 2 x               | 3 x               | 4 x               | 5 x               |
| Dry matter (DM)       | 47.74±2.98<sup>a</sup> | 48.74±9.73<sup>a</sup> | 53.64±6.54<sup>a</sup> | 60.12±5.17<sup>b</sup> |
| Organic matter (OM)   | 51.46±2.41<sup>a</sup> | 51.51±9.44<sup>a</sup> | 55.96±6.18<sup>a</sup> | 61.64±4.95<sup>b</sup> |
| Extract ether (EE)<sup>ns</sup> | 70.92±1.45 | 70.61±5.95 | 72.34±3.79 | 73.28±3.20 |
| Crude fiber (CF)      | 65.88±1.70<sup>a</sup> | 66.04±6.50<sup>a</sup> | 69.71±4.28<sup>ab</sup> | 73.66±3.29<sup>b</sup> |
| Crude protein (CP)    | 28.13±4.20<sup>a</sup> | 31.00±13.88<sup>a</sup> | 40.51±9.21<sup>a</sup> | 61.31±5.36<sup>b</sup> |
| Nitrogen free extract (NFE) | 43.59±2.87<sup>a</sup> | 43.69±10.78<sup>a</sup> | 50.26±6.96<sup>ab</sup> | 55.28±5.64<sup>b</sup> |
| Total digestible nutrients (TDN) | 48.99±12.34<sup>a</sup> | 48.50±8.28<sup>a</sup> | 52.93±5.25<sup>ab</sup> | 56.73±4.12<sup>b</sup> |

<sup>a,b</sup> superscripts on the same rows indicate significant difference (P <0.05)

Observation of the *H. contortus* egg number is shown on Figure 1. This study is a laboratory model of an actual condition. The parasite infection in the actual condition was manifested by artificially infecting the sheep with infective larvae (orally, weekly for 6 weeks). The observation was performed 6 weeks after the infection.

![Figure 1. *H. contortus* egg number during observation. The symbol represent A served as control – feeding frequency treatment 2 times per day (●); the second group (B) were fed 3 times per day (■); the third group (C) were fed 4 times per day (▲); the fourth group (D) were fed 5 times per day (×).](image-url)

All animals were fed with field grass-based feed, obtained from pasture land where normally sheep and goat are being grazed. This approach was taken since the contamination of parasite egg that will
hatch into infective larvae is on high level of occurrence. Different feeding frequency among groups were a manifestation of an actual condition in which greater feeding frequency with contaminated grass will expand the infection possibility [1].

The number of EPG, that was performed in the last week of observation on A, B, C, and D groups, were 1.613; 2.263; 2.288, and 2.525, respectively. Group that fed more frequently (5 times/day) had the highest number of EPG. It might be a result from high intensity of larvae infection on the group.

4. Conclusion
Increasing the feeding frequency on the sheep kept on intensive rearing management increases the nutrient consumption and digestibility. On the other hand, intensity of infective larvae infection per oral on the sheep leads to the greater possibility of parasite infection on the sheep.

Acknowledgments
We would like to thanks of Directorate of Research Universities of the Universitas Gadjah Mada (UGM) through Rekognisi Tugas Akhir (RTA) 2019 with Contract number: 3268/UN1/DITLIT/DIT-LIT/LT/2019.

References
[1] Yisehak K, Kibreab Y, Taye T, Ribeiro Alves Lourenço M and Janssens G P J 2016 Trop. Anim. Health Prod. 48 125–31
[2] Gasser R B, Schwarz E M, Korhonen P K and Young N D 2016 (Elsevier Ltd)
[3] Pathak A K, Dutta N, Banerjee P S, Goswami T K and Sharma K 2016 J. Parasit. Dis. 40 100–5
[4] Eguale T, Tilahun G, Debella A, Feleke A and Makonnen E 2007 J. Ethnopharmacol. 110 428–33
[5] Coles G C, Bauer C, Borgsteede F H M, Geerts S, Klei T R, Taylor M A and Waller P J 1992 Vet. Parasitol. 44 35–44
[6] AOAC 2005 (The Association of Official Analytical Chemists, Maryland. USA)
[7] da Silva J, Carrara T V B, Pereira M C S, de Oliveira C A, Batista Júnior I C, Watanabe D H M, Rigueiro A L N, Arrigoni M D B and Millen D D 2018 Sci. Agric. 75 121–8
[8] Shabi Z, Bruckental I, Zamwell S, Tagari H and Arieli A 1999 J. Dairy Sci. 82 1252–60