

# DRY MATTER DIGESTIBILITY, VFA AND NH<sub>3</sub> PRODUCTION IN VITRO OF SHEEP RATIONS SUPPLEMENTED SWEET ORANGE WASTE

Elvia Hernawan<sup>1\*</sup>, Lovita Adriani<sup>1</sup>, U. Hidayat Tanuwiria<sup>1</sup>

<sup>1</sup>University Padjadjaran, Bandung Indonesia

## Abstract

The nutrients content of sweet orange waste are high but it never fully utilized, especially in animal feed industries in Indonesia. Besides, the sweet orange waste also contains high active components such as antimicrobial, antibacterial, antifungal, antioxidant, etc. Meanwhile there is a part of sheep rumen that has a strategic function in transforming raw fiber into various fermentations which are conducted by rumen microbes. The active components in sweet orange waste are predicted to affect digestive process in rumen. This research was aimed to obtain information about digestibility of dry matter, VFA and NH<sub>3</sub> in sheep ration with sweet orange waste addition. The research was conducted using a completely randomized design with 4 treatments SOW level in the ration, i.e. 0, 6, 9, 12% and 5 replicates, so there are 20 units of study. The variables measured were dry matter digestibility, production of Volatile fatty acids (VFA) and NH<sub>3</sub>. The result showed that the provision of SOW above 6% resulted declining trends of dry matter digestibility values, VFA production and NH<sub>3</sub> production, but the four treatments (0, 6, 9, 12% SOW) were not significantly different statistically. In conclusion, provision of sweet orange waste in the ration up until 12% gives same effect on the value of dry matter digestibility, VFA and NH<sub>3</sub> production.

**Key words:** Sweet Orange Waste (SOW), in vitro, digestibility, VFA, ammonia (NH<sub>3</sub>)

## INTRODUCTION

Indonesia is one of the highest orange production country with the largest production of sweet orange (*citrus sinensis*) is 1.611.784 tons [3]. The waste comes from sweet orange industry still not optimally utilized in spite of the sweet orange waste (SOW) nutrient content which is considered high enough to be used. The problem is there is not a stable availability of SOW. There should be a compromise with factories that used sweet orange as raw material of its product such as orange drinks factories.

SOW contains 6.5% of crude protein, 12.8% of crude fiber and 59.7% of NFE [15]. It also has some active components with several function such as antimicrobial, anti-inflammatory and antioxidant. Beside of that, there are also tannins, saponins and pectins [14].

The active component can reduce cholesterol and fat on blood of animal experiments as well as humans according to many academic publish. SOW has potential to be used as ration in order to reduce fat and cholesterol level on lamb [1], but the active components such as antimicrobial must be examined further first. That is because sheep is one of ruminantia that utilize crude fiber into main energy source with a help of rumen's microbe such as bacteria, protozoa, and fungi that can extract cellulolytic enzyme [2,4].

## MATERIAL AND METHOD

This research was done experimentally in Animal Husbandry Faculty of Padjadjaran University. Completely randomized design was used with 4 treatments of SOW provision in ration (non SOW, 6%, 9%, and 12%) which repeated 5 times. This research used batch culture in vitro technique according to [22]. Rumen's fluid obtained from slaughtering house in Tanjungsari, Sumedang. The ration consists of 40% concentrate in which the

\*Corresponding author:

hernawan.elvia@yahoo.com

The manuscript was received: 05.12.2014

Accepted for publication: 20.01.2015

ingredients can be seen at Table 1 and 60% forage. The used forage was a mixture between *Brachiaria brizantha* grass lawn and *Pennisetum purpureum*.

The measured variables were:

1. Total VFA concentration (mM) that was measured by using steam distillation technique (General Laboratory Procedures, 1966).

2. NH<sub>3</sub> concentration (mM) that was measured by using Conway micro-diffusion method (General Laboratory Procedures, 1966).

3. Dry matter digestibility coefficient that was measured by using Tilley and Terry method (1963).

Table 1 Concentrate's Ingredients

Concentrate's Ingredients	Treatment A (Non SOW)	Treatment B (6% SOW)	Treatment C (9% SOW)	Treatment D (12% SOW)
Dry Matter	81.88	84.29	84.32	84.32
Ash	8.80	9.47	9.64	9.81
Crude Protein	12.45	12.33	12.23	12.12
Crude Fiber	16.15	16.09	16.07	16.04
Crude Lipid	5.51	5.38	5.31	5.24
NNFE	42.10	44.53	44.76	44.99
TDN	68.11	70.10	70.08	70.04

## RESULTS AND DISCUSSIONS

### Total Volatile Fatty Acid (VFA) Concentration

About 75% carbohydrate of ruminantia's ration was forage in form of crude fiber and about 60 until 75% of it will be digested in fermentation digestion in rumen. Fermentation of carbohydrate will produce Volatile Fatty Acid that will be used as main

energy source for the animal. The value of VFA, whether high or low, will be determined by ratio between concentrate and forage. In this research, the ration and the forage were relatively the same, the only different things were the concentration of active components especially the one that derived from SOW.

Table 2 Total VFA Production, NH<sub>3</sub> and Dry Matter Digestibility in SOW Provision on Ration

Variables	Treatment A	Treatment B	Treatment C	Treatment D
	(Non SOW)	(6 % SOW)	(9% SOW)	(12% SOW)
Total VFA (mM/L)	162.13	191.23	141.9	103.07
NH <sub>3</sub> (mM/L)	3.7	3.26	3.41	3.42
Dry matter digestibility (%)	53.77	59.96	47.76	43.44
pH	8.67	8.58	8.59	8.73

As can be seen from Table 2, the highest VFA production was resulted by treatment B (6% SOW) meanwhile the lowest was resulted by treatment D (12% SOW). Descriptively, the treatment B (6% SOW) showed the highest

VFA increase i.e. 17.95% compared to treatment A (Non SOW).

On the other hand, treatment C (9% SOW) and treatment D (12% SOW) showed decreases in VFA production i.e. 12.48%

(treatment C) and 36.43 (treatment D) compared to treatment A (0% SOW). Statistic calculation showed that the treatments were not significantly different, so that the in vitro observation of VFA production was not affected by SOW provision.

Fermentation process of crude fiber on rumen that was resulted by microbe's activity [23] would be used as indicator of carbohydrate fermentation on rumen. Total VFA productions from all treatments were about 103.07 – 191.25 mM/L. That was still in the optimum standard of microbe's growth i.e. 80-160 mM.L, [20]. Treatment with non SOW provision showed high VFA production as well as the treatments with SOW provision. The value of VFA production in treatment B (6% SOW) was assumed a result of active components contribution such as pectin.

Pectin is a polysaccharide compound that has a high molecular weight consist of polymer from D-galacturonat combines with  $\alpha$ -1,4 linkages such as galacturonic acid, arabinans, galactans and arabinogalactan so that the bond in pectin can easily hydrolyze compare to cellulose or hemiceluloses, [6]. It

is a polysaccharide that build one third of cell's wall of plant which is consist of 60% water and 40% polymer. It is located in the centre of lamella on cell's wall and functioned as adhesive for cells[11] It can be quickly hydrolyzed into glucoronat acid and glucose which are ready to be fermented, [6].

Therefore, the availability of carbohydrate especially the one that can be easily hydrolyzed was likely to be the reason of high VFA production. Ration with SOW provision contained relatively high pectin i.e. 14.62%[14], 20-35% [11]. This condition was suspected to happen because the SOW provision in ration would contribute to provide energy for rumen's microbes.

High production of VFA (in vitro) was suspected because there was not any VFA absorption by the rumen's wall so that the VFA only used for living by microbes [19]. In vitro observation is a closed system that has only one intake and there isn't absorption or excretion of metabolism residue. It is different from in vivo observation that is an open system so there are nutrient and oxygen intake everytime and also nutrient and metabolism residue absorption [21].

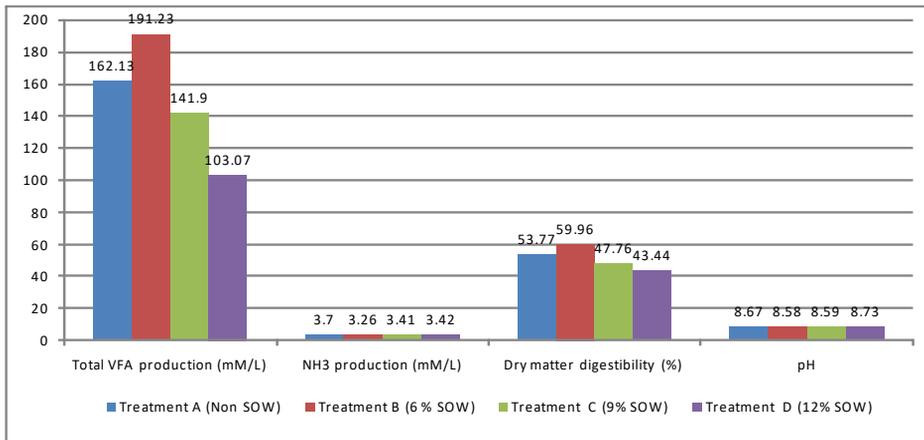


Figure 1 Total VFA Production, NH3 Production, Dry Matter Digestibility and pH in SOW Provision on Ration (in vitro)

There is a decreasing trend on VFA production as showed in Figure 1. This trend was suspected to happen because of hygroscopic nature of pectin [7]. It caused pectin breaker enzyme to convert pectin into

pectin acid that would form gel with calcium ion [6] This condition would disrupt the activity of rumen's microbes.

The presence of tannin in SOW did not negatively affect the VFA production. This

happened because SOW had structural carbohydrate that would be restrained from hydrolyzing by tannin.

The same happened to saponin and flavonoid even after the 12% SOW provision. They did not show negative effect on VFA production. Saponin is a detergent that has a role to increase permeability of membrane even make pores on it [8]. But in this research, concentration of saponin (20%) was still below the maximum allowable limit for animal [24]. Meanwhile, flavonoid functions as antioxidant that will work by donating its hydrogen atom [17]. It also functions as antibacterial that can form complex components on extracellular protein that potentially will disrupt integrity of cell's membrane of the bacteria [13]. Essential oil of SOW has a sweet smell and tart taste [10]. It can restrain spore production and colony of fungi (*Aspergillus sp*) growth because it contains  $\alpha$ -citral (*geraniol*) and  $\beta$ -citral (*neral*) [5].

#### Ammonia (NH<sub>3</sub>) Production

Ammonia is main source of nitrogen and very important in synthesis process of microbes protein in rumen. Therefore, concentration of ammonia in rumen is a very important variable to control because it related to growth of rumen's microbe, [2]. Ammonia can be derived by hydrolysis process of protein to oligoprotein and amino acid by rumen's microbe enzyme. Then it will go through deamination process to obtain keto acid, VFA and NH<sub>3</sub> [16]. As showed on Figure 1, it showed that the lowest average of NH<sub>3</sub> production was treatment C (9% SOW) with 3.41 mM/L meanwhile the highest was treatment A (Non SOW) with 3.70 mM/L. The standard of NH<sub>3</sub> production for rumen's microbes growth were in a range of 3.57 – 7.14 mM/L, [20] or 6 – 21 mM/L [12]. The experiment showed that all of treatments were below the optimum standard (3.41 – 3.70 mM/L). Descriptively, NH<sub>3</sub> production showed a decrease trend along with the increase of SOW provision on ration. Treatment A (Non SOW) showed higher NH<sub>3</sub> production compared to others treatments.

Statistic analysis showed that NH<sub>3</sub> production from all treatments was not significantly different ( $P > 0.05$ ). This meant that NH<sub>3</sub> production did not affected by SOW concentration on ration. Average of NH<sub>3</sub> production from all treatments was in a narrow range. The time of NH<sub>3</sub> measurement was suspected to be done simultaneously with incorporation process of ammonia to microbe body for body protein synthesis so that the concentration of NH<sub>3</sub> on rumen became low [12]. This condition was supported by presence of VFA as main energy source in relatively high amount (103.07 – 191.25 mM/L). Incorporation of NH<sub>3</sub> to body would require energies that derived from VFA. Microbe's protein synthesis would become constant after 3 hours so that NH<sub>3</sub> absorption would decrease and tended to accumulate, [12]. Another cause of the narrow range was suspected to be presence of tannin with concentration below 2-3%. It would positively contribute to protect protein of ration from any protein's degradation activity by the rumen's microbes [17]. Therefore the NH<sub>3</sub> production was relatively low but it was still able to fulfill microbe's protein synthesis requirement.

#### Dry Matter Digestibility

Digestibility is used to measure physical and chemical change of the ration on digestive tract. The change includes materials change into smaller particles or materials change from complex compound into simpler one. On ruminantia, ration will go through the fermentative change until the chemical properties of it become another compound with different chemical properties

Provision of SOW on the ration descriptively showed that the highest digestibility was treatment B (6% SOW) with 59.96% meanwhile the lowest was treatment D (12% SOW) with 43.44%. Six percent of SOW provision could increase dry matter digestibility value as much as 11.5% compared to the treatment A (Non SOW). Provision above 6% level i.e. 9% and 12% tended to decrease dry matter digestibility value as much as 11.2% and 19.2%

respectively compared to the treatment A (Non SOW).

Statistic analysis showed that SOW provision on ration was not significantly different ( $P>0.05$ ). This meant that SOW provision did not affect dry matter digestibility. Digestibility could be used to determine nutrient contained by the ration that could be used potentially for livestock production [9] (Therefore dry matter digestibility of every treatment has the same potential with treatment A (Non SOW)).

Digestibility above 50% shows a good dry matter quality of the ration, meanwhile digestibility below 50% shows a low digestibility level. SOW provision above 6% descriptively showed a decreasing trend in digestibility. Tannin was suspected to be the cause of this because it could react with protein on digestive tract. Besides, tannin that derived from plant caused unpalatable sensation by precipitating salivary protein. It also would reduce permeability of intestine wall when it reacted with cells outside the intestine wall so that this condition would reduce nutrients that were passing through intestine wall [6]. Tannin could also form a bond with protein; this would result to blockage of digestive enzyme. The bond between tannin and protein would protect protein from degradation of rumen's microbes but it would also be difficult to digest [6].

Tannin concentration on every treatment was below of 2-3%. It would give positive effect for protein on rumen so that protein would protected from degradation by rumen microbes. However, the longer incubation time the more it would cause decrease to VFA production. It eventually would also decrease availability of energy for rumen's microbes.

On post rumen process, complex bond between tannin and protein would detach on low pH environment in abomasum pH but tannin would coat instestinal's cell surface that would potentially decrease nutrient absorption [6,24]. This condition would result a low digestibility level of nutrient or dry matter even though the decrease were not significant compared to non SOW treatment.

## CONCLUSIONS

Sweet orange waste (SOW) provision until 12% on sheep's ration (in vitro) physiologically gives no negative effect on VFA production, NH<sub>3</sub> production or dry matter digestibility. Meanwhile, it suggested that 6% SOW provision result suitable VFA production, NH<sub>3</sub> production and dry matter digestibility for rumen's microbes needs. This will eventually result positive effects on livestock production.

## ACKNOWLEDGEMENTS

Thank you to Padjadjaran University Rector who has funded this research through BOPTN Dikti 2013. Another gratitude would also given to Lalan Jaelani, Satrio Budi Santoso, Michelle Roselyn and Galih Ahmad who have helped this research.

## REFERENCES

- [1] Adriani L., E. Hernawan and Ujang Hidayat, Decreasing cholesterol and triglyceride level on Blood by adding orange(Citrus-sinensis) waste on Padjadjaran 1sheep, Scientific Papers series D, Animal Science, Vol LVII, 2014,
- [2] Arora, S.P. 1995. Pencernaan mikroba pada ruminansia. Yogyakarta: Gadjah Mada Press.
- [3] BPS, Produksi Buah-buahan dan Sayuran Tahunan di Indonesia, 1995-2012. diunduh dari [http://bps.go.id/tab\\_sub/view.php?kat=3&tabel=1&daftar=1&id\\_subyek=55&notab=5](http://bps.go.id/tab_sub/view.php?kat=3&tabel=1&daftar=1&id_subyek=55&notab=5) pada tanggal 20 desember 2013 pukul 20.00 WIB. 2012.
- [4] Church, D. C., The Ruminant Animal Digestive Physiology and Nutrition. Prentice hall, Englewood Cliffs, New Jersey, 1988
- [5] Ella, Maria Ulfa., Ketut Sumiartha., Ni Wayan Suniti., I Putu Sudiarta dan Nyoman Semadi Antara. Uji Efektivitas Konsentrasi Minyak Atsiri Sereh Dapur (Cymbopogon Citratus (DC.) Stapf) terhadap Pertumbuhan Jamur Aspergillus Sp. secara In Vitro. E-Jurnal Agroekoteknologi Tropika : Vol. 2, No. 1, Januari 2013
- [6] Fahey, G.C. and L.L. Berger, Carbohydrate Nutrition Of Ruminants .in The Ruminant Animal Digestive Physiology And Nutrition Ed. D.C. Church, A. Reston Book. Prentice Hall, Englewood Cliffs, New Jersey., 1988, p 283 -285.
- [7] Figuerola, F., Hurtado, M.L., Estevez, A.M., Chiffelle I., and Asenjo, F.2005. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chem. 91, 2005, 395-401.
- [8] Francis, 2002 Francis G, Zohar K, Harinder P.S. Makkar HP, and Becker K. 2002. The

biological action of saponin in animal systems : A review. J. Nutr. British 88: 587-605.

[9] Guenther, Ernest., The essential Oils. Vol. 1 History-Origin Plants Production-analysis. D. Van Nostrand Company, Inc. New York. 1948

[10] Ginting, S. P., dan J. Elisabeth. 2002. *Teknologi Pakan Berbahan Dasar Hasil Simpangan Perkebunan Kelapa Sawit*. Prosiding Lokakarya Nasional: Sistem Integrasi Kelapa Sawit-Sapi. Bengkulu 9-10 September 2002

[11] Hariyati, M. N. Ekstraksi dan Karakterisasi Pektin dari Limbah Proses Pengolahan Jeruk Pontianak. Fakultas Teknologi Pertanian, Institut Pertanian Bogor: Bogor., 2006.

[12] Jayanegara, A.,A.S. Tjakradidjaja & T. Sutardi, Fermentabilitas dan Kecernaan *in Vitro* Ransum Limbah Agroindustri yang disuplementasi Kromium Anorganik dan Organik, [13] Media Peternakan, Agustus, 2006, p 54-62

Juliantina, Farida R., Dewa Ayu Citra M., Bunga Nirwani., Titis Nurmasitoh., dan Endrawati Tri Bowo, Manfaat Sirih Merah (*Piper crocatum*) Sebagai Agen Anti Bakterial Terhadap Bakteri Gram Positif dan Gram Negatif. JKKI Vol.1 No. 1 April 2009 Jurnal Kedokteran dan Kesehatan Indonesia, 2009.

[14] Laboratorium Kimia Organik. Analisis Kimia Tepung Limbah Jeruk manis (*Citrus sinensis*), Sumedang: Fakultas MIPA, Universitas Padjadjaran, 2013

[15] Laboratorium Nutrisi Ternak Ruminansia dan Kimia Makanan Ternak.. Analisis Proksimat Tepung Limbah Jeruk manis (*Citrus sinensis*). Sumedang: Fakultas Peternakan, Universitas Padjadjaran, 2010

[16] McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, Animal Nutrition sixth edition. Longman, Harlow, UK, 2002

[17] Oluremi, O I A., J Ngi., and I A Andre., Phytonutrients in citrus fruit peel meal and nutritional implication for livestock production. University of Agriculture, Makurdi, Benue State. Nigeria. Livestock Research for Rural Development 19 (7) 2007.

[18] Perry, Tilden Wayne., Beef Cattle Feeding and Nutrition. Academic Press, Inc. San Diego, California. 1980.

[19] Silalahi, R. E., Uji fermentabilitas dan kecernaan *in vitro* suplemen Zn anorganik dan Zn organik dalam ransum ruminansia, Skripsi, Fakultas Peternakan. Institut Pertanian Bogor, Bogor. 2003

[20] Sutardi, T., Ketahanan protein bahan makanan terhadap degradasi oleh mikroba rumen dan manfaatnya bagi peningkatan produksi ternak, Bulletin Makanan Ternak, IPB. 5(1): 1979, p 1-7

[21] Suwandystuti. S.N.O., Produk Metabolisme Rumen pada Sapi Peranakan Ongole Fase Tumbuh, Agripet : Vol (13) No. 1, 2013, p 31-35

[22] Telley, J.M.A. and R.A.Terry, A two stage technique for the *in vitro* digestion of forage crops, J.Br.Grassland Soc.18, 1963, p 104-111

[23] Van Soest, P. J. Estimation of true Digestibility of forages by the *in vitro* of cell walls. Proc. 10th international Grassland Congress, 1982

[24] Widodo,W., Widodo, Wahyu. *Tanaman Beracun Dalam Kehidupan Ternak*. UMM press. 2005.