THE EFFECTS OF FEEDING WHOLE COTTONSEED AS A SUPPLEMENT FOR SHEEP FED A BASAL DIET GRASS HAY ON FEED DIGESTIBILITY AND THE RUMEN FERMENTATION

(Pengaruh Pemberian Biji Kapas sebagai Suplemen pada Ternak Domba yang Diberi Ransum basal Hay terhadap Kecernaan Pakan dan Fermentasi Rumen)

Ismartoyo

Faculty of Animal Science, Hasanuddin University, Makassar, 90245.
Email:ismartoyo@gmail.com

ABSTRACT

This experiment was conducted in vivo to investigate the effects of feeding WCS as a supplement for sheep fed GH on the apparent feed digestibility, and the rumen fermentation. Four different diets (T1-T4) were used, GH alone, or GH plus 150, 300 or 500 g WCS d-1 (fresh basis). The main objectives of the in vivo experiment was to examine digestibility and rumen characteristics (pH, ammonia and VFA concentration) of sheep fed the four diets. The results of this experiment indicated that supplementation of GH with WCS at level of 500 g d-1 (0.37 of the diet) reduced DM, ADF and NDF digestibility. There was no statistically significant effect on total rumen VFA, but the molar proportions of acetate, propionate and butyrate were altered by the highest rate of inclusion of WCS. These results suggested that WCS might have reduced the numbers or activities of cellulolytic rumen microorganisms.

Key words : Whole cottonseed, Gossypol, Feed digestibility, and Rumen characteristics.

ABSTRAK

Penelitian ini dilakukan untuk menguji pengaruh beberapa level supplemen biji kapas terhadap daya cerna bahan kering, ADF dan NDF ransum, dan fermentasi dalam rumen domba jantan dewasa yang diberi pakan basal hay. Dengan menggunakan rancangan percobaan Latin Square (4x4) domba dialokasikan pada 4 perlakuan ransum T1, T2, T3, and T4. Ke 4 ransum tersebut adalah T1 = hay, T2 = hay + 150 g biji kapas, T3 = hay + 300 g biji kapas, dan T4 = hay + 500 g biji kapas. Hasil penelitian ini menunjukkan bahwa suplementasi biji kapas 500 g/hari menurunkan daya cerna bahan kering, ADF dan NDF ransum. Biji kapas yang digunakan dalam penelitian ini mengandung gossypol 1.93 g/kg. Pemberian suplemen biji kapas tidak berpengaruh terhadap karakteristik rumen (pH, ammonia, total konsentrasi VFA), tetapi berpengaruh terhadap proporsi molar asam asetat, asam propionat, dan asam butirat. Suplementasi biji kapas meningkatkan proporsi molar asam butirat yang menyarankan bahwa suplementasi biji kapas menurunkan aktivitas selulolysis dari mikroba rumen.

Kata kunci : Biji kapas, gossypol, daya cerna pakan, karakteristik rumen.

INTRODUCTION

In Indonesia and other tropical countries wherever cotton is grown whole cottonseed (WCS) has been utilised by farmers as feed supplement for ruminants (Preston and Leng, 1987: Ely and Guthrie, 2012). Coppock *et al.*, (1987) reported that WCS is rich in energy, protein and crude fibre content which is similar to that of peanut kernels with skins and hulls. It was suggested that inclusion of WCS up to 25% in the diets of dairy cows increased net energy for lactation and milk fat percentage. However other studies *in vivo* indicated that inclusion of WCS in the diets of steers (Moore *et al*, 1986; Ely and Guthrie, 2012) or sheep (Bird and Dicko, 1987) reduced dry matter and fibre digestibility and numbers of rumen microbes. It was not clear whether fat content or gossypol and/or a combination of both fat and gossypol contributed to the decreased in the dry matter and fibre digestibility. It has been reported that the presence of gossypol might have contributed to the reduction of the number of rumen microbes and degradation of grass hay (GH) in the consecutive batch culture (Ismartoyo *et*

al, 1993). The fermentation of GH by rumen protozoa (Ismartoyo *et al*, 1994), and attachment to and degradation of cellulose by rumen fungi in culture (Ismartoyo *et al*, 1995a) were reduced in the presence of gossypol. Feeding studies *in vivo* with whole cottonseed (Ismartoyo *et al*, 1995b) showed that supplementation of whole cottonseed (WCS) up to 500 g d^{-1} (0.37 of the diet) for sheep fed GH resulted in a significant reduction in the numbers of protozoa and fungi in the sheep rumen.

In the present study, an experiment was conducted *in vivo* to examine the effects of feeding WCS as a supplement for sheep fed GH on the apparent feed digestibility, and the rumen fermentation. Four different diets (T1-T4) were used, GH alone, or GH plus 150, 300 or 500 g WCS d^{-1} (fresh basis). The main objectives of the *in vivo* experiment was to examine digestibility and rumen characteristics (pH, ammonia and VFA concentration) of sheep fed the four diets.

MATERIALS AND METHODS

Animals

Four male mature castrated sheep of average weight (80.7 $\pm$ 4.6) kg, each fitted with rumen canulae of 40 mm internal diameter, were used. The sheep were housed indoors in separate pens and were chosen from six animals on the basis of their ability to consume the WCS offered.

Diets management and experimental design

Four diets were offered to the sheep according to a 4 x 4 Latin square design (Table 1) with periods of four weeks. The diets consisted of grass hay (GH) alone, or 150, 300 or 500 g d^{-1} whole cottonseed (WCS) plus GH (diets T1-T4 respectively). The amount of GH offered was that which the animals would eat, leaving little or no residue. This was achieved by adjusting the amount of GH offered during the first 3 weeks of each period (adjustment stage), and a fixed amount as determined in the initial stage during the 4th week when observations were made. Each diet was offered as two equal meals at 08.00 and 16.00 h. The WCS was always offered first, and the GH once the WCS had been consumed. Thus intake of hay was restricted, although the amount of hay consumed varied between sheep. The average intakes of feed eaten are given in Table 2.

Water was freely available to sheep throughout the experiment. WCS was offered in a separate small bucket so that intakes of GH and WCS could be measured.

Measurement of rumen characteristics

Rumen liquor samples were taken through the rumen fistula 2 h after the morning feed for 2 days in each observation period for measurement of rumen characteristics (pH, NH_3-N and VFA concentration). Rumen fluid was immediately transferred to a beaker for pH measurement using a portable pH meter (Model M-80, Hanna Instruments Ltd., UK).

Rumen VFA

Samples of rumen fluid for VFA analysis were collected at the same time as that for pH measurements. The rumen sample was immediately strained through a double layer of muslin and transferred to plastic bottles (100 ml) and frozen at -20^{o}C. Volatile fatty acid (VFA) concentrations in the rumen samples were analysed by HPLC according to the method of Rooke *et al.* (1990).

Rumen NH_3-N

Frozen rumen fluid was thawed at room temperature overnight and the supernatant

Tabel 1. Allocation of diets (T1-T4) and animals according to 4 x 4 Latin square experimental design

Periods of observation	Identity numbers of sheep and diets offered			
	sheep 8	sheep 5	sheep 4	sheep 9
I (21 days)	T1	T2	T3	T4
II (21 days)	T3	T4	T2	T1
III (21 days)	T2	T1	T4	T3
IV (21 days)	T4	T3	T1	T2

T1 = GH alone, T2 = GH + 150 g WCS, T3 = GH + 300 g WCS and T4 = GH + 500 g WCS.
GH = grass hay, WCS = whole cottonseed

Tabel 2. Intake, digestibility of feed and rumen characteristics of sheep fed diets T1-T4

Measurements	T1	T2	T3	T4	SED
Feed intakes (g d-1) :					
Dry matter of GH	1009	879	727	645	na.
Dry matter of WCS	0	132	264	382	na.
Total dry matter	1009	1011	985	1030	46.8; ns.
Organic matter	949	953	935	973	17.6; ns.
Total protein	58[a]	81[b]	103c	126[d]	4.5
NDF	621	608	582	593	26.8; ns.
ADF	407	421	404	422	47.2; ns.
Digestibility (g kg-1) :					
Dry matter	681[a]	717[a]	679 [a]	633[b]	18.4
Organic matter	676[ab]	700[a]	696[a]	635[b]	20
Protein	889[a]	920[ab]	947[b]	948[b]	19.1
NDF	674[a]	695[a]	677[a]	602[b]	29.0
ADF	685[a]	712[a]	681[a]	614[b]	16.3
ME (MJ kg-1DM)*	9.65	10.05	9.99	9.00	na.
Rumen fluid :					
pH	6.6	6.4	6.2	6.5	0.14; ns.
NH3-N (mM)	4.4[a]	9.8[b]	8.7[b]	9.3[b]	1.4
Acetic acid (mM)	68.9	72.9	70.2	59.4	9.3; ns.
Propionic acid (mM)	23.8	26.9	28.8	27.0	5.2; ns.
Butyric acid (mM)	6.0[ab]	7.1[a]	6.9[ab]	4.9[b]	0.9
Total VFA (mM)	99.7	107.0	106.2	91.3	18.6; ns.

Means with different subscripts in the same rows are significantly different ($p<0.05$). T1 = GH alone, T2 = GH + 150 g WCS, T3 = GH + 300 g WCS and T4 = GH + 500 g WCS. * Estimated ME = -1.15 + 0.16 DOM (Menke and Steingass, 1988), DOM = digestible organic matter, na. = no statistical analysis; ns. = not significant ($p>0.05$). WCS = whole cottonseed, GH = grass hay, VFA = volatile fatty acids.

(1 ml) was transferred to a small vial to which was added 1 ml sulphuric acid (10%, v/v, 10 ml concentrated sulphuric acid in 100 ml distilled H_2O). Rumen samples were centrifuged at 300*g for 5 min and the supernatant was transferred to tubes for NH_3-N determination. NH_3-N concentrations in the rumen fluid was analysed using an autoanalyser (TechniconInstruments) by the Central Analytical Unit, SAC, Aberdeen, according to the methods of Weatherburn (1967) by complexing with salicylate in the presence of nitroprussidehypochloride (a source of chlorine), in a buffered alkaline solution at a pH of 12.8-13. The absorbance of the ammonia-salicylate complex was read spectrophotometrically at 660 nm.

RESULTS AND DISCUSSIONS

The feed intake and digestibility, and the rumen characteristics (pH, NH_3-N and VFA concentrations) are shown in Table 2.

The data in Table 2 show the substitution of GH by WCS. The DM, NDF and ADF digestibility for supplemented diet T4 were significantly ($p<0.01$) reduced compared to T1, T2, and T3. There were no significant differences ($p>0.05$) in the total VFA concentrations between the diets. When the VFA concentrations were calculated as molar proportions of the total VFA, the molar proportion of propionic acids for T1, T2, T3 and T4 were 0.23, 0.25, 0.26, and 0.29 respectively ($p<0.05$, SED= 0.024) suggesting that supplementation with WCS at 500 g d^{-1} tended to increase the proportion of propionic acid. There were no significant ($p>0.05$) differences in the molar proportions of acetic and butyric acid between rumen liquor from sheep fed the different diets.

Figure 1 shows that the DM, NDF and ADF digestibility decreased as the the proportion of WCS in the diet increased. This was supported by the slopes of regressions between the feed digestibility against the proportion of WCS in

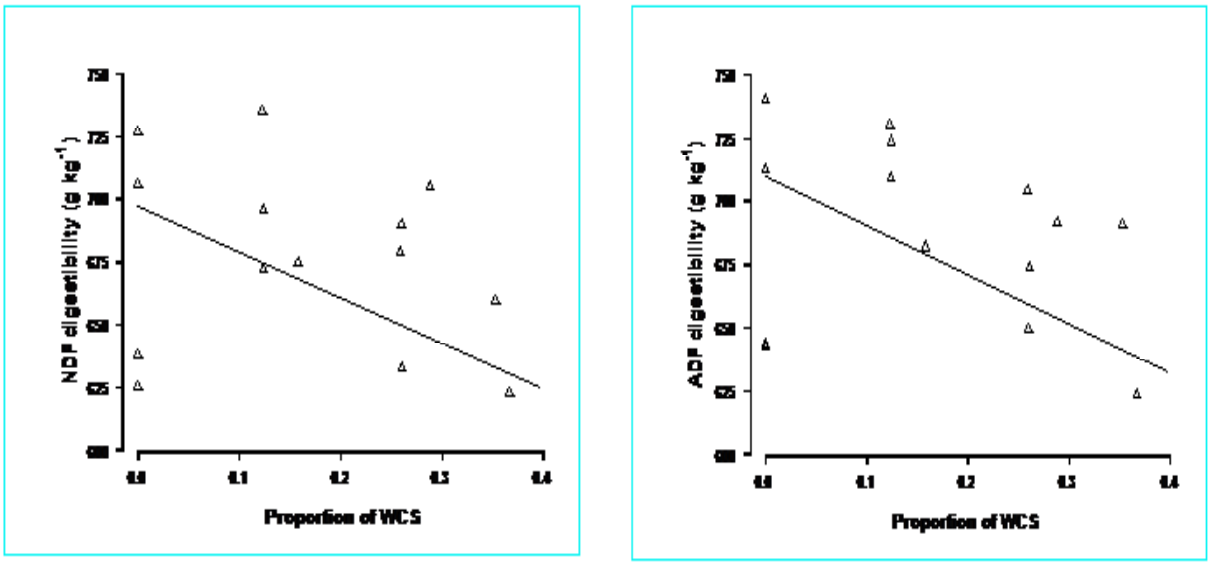

Figure 1. The regressions between feed digestibility vs the proportion of WCS supplement in the diet of sheep fed GH. WCS = whole cottonseed, GH = grass hay, DM = dry matter, OM = organic matter, NDF = neutral-detergent fibre, ADF = acid-detergent fibre

the diets (see Table 3). The reduced NDF and ADF digestibility of T4 indicated a possible depression of the activity or numbers of cellulolytic rumen microbes, and suggested that the microbial degradation of cellulose was affected by the presence of WCS.

The dietary lipid content of T4 of 79 g kg^{-1}, is higher than the level of dietary lipid in other studies (Moore *et al*, 1986; Bird and Dicko, 1987) from which it was concluded that a lipid content of 66 g kg^{-1} (derived from an inclusion of WCS) in the diet of steers or an addition of 60 to 90 g kg^{-1} cottonseed oil in the diet of sheep decreased DM and ADF digestibility. It is unclear whether the oil content of WCS might be involved in the reduction of the DM and fibre digestibility in the rumen of sheep fed T4.

Gossypol content of WCS used in this study was 1.93 g kg^{-1} (Ismartoyo, 1999) and the gossypol was analysed using methods of Botsoglou (1992). WCS is also known to contains other antinutrients compounds such as tannins (Bailey, 1948; Acamovic, 1994; Ismartoyo, 1999) and various pigments glands (Jones, 1969; Lyman *et al*, 1963; Risco and Chase, 1997) which might contributed low rumen fermentation. The results from the earlier study *in vitro* (Ismartoyo *et al*, 1993) indicated that removal of oil from WCS did not increase its fermentability suggesting that the oil content might not the main factor causing the reduction of the DM and fibre digestibility. Other factors affecting the degradability and fermentation characteristics of WCS such as the degradability and fermentability of NDF and ADF, the presence of lignin and of the presence of antinutritional factors might contribute to the low DM and fibre digestibility(Nunung Akhirany, dkk, 2013; Rohmyatul Islamiyati,

Table 3. Regression equations of feed digestibility (g kg-1) vs the proportion of whole cottonseed in the diet of sheep fed grass hay (n = 16)

Feed digestibility	Regression equations	r^2 (%)
DMD	DMD = (705 + 15.21) - (145 + 63.6) WCS	27.1
OMD	OMD = (695.9 + 17.8) - (102.5 + 74.3) WCS	11.9
NDFD	NDFD = (697.4 + 19.5) - (181.5 + 81.5) WCS	26.1
ADFD	ADFD = (708 + 18.8) - (189.6 + 78.7) WCS	29.4

WCS = the proportion whole cottonseed in the diet of sheep fed GH, GH = grass hay, DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral-detergent fibre digestibility, ADFD = acid-detergent fibre digestibility, r^2 = coefficient of regression

dkk, 2013; Jamila, dkk, 2013), and probably the activity of the cellulolytic microbes in the rumen of sheep fed T4. The low nitrogen content of GH might also affect the NDF and ADF digestibility of GH basal diet.

CONCLUSION

The most significant findings from this experiment taken together were as follows. Supplementation of GH with WCS at level of 500 g d^{-1} (0.37 of the diet) reduced DM, ADF and NDF digestibility. There was no statistically significant effect on total rumen VFA, but the molar proportions of acetate, propionate and butyrate were altered by the highest rate of inclusion of WCS. These results suggested that WCS might have reduced the numbers or activities of cellulolytic rumen microorganisms.

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