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Effect of feeding whole cottonseed as a supplement on digestibility and rumen fermentation characteristics of sheep

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Abstract

An experiment was conducted in vivo to investigate the effects on the apparent feed digestibility and rumen fermentation of feeding whole cottonseed (WCS) as a supplement for sheep fed grass hay (GH). Four different diets (T1, T2, T3 and T4) were used as GH alone, GH plus 150, 300 and 500 g WCS d-1 on fresh basis. The main objectives of the in vivo experiment were to examine digestibility and rumen fermentation characteristics (pH, ammonia and VFA concentration) of sheep fed four diets. The results of this experiment indicated that supplementation of WCS at a level of 500 g d-1 (0.37 of the diet) with GH 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 addition of highest rate of WCS. These results suggested that WCS might have reduced the number or activity of cellulolytic rumen microorganisms.

Key words: whole cottonseed, gossypol, feed digestibility, rumen fermentation characteristics

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Introduction

Whole cottonseed is an excellent feed and should be used extensively whenever practical. It is an excellent source of crude protein (20%), crude fiber (22%) and TDN (87%) (Lane O. Ely and Larry D. Guthrie, 2012). 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). Coppocket 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) 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 and Acamovic, 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 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. Cottonseed is a product of the cotton-fiber industry that is extensively used as an energy and protein source in dairy cattle diets. It provides a unique blend of protein, energy, and fiber compared with other feedstuffs. Cottonseed is fed to dairy cows at 10 to 15% of the total diet dry matter (DM). Diets containing more than 15% cottonseed generally are high in fat and may contain undesirable concentrations of gossypol (José Eduardo P. Santos, 2011).

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 characteristics (pH, ammonia and VFA concentration) of sheep.

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Materials and Methods

Animals

Four male mature castrated sheep of average weight (80.7 + 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 three weeks. The diets consisted of GH alone, or 150, 300 or 500 g d-1 of 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 fully consumed. Thus, intake of hay was restricted, although the amount of hay consumed varied between sheep.

Measurement of rumen fermentation characteristics

Rumen liquors were taken through the rumen fistula 2 h after the morning feed for 2 days in each observation period for measurement of rumen fermentation characteristics (pH, NH3-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 volatile fatty acid (VFA)

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

Rumen ammonia nitrogen (NH3-N)

Frozen rumen fluid was thawed at room temperature overnight and the supernatant (1 ml) was transferred to a small vial. To it 1 ml sulphuric acid (10%, v/v, 10 ml concentrated sulphuric acid in 100 ml distilled H2O) was added. The samples were then centrifuged at 300*g for 5 min and the supernatant was transferred to a tubes for NH3-N determination. NH3-N concentration in the rumen fluid was analysed using an auto analyser (Technicon Instruments) 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 spectro-photometrically at 660 nm.

Table 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. Fresh and clean water was available ad libitum to sheep throughout the experiment. WCS was offered in a separate small bucket so that intakes of GH and WCS could be measured.

Result and Discussion

The feed intake, digestibility, and rumen characteristics (pH, NH3-N and VFA concentrations) are shown in Table 2. The data in Table 2 showed 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 different diets.

Figure 1 showed that the DM, NDF and ADF digestibility decreased as the proportion of WCS in the diet increased. This was supported by the slope of the regressions of feed digestibility against the proportion of WCS in the diet (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 (Ismartoyo et al, 1993).

Table 2. Intake, digestibility of feed and rumen fermentation 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 crude protein	58a	81b	103c	126d	4.5
NDF	621	608	582	593	26.8; ns.
ADF	407	421	404	422	47.2; ns.
Digestibility (g kg^{-1}) :					
Dry matter	681a	717a	679a	633b	18.4
Organic matter	676ab	700a	696a	635b	20
Crude protein	889a	920ab	947b	948b	19.1
NDF	674a	695a	677a	602b	29.0
ADF	685a	712a	681a	614b	16.3
ME (MJ kg^{-1}DM)*	9.65	10.05	9.99	9.00	na.
Rumen fluid :					
pH	6.6	6.4	6.2	6.5	0.14; ns.
NH_3-N (mM)	4.4a	9.8b	8.7b	9.3b	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)	6.0ab	7.1a	6.9ab	4.9b	0.9
Total VFA (mM)	99.7	107.0	106.2	91.3	18.6; ns.

Means with different subscripts in the same row 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.

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 of 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.

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 (79 g kg-1). Whereas, the results from the earlier in vitro study (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.

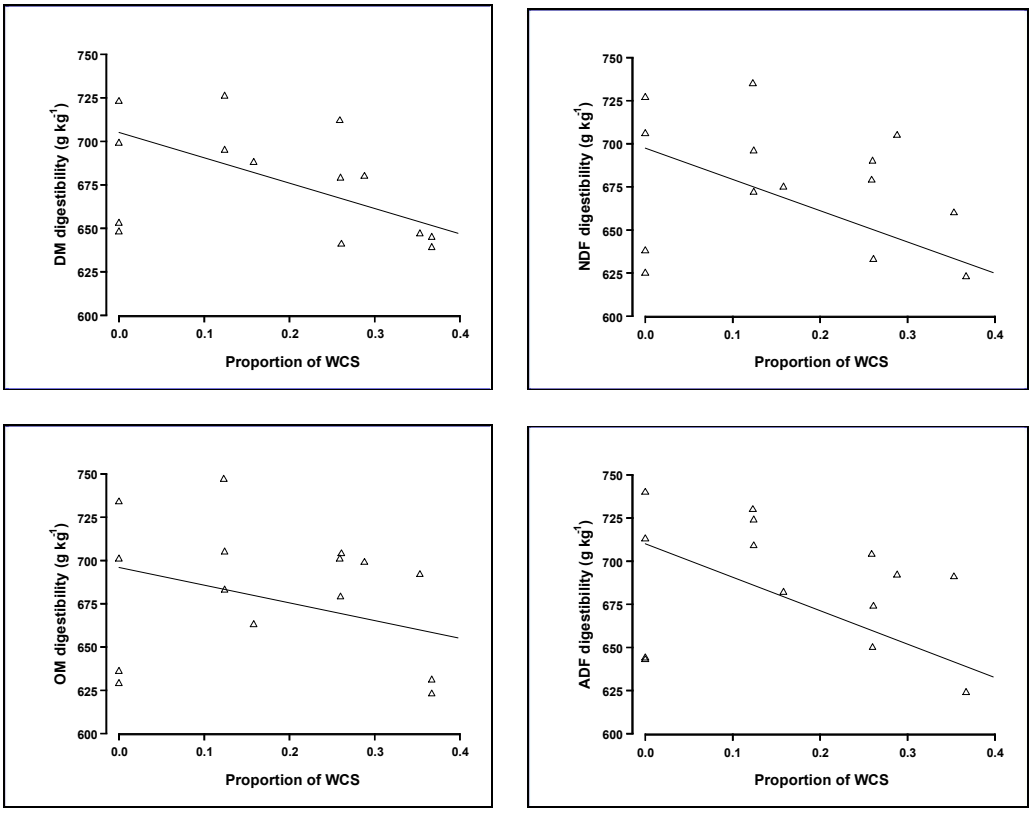

Figure 1. Regressions between feed digestibility vs the proportion of WCS supplement in the diet of sheep fed GH. WCS = whole cottonseed, GH = grass hay, DM = dry matter, OM = organic matter, NDF = neutral-detergent fibre, ADF = acid-detergent fibre.

On the other hand, gossypol content of WCS used in this study was 1.93 g kg-1 (Ismartoyo, 1999). WCS is also known to contain other anti-nutritional compounds such as tannins (Bailey, 1948; Acamovic, 1994) and various pigments (Jones, 1979; Lyman et al, 1963; Risco and Chase, 1997) which might contribute to low rumen fermentation. 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 the presence of antinutritional compounds might contribute to the low DM and fibre digestibility, and probably the activity of the cellulolytic microbes (Ismartoyo et al, 1994a; Ismartoyo et al, 1994b) in the rumen of sheep fed T4 (diet T4 contains the highest antinutritional compounds compared to that of T3 and T2). The low nitrogen content of GH might also affect the NDF and ADF digestibility of GH basal diet (Preston and Leng, 1987)

Conclusion

Supplementation of WCS at level of 500 g d-1 (0.37 of the diet) with GH reduced DM, ADF and NDF digestibility. There was no statistically significant effect on the total rumen VFA concentration, but the molar proportions of acetate, propionate and butyrate were altered by the increased rate of inclusion of WCS.

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References

Acamovic T (1994). The advantages and disadvantages of xenobiotic in plant foods and feeds. In: Development and ethical considerations in toxicology. (Ed. M.I.Weitzner) *Royal Society of Chemistry*:129-138.

Bailey AE (1948). Cotton seed. Inter scholastic Publishing Inc., New York, USA. p.522.

Bird SH and M Dicko (1987). Cottonseed supplement for sheep. *In*: Recent advances in animal nutrition in Australia. (Ed. D.J. Farrel). *Department of Biochemistry, Microbiology and Nutrition, University of New England*, Amidala, Australia, Pp. 80-88.

Botsoglou NA (1992). Liquid chromatographic determination of unbound and acetone-soluble bound gossypol in cottonseed meals and mixed feeds. *Journal of AOAC* 75:815-822.

Coppock CE, JK Lanham and JI Horner (1987). A Review of the nutritive value and utilization of whole cotton seed, cotton seed meal and associated by-products by dairy cattle. *Animal Feed Science and Technology* 18:89-129.

Eduardo J and P Santos (2011). Gossypol Intake from Cottonseed Feedstuffs – A Performance Concern for Dairy Cattle. Extension, issues, innovation, impact. May 23, 2011.Department of Animal Sciences University of Florida, Gainesville.

Ismartoyo (1999). Analysis of gossypol in whole cottonseed and rumen liquor of sheep fed a basal diet grass hay supplemented with whole cottonseed. BuletinIlmuPeternakandanPerikanan. Vol. V (13), April 1999, Pp 11-31.

Ismartoyo and T Acamovic (1994). The effect of gossypol on animal cells in culture. ***In*** Plant-Associated Toxins; Agricultural, Phytochemical and Ecological Aspects. (Eds. S.M. Colegate and P.R. Dorling). CAB International, Wallingford, United Kingdom, pp. 201-206.

Ismartoyo, CS Stewart, T Acamovic, and AJ Richardson (1995a). The effect of gossypol on rumen fungal attachment to and degradation of cellulose in culture. *British Society of Animal production, Proceedings of the Winter Meeting, Animal production:*153.

Ismartoyo, CS Stewart, WJ Shand and T Acamovic (1994a). The effect of gossypol on the rumen protozoal degradation of grass hay (GH) *in vitro*. *VIII International Symposium on ruminant Physiology*. 25-30 September 1994, Willigen, Germany. p. 205.

Ismartoyo, CS Stewart, WJ Shand and T Acamovic (1994b). *In vitro* rumen microbial degradation of a selection oilseeds and legume seeds under consecutive batch culture (CBC). Animal Production 58**:**453.

Ismartoyo, T Acamovic and CS Stewart (1993). The effect of gossypol on the rumen microbial degradation of grass hay under consecutive batch culture (CBC). Animal Production 56: (Suppl.1).462 (A.).

Ismartoyo, T Acamovic, CS Stewart, AJ Richardson, SH Duncan and WJ Shand (1995b). The effect of feeding WCS (whole cottonseed) as a supplement for sheep fed GH (grass hay) on the rumen fermentation and defaunation of rumen microorganism. *Conference on the Evaluation of Tropical Forages for Ruminant Utilisation*. Held in University of Zimbabwe, August 27 to September 1st, 1995.

Jones LA (1991). Definition of gossypol and its prevalence in cottonseed products. In: Cattle Research with gossypol containing feeds: A collecting of papers addressing gossypol effects in Cattle. (Eds L.A. Jones, D.H. Kinard and J.S. Mills). Published by National Cottonseed Products Association, Memphis, Tennessee. Pp 1-18.

Jones LA (1979). Gossypol and some other terpenoids, flavanoids, and phenols that affect quality of cottonseed protein. *Journal of American Oil Chemistry Society* 56:727-730.

Lane EO and LD Guthrie (2012). Feeding Whole Cottonseed to Dairy Cows and Replacements. College of Agricultural and Environmental Science, The University of Georgia.