Indonesian Journal of Tropical Agriculture

Volume 10 Number 1, January 2001 ISSN: 0852-5927

Published by :

Research Institute - Institut Pertanian Bogor
Bogor - Indonesia

Indonesian Journal of Tropical Agriculture

Volume 10, Number 1, January 2001 **ISSN: 0852-5927**

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Indon. J. Trop. Agric. 10 (1) : 1 - 3. 2001

POPULATION OF SECRETORY CELLS AND SYNTHETIC ACTIVITIES IN MAMMARY GLAND OF LACTATING SHEEP AFTER CONSUMING *Sauropus androgynus* (L.) Merr. LEAVES

A. Suprayogi*, U. ter Meulen**, T. Ungerer***, & W. Manalu***

ABSTRACT

Thirty-five lactating ewes were divided into four groups and given *Sauropus androgynus* (SA) leaf extract solution at 1.89 g/day orally twice a day (SAx-group), SA leaf powder solution at 7.44 g/day (SAp-group), distilled water (control-group), and untreated group. Milk yield was measured. After 14 and 35 days, half of the udders were excised and the DNA and RNA determined. SAp administration increased total milk yield for 35 days administration (P>0.05), compared to the control values. The population of secretory cells (total DNA) and synthetic activities (total RNA) were also increased significantly (P<0.05) by SA leaves administration for 14 days.

Keywords : Sauropus androgynus, milk synthesis, DNA, RNA

Sauropus androgynus (SA), a member of *Euphorbiaceae* family, is a leafy shrub found in Malaysia, Indonesia, South-west China and Vietnam (Padmavathi & Rao, 1990). In Indonesia, the leaves of this plant are commonly used as a vegetable and have properties as stimulants in mother's breast feeding (Soeparto, 1994).

The SA leaves can also stimulate milk production in lactating ruminants (Suprayogi, 1993). It has however not been established if the SA leaves influence milk synthesis process through improving the population of secretory cells and synthetic activities in the mammary gland or through an improvement in the nutrient supply to the mammary gland. Besides, the chemical investigation on the active compounds in SA leaves is still scarce.

This experiment was carried out to determine the influence of either the powder of SA leaves (SAp) or SA leaf alcohol extract (SAx) on the population of secretory cells (as indicated by total deoxyribonucleic acid, DNA) and synthetic activities (as indicated by total ribonucleic acid, RNA) in the mammary gland, and its effect on milk yield.

METHOD

Animals and Housing

This experiment was carried out from March 1998 until July 1999 at the Faculty of Veterinary Medicine and Life-Sciences Interuniversity Center, Bogor Agricultural University, Bogor-Indonesia. This experiment was conducted during the hot (25.50 ± 3.02 °C) and humid (69.85 ± 9.05 % relative humidity) season.

Thirty-five first lactating javanese Thin-Tailed ewes with a mean body weight of 20.20 + 2.03 kg and age of 1.5 to 2.0 years at the first week of lactation were used. All ewes had twins at parturition, and were placed in individual cages. The Javanese thin-Tailed sheep is normally used as a model of lactating ruminants (Sumaryadi, 1997; Manalu & Sumaryadi, 1998), although it is a meat-type, it is well recognised for its high prolificacy (Bradford *et al*., 1986; Sutama *et al*., 1988).

Animal Feeding

Each ewe in the individual cage was fed a mixed concentrate and dry chopped elephant grass *ad libitum*. Water was available freely. Elephant grass was given at 06:30 h until mid-day and then was replaced with concentrate until late afternoon, and finally it was replaced again with the elephant grass overnight.

Preparation of SA Leaf Powder and Extract Solution

Fresh SA leaves from the local markets around Bogor were dried in an automatic oven at 60°C overnight. The dry leaves were ground to powder (SAp), and the powder was extracted to produce the thick SA leaf extract (SAx).

Extraction method of SA leaves was as described by Santoso *et al*. (1997), using 70 % alcohol as a solvent. The extract was evaluated using the pharmaceutical test standard (Yuliani & Marwati, 1997), and was found to have no toxic effects on the laboratory animal from acute and subacute doses (Santoso *et al*., 1997).

This method uses a maceration technique, as follows: 88 grams of SA leaf powder were mixed with 1 litter of 70 % alcohol, stirred for up to 9 hours and then the mixture was stored for 24 hours. The mixture was filtered and the liquid extract was evaporated using a rotary-evaporator at the temperature of 50°C to produce the thick extract.

SA leaf extract solution (5 %) was made by dissolving 5 g of SA leaf extract in 100 ml distilled water and SA leaf powder solution (18 %) was made by dissolving 18 g of SA leaf powder in 100 ml distilled water.

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Experimental Design

Thirty-five lactating ewes were divided into four groups and fed concentrate and dry elephant grass for 35 days. Each group was given either SA leaf extract solution at 1.89 g/day orally twice a day (SAx-group; 10 ewes), SA leaf powder solution at 7.44 g/day (SAp-group; 10 ewes), distilled water (control-group; 10 ewes), or untreated (5 ewes). The experimental design was completely randomised (Steel & Torrie, 1980).

Milk Yield Measurement

Milk yield was measured using a glass-measuring cylinder. The ewes were hand milked, twice a day at 07:00 h and at 16:00 h.

Mammary Total DNA and RNA Measurement

After 14 and 35 days the ewes were slaughtered, half of the udders were excised. The mammary gland was isolated by trimming the skin, subcutaneous fat, and removing milk inside the gland. The isolated mammary gland was frozen to facilitate slicing. The thinly sliced mammary gland samples were soaked in 90 % ethanol for 48 h., and then with diethyl-ether for a further 48 hours until the glands became free of fat. The fat-free sliced mammary gland samples obtained were dried at 52°C for 48 h., and then ground to make a fine powder for use in the determinations of mammary chemical indices.

DNA of mammary gland was determined by *p*-nitrophenylhydrazine (Sigma: N-8880, 1996) reaction. RNA of mammary gland was determined by the orcinol (Sigma: O-1875, 1996) reaction as described by Manalu & Sumaryadi (1998).

Data Analysis

Analysis of variance (ANOVA) was used to determine the difference between the treatment means (Snedecor & Cochran, 1982). A probability (P) value less than 0.05 was accepted as significantly different. Duncan's multiple range test (Steel & Torrie, 1980) was used to determine differences between the treatment means.

RESULTS AND DISCUSSION

The concentrate and elephant grass intakes were 880.50 (±45.75) and 460.80 (± 38.35) g of dry matter, respectively. The daily nutrient intake was close to the nutrient requirements of ewes per day, according to NRC (1985) recommendations for lactating ewes suckling twins in the first 6-8 weeks of lactation.

SAp and SAx administration increased total milk yield for 35 days by 7.75 % and 0.89 % (P>0.05), respectively compared to the control values of 17.89 litres (Table 1). The mammary cell numbers (total DNA) and synthetic activities (total RNA) were also increased significantly (P<0.05) by SA leaf administration for 14 days. The SAp administration had a higher contribution to the increase in total DNA and RNA than SAx administration of respectively, 72.84 % vs 25.93 % in DNA and 112.97 % vs 47.28 % in RNA, compared to the control values of 0.81 g in total DNA and 2.39 g in total RNA (Table 2).

The possible reason of the enhancement of milk yield was due to the increase in the proliferation of mammary gland cells and their synthetic activities as indicated by an increase in total mammary DNA and RNA (Manalu & Sumaryadi, 1998). Probably, the SA-leaf active compounds might directly or indirectly modulate, prior to lactogenesis and lactation, hormones such as prolactin (PRL), growth hormone (GH), glucocorticoids, thyroid hormone, prostaglandin, and oxytocin. These hormones directly stimulate the synthesis of DNA and RNA in the lactating mammary secretory cells (Shiu & Friesen, 1980). Further studies should be carried out to identify chemical substances in the SA leaves, which play an important role directly or indirectly in milk synthesis.

Table 1. Weekly mean milk yield, total yield for 35 days, and percentage increase in yield of lactating ewes given SA leaf extract (SAx) and SA leaves powder (SAp).

Mean Weekly Yield	Mean daily milk yield per ewe (litres ± SD)		
	Control Group	SAx-Group	SAp-Group
0 week*	0.43±0.06	0.43±0.09	0.42±0.05
1st week	0.50±0.06	0.50±0.09	0.49±0.04
2nd week	0.53±0.07	0.51±0.08	0.56±0.07
3rd week	0.52±0.05	0.51±0.09	0.57±0.06
4th week	0.50±0.10	0.52±0.08	0.55±0.05
5th week	0.44±0.10	0.48±0.10	0.52±0.05
Total Milk Yield For 35 days	17.89 ± 2.36	18.05 ± 3.12	19.27 ± 1.50
(%) Increase in Milk Yield	0.00	0.89	7.75

* Mean milk yield before starting treatment, or mean milk yield for the first week of lactation.

Table 2. Mean mammary total deoxyribonucleic acid (DNA), and total ribonucleic acid (RNA) of lactating ewes given SAx, SAp, and PPV

Duration of treatment	Group	Mean mammary total DNA and RNA (g ± SD)*	
		DNA	RNA
Untreated (1st week lactation)		0.60 ± 0.19	1.01 ± 0.73
14 Days (3rd weeks - lactation)	Control	0.81 ± 0.05[a]	2.39 ± 0.82[a]
	SAx	1.02 ± 0.26[a]	3.52 ± 1.10[a]
	SAp	1.40 ± 0.43[b]	5.09 ± 1.66[b]
35 Days (8th weeks - lactation)	Control	0.96 ± 0.20	4.57 ± 2.76
	SAx	0.98 ± 0.22	4.58 ± 1.60
	SAp	1.10 ± 0.27	4.88 ± 2.38

* Means with no common superscript ([a], [b]) in the same column are significantly different (P<0.05)

The reason for the lower milk yield by SAx administration compared to SAp administration is that the chemical active compounds in the 70 % alcohol extract are not quantitatively and qualitatively completely available as in the powder form. Therefore the biological effect of the active compounds, in the SAx were not maximal. Probably, addition of higher doses of the SAx could improve biological response.

CONCLUSIONS

The enhancement of milk yield in the lactating mammary gland can be due to an increase in the population of secretory cells (total DNA) and the synthetic activities (total RNA). The higher contribution on the biological effect occurred from SAp administration than SAx administration. Further studies on the influence of the SA leaves administration on the improvement of nutrient supply to the mammary gland needs to be conducted.

ACKNOWLEDGEMENTS

The authors would like to express our sincere and heartfelt gratitude to the late Prof. Dr. Soewondo Djojosoebagio (Former Director of Life-Science Interuniversity Center, LS-IUC, Bogor Agricultural University, Bogor, Indonesia) for having arranged the University Research-Graduate Education (URGE-World Bank, Loan No. 3754-IND) project for this research funding. His interest, support and enthusiasm stimulated me to embark on this research.

We also thank from Indonesia: Prof. Dr. Dodi Nandika (Current Director of LS-IUC, IPB, Bogor, Indonesia) and Prof. Dr. Eddie Gurnadi (Coordinator of cooperation between IPB and Georg-August University of Göttingen), and from Germany: Dr. Diethard Mai (Managing Director of Tropical and Sub-Tropical Center, Georg-August University of Göttingen) for ensuring the smooth running of the project.

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THE PROFILES OF WEEKLY PROGESTERONE AND STRADIOL CONCENTRATIONS DURING PREGNANCY IN EWES : 2. THEIR CORRELATIONS WITH MAMMARY GROWTH INDICES AT PARTURITION

M.Y. Sumaryadi* & W. Manalu**

ABSTRACT

Fifteen pregnant (9 and 6 carrying a single and multiple fetuses, respectively) and 5 nonpregnant ewes, as a control, were used to study the correlations of weekly maternal serum progesterone and estradiol concentrations during pregnancy with mammary growth indices at parturition. Blood samples were drawn weekly (weeks 0 to 20) during gestation period for determination of progesterone and estradiol concentrations. The experimental ewes were sacrificed at parturition to determine mammary growth indices (mammary dry fat-free tissue [DFFT], DNA, RNA, and collagen). Concentrations of progesterone at week 1 and weeks 3 to 19 of pregnancy positively correlated with mammary DFFT, DNA, and RNA at parturition. Maternal serum progesterone concentrations at weeks 1, 3 and weeks 6 to 20 of pregnancy positively correlated with mammary collagen at parturition. Concentrations of estradiol at weeks 5, 7, 9, and 11 to 20 positively correlated with the mammary DFFT, DNA, RNA, and collagen at parturition. The higher the maternal serum progesterone and estradiol concentrations during pregnancy the greater the mammary growth and development at parturition.

Keywords : progesterone, estradiol, mammary growth, pregnancy, sheep

Secretions of estradiol and progesterone increase dramatically with the changes in ovarian activity during the estrous cycle and pregnancy. In ovine, secretion of estradiol increases during proestrous along with the maturation of the follicle in the ovary, then decreases significantly during the embryonal stage of pregnancy, and increases precipitously during the fetal stage of pregnancy until parturition (Umo *et al.*, 1976; Pant *et al.*, 1977; Manalu *et al.*, 1996). Progesterone increases 2 days after ovulation, and it shows a marked rise from day 5 to a peak between days 7 and 13 (Umo *et al.*, 1976; Pant *et al.*, 1977). It remains almost stable during the first 7 weeks of pregnancy and increases dramatically after week 8 of pregnancy (Butler *et al.*, 1981; Manalu *et al.*, 1996).

During the luteal phase of estrous cycle and the embryonal stage of pregnancy, maternal serum progesterone concentrations are positively correlated with the number of corpora lutea (Quirke *et al.*, 1979). During the fetal stage of pregnancy in goats and sheep (when the placenta is functional), progesterone and estradiol (Manalu *et al.*, 1996), and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Butler *et al.*, 1981) also increases with the increased fetal number.

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Mammary gland growth and development during pregnancy in ovine starts during the estrous cycle, slowly progresses until the first three months of gestation and dramatically increases during the last two months of gestation (Anderson, 1975; Anderson *et al.*, 1981) around the time when the placenta significantly secretes progesterone (Ricketts & Flint, 1980; Sheldrick *et al.*, 1981), and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Butler *et al.*, 1981).

Temporal changes in mammogenic hormones secretions relating to pregnancy seem to correlate well with the mammary growth pattern in ovine. The present study was designed to determine temporal changes in maternal serum progesterone concentration during pregnancy and mammary gland growth and development at parturition in ewes.

METHODS

Experimental Conditions and Animals

This experiment was conducted during the hot (25 to 32°C) and wet (70 to 80% relative humidity) season in the humid tropics of Indonesia. Experimental animals were 20 Javanese thin-tail ewes (5, 9, 4, and 2 ewes carrying 0, 1, 2, and 3 fetuses, respectively) with similar body weight (20 to 22 kg) and age (2 to3 years) at breeding. Javanese thin-tail sheep is a meat-type indigenous breed well recognized for its high prolificacy (Bradford *et al.*, 1986; Sutama *et al.*, 1988). The experimental ewes were injected twice with $PGF_{2\alpha}$ (i.m) at an 11-day interval. Three days after the last injection, 15 ewes were mated naturally by group breeding.

Blood Sampling and Processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein between 0900 and 1000 h. The first blood samples were taken one day after the last prostaglandin injection (week 0 of pregnancy), and 10 days later (seven days after the predicted ovulation, as the end of week 1 of pregnancy). Additional blood samples were drawn weekly on Thursday until parturition. Blood samples were allowed to clot in a cool ice box, centrifuged to separate serum, which was then frozen for estradiol and progesterone analyses. At parturition the experimental ewes were sacrificed for determination of mammary gland growth and development at the beginning of lactation.

Progesterone, Estradiol, and Mammary Gland Analyses

The concentration of serum progesterone was measured in duplicate by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-progesterone as a tracer, with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu *et al*., 1996). The progesterone assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-progesterone-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 0.1 and 20 ng/ml, respectively. Therefore, the concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 ml serum was used in the assay of samples with progesterone concentrations ranged from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 ml. For samples with progesterone concentrations higher than 20 ng/ml, sample volume was reduced to 50 ml. Inter- and intra-assay coefficients of variation were 6, and 4%, respectively. Concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 ml.

The concentration of serum estradiol was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125}-estradiol as a tracer, with a slight modification to accommodate wide ranges of estradiol concentrations in pregnant ovine (Manalu *et al*., 1996). The estradiol assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-estradiol-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 20 and 150 pg/ml, respectively. Therefore, the concentrations of standard estradiol used to construct the standard curve ranged from 20 to 150 pg/ml. A sample volume of 100 ml serum was used in the assay for samples with estradiol concentrations ranged from 20 to 150 pg/ml. For samples with estradiol concentrations lower than 20 pg/ml, sample volume was increased to 200 to 300 ml to bring the estradiol concentrations to the range of standard used. For samples with estradiol concentrations higher than 150 pg/ml, sample volume was decreased to 50 ml to bring the estradiol concentrations to the range of standard used. All samples' estradiol concentrations were within the range of concentrations of standard estradiol used to construct the standard curve. Inter- and intra-assay variations coefficients were 7 and 5.0%, respectively. The concentrations of estradiol were parallel in the sample volumes of 50, 100, 200 and 300 ml.

Dry fat-free tissue (DFFT), DNA, RNA and collagen are indices used to determine mammary gland growth and development. Dry fat-free tissue (DFFT) was measured by modification of method described by Anderson (1975). Half the udder was excised and the mammary gland was isolated by trimming skin and subcutaneous fat and removing milk inside the gland. The isolated mammary gland was frozen for easy slicing. The thinly sliced mammary gland was soaked in ethanol for 48 hr and then with diethyl ether (48 hr) until the glands became free of fat, and then dried at 50°C for 24 h to obtain DFFT. The DFFT was then ground to make a fine powder to be used for mammary chemical indices determinations. Mammary DNA was determined by p-nitrophenylhydrazine reaction (Webb & Levy, 1955), RNA by orcinol reaction (Albaum & Umbreit, 1947), collagen by measuring hydroxyproline (Woessner, 1961).

Statistical Analyses

Weekly estradiol and progesterone concentrations during pregnancy were regressed with mammary DFFT, DNA, RNA and collagen at parturition using simple regression and correlation analyses (Neter *et al*., 1985).

RESULTS

The profile of maternal serum progesterone concentration during pregnancy in the experimental ewes is setout in Figure 1. Progesterone concentration slowly increased during the first 8 weeks of pregnancy. After that, it increased rapidly and reached peak concentrations around weeks 12 to 16 of pregnancy and then it decreased near parturition. In the ewes carrying multiple fetuses, concentrations of progesterone were distinctly higher than in those carrying a single fetus, and the greatest differences occurred during weeks 12 to 16 of pregnancy.

The profile of estradiol concentrations in the maternal serum during pregnancy in the experimental ewes is depicted in Figure 2. The concentrations of estradiol were constantly low during the first 8 weeks of pregnancy. After week 8 of pregnancy, estradiol concentration in the pregnant ewes constantly increased until parturition, and being higher in the higher litter size. In the ewes carrying multiple fetuses, the concentrations of estradiol were distinctly higher than in those carrying a single fetus, and the greatest differences occurred during weeks 12 to 20 of pregnancy.

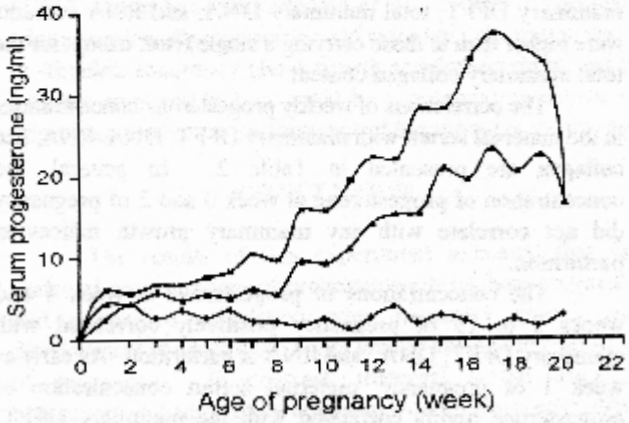

Figure 1. Weekly serum progesterone concentrations during pregnancy in the nonpregnant ewes (n), ewes carrying a single (D) and multiple (n) fetuses in Javanese thin-tail sheep.

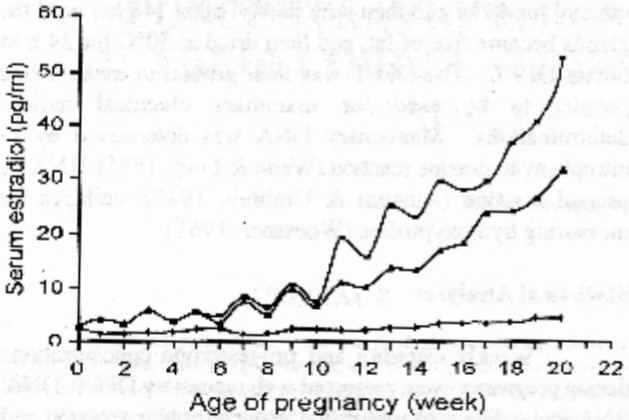

Figure 2. Weekly serum estradiol concentrations during pregnancy in the nonpregnant ewes (u), ewes carrying a single (D) and multiple (n) fetuses in Javanese thin-tail sheep.

Table 1. Mean (±SE) mammary DFFT and total mammary DNA, RNA, and collagen contents at parturition in the nonpregnant ewes, ewes carrying a single and multiple fetuses

Litter size	N	Total mammary chemical indices contents (g)			
		DFFT	DNA	RNA	Collagen
0	5	2.34[c]	0.10[c]	0.03[c]	0.11[b]
		0.21	0.01	0.003	0.01
1	9	26.39[b]	0.92[b]	0.30[b]	0.30[a]
		2.02	0.09	0.05	0.04
>1	6	45.88[a]	1.51[a]	0.94[a]	0.35[a]
		10.56	0.30	0.23	0.04

[a,b,c]Means with different superscripts in the same column are significantly different ($P < 0.05$).

Average mammary DFFT and its component were absolutely lower in nonpregnant than in pregnant ewes (Table 1). However, in pregnant ewes carrying multiple fetuses, mammary DFFT, total mammary DNA, and RNA contents were higher than in those carrying a single fetus, except for the total mammary collagen content.

The correlations of weekly progesterone concentrations in the maternal serum with mammary DFFT, DNA, RNA, and collagen are presented in Table 2. In general, the concentration of progesterone at week 0 and 2 of pregnancy did not correlate with any mammary growth indices at parturition.

The concentrations of progesterone at week 1 and weeks 3 to 19 of pregnancy positively correlated with mammary DFFT, DNA, and RNA at parturition. As early as week 1 of pregnancy, maternal serum concentration of progesterone highly correlated with the mammary DFFT, DNA, and RNA at parturition. The higher the maternal serum concentrations at weeks 3 to 19 the higher mammary DFFT, DNA, and RNA contents indicating a greater mammary growth and development, mammary cell number and cellular activities at parturition. The highest correlations of mean serum progesterone concentrations with mammary DFFT, DNA, and RNA were occurred at week 12 of pregnancy.

The concentrations of progesterone at weeks 1 and 3, and weeks 7 to 20 of pregnancy positively correlate with mammary collagen at parturition. As early as week 1 of pregnancy, maternal serum concentration of progesterone was highly correlated with the mammary collagen at parturition. The higher the maternal serum concentrations at weeks 1 and 3, and weeks 7 to 20 of pregnancy the higher mammary collagen contents implying the greater mammary ductal growth and branching at parturition. The highest correlation of mean serum progesterone concentrations with mammary collagen was occurred at week 14 of pregnancy.

Table 2. Coefficients of correlation between weekly maternal serum progesterone concentrations during pregnancy and mammary gland indices (DFFT, DNA, RNA and collagen) at parturition in Javanese thin-tail ewes

Weeks of pregnancy	Mammary gland indices			
	DFFT	DNA	RNA	Collagen
0	0.01	0.04	0.03	0.21
1	0.66**	0.64**	0.65**	0.54*
2	0.04	0.11	0.15	0.08
3	0.65**	0.68**	0.56*	0.49*
4	0.47*	0.51*	0.44*	0.24
5	0.46*	0.51*	0.47*	0.26
6	0.58*	0.69**	0.59*	0.49*
7	0.67**	0.77**	0.73**	0.57*
8	0.62*	0.70**	0.60*	0.68**
9	0.78**	0.84**	0.78**	0.62*
10	0.73**	0.76**	0.68**	0.67**
11	0.77**	0.82**	0.75**	0.67**
12	0.85**	0.89**	0.84**	0.60*
13	0.69**	0.74**	0.72**	0.61*
14	0.57*	0.60*	0.51*	0.84**
15	0.79**	0.81**	0.66**	0.74**
16	0.72**	0.76**	0.78**	0.69**
17	0.80**	0.84**	0.78**	0.80**
18	0.71**	0.76**	0.70**	0.82**
19	0.74**	0.74**	0.69**	0.72**
20	0.43	0.41	0.44	0.78**

* $P < 0.05$.
** $P < 0.01$.

The correlations of weekly estradiol concentrations in the maternal serum with mammary DFFT, DNA, RNA, and collagen are presented in Table 3. In general, the concentration of estradiol at early stage of pregnancy (weeks 0 to 4) did not correlate with any mammary growth indices at parturition. The correlations of estradiol with DFFT, DNA and RNA, and with collagen, became evident from weeks 11 to 20 and weeks 12 to 20 of pregnancy, respectively, as concentrations of estradiol in the maternal circulation became significantly increase. These data indicated that the higher the concentrations of estradiol in the maternal circulation during weeks 11 to 20 of pregnancy, the greater the mammary gland growth and development at parturition.

Table 3. Coefficients of correlation between weekly maternal serum estradiol concentrations during pregnancy and mammary gland indices (DFFT, DNA, RNA and collagen) at parturition in Javanese thin-tail ewes

Weeks of pregnancy	Mammary gland indices			
	DFFT	DNA	RNA	Collagen
0	0.13	0.24	0.08	0.09
1	0.13	0.06	0.12	0.35
2	0.12	0.15	0.16	0.09
3	0.25	0.31	0.26	0.39
4	0.07	0.09	0.10	0.32
5	0.48*	0.48*	0.44*	0.57**
6	0.10	0.08	0.28	0.02
7	0.49*	0.46*	0.39	0.31
8	0.39	0.38	0.16	0.42
9	0.55**	0.53*	0.56**	0.55**
10	0.16	0.16	0.02	0.29
11	0.65**	0.65**	0.71**	0.38
12	0.63**	0.68**	0.61**	0.57**
13	0.50*	0.60**	0.52*	0.55**
14	0.67**	0.68**	0.63**	0.55**
15	0.63**	0.62**	0.59**	0.69**
16	0.61**	0.62**	0.57**	0.76**
17	0.60**	0.56**	0.49*	0.63**
18	0.48*	0.50*	0.42*	0.71**
19	0.63**	0.58**	0.57**	0.66**
20	0.62**	0.64**	0.57**	0.64**

* $P < 0.05$
** $P < 0.01$

DISCUSSION

Progesterone and estradiol are traditionally considered as hormones function in maintaining of pregnancy and parturition, respectively. The patterns of progesterone and estradiol profiles during pregnancy in sheep and goats have been explained previously (Manalu *et al.*, 1996; Manalu & Sumaryadi, 1998). The increased maternal serum progesterone and estradiol with the advance of pregnancy, especially during the fetal stage of pregnancy, is far beyond the levels required to maintain pregnancy. Previous report states that the increased maternal serum progesterone, and probably estradiol, with the advance of pregnancy has some farther functions in stimulation of mammary growth during pregnancy in preparation of nutrients secretion required by the newborn offsprings (Manalu & Sumaryadi, 1996).

Observations in sheep and goats (Anderson, 1975; Anderson *et al.*, 1981) suggest that mammary gland growth and development during pregnancy are parallel with the temporal changes in hormonal secretion during gestation. Among hormones that their secretions increase during pregnancy are estradiol, progesterone, relaxin, and placental lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Ricketts & Flint, 1980; Butler *et al.*, 1981; Sheldrick *et al.*, 1981). These hormones are generally grouped as mammogenic hormones i.e., hormones stimulating mammary growth and development. Exogenous administrations of progesterone, estradiol and relaxin in ovariectomized and nonpregnant mice have been shown to stimulated mammary gland growth and development as indicated by the increase in DFFT, DNA, RNA and collagen contents of the glands (Harness & Anderson, 1977a; Harness & Anderson, 1977b; Hayden *et al.*, 1979; Wright & Anderson, 1982; Wahab & Anderson, 1989).

The results of this experiment showed that the increased maternal concentrations of progesterone and estradiol during pregnancy had a positive correlation with mammary growth and development at parturition. Higher concentrations of progesterone and estradiol during pregnancy associated with the greater mammary growth and development at parturition. The correlation of maternal serum progesterone with mammary gland growth and development was evident since the beginning of pregnancy. However, the correlation of maternal serum estradiol with mammary gland growth and development was more evident during the fetal stage of pregnancy. The difference in temporal correlation of maternal serum concentrations of estradiol and progesterone during pregnancy with mammary gland growth at parturition was probably related to the temporal difference in secretion of both hormones, and the fluctuation in the secretion of the hormones during pregnancy. Maternal serum progesterone concentration increased slowly during weeks 1 to 8 of pregnancy, then increased dramatically until week 17, and then slowly decreased approaching parturition. Maternal serum estradiol tended to decrease from estrous to pregnancy (not shown in the Figure 2) and relatively stable during the first 7 weeks of pregnancy, and slowly increased until week 10 of pregnancy. Estradiol increased dramatically at week 11 of pregnancy until parturition.

Regardless of the difference, the results implied that increasing maternal serum progesterone and estradiol during pregnancy, either by exogenous administration or by increasing their endogenous secretion by superovulation, could be utilized to improve mammary gland growth and development at the beginning of lactation and milk production during lactation. Preliminary result shows that superovulated ewes have higher progesterone concentrations and greater mammary growth and development during pregnancy (Manalu *et al.*, 1999) and correspondingly higher milk production (60%) during lactation (Manalu *et al.*, 2000). Exogenous administration of progesterone and estradiol during pregnancy to stimulate mammary gland growth and development, and to increase milk production could be a potential technique in improving production performance of the animal in the tropics.

CONCLUSION

The results of this experiment indicated that the maternal concentrations of progesterone throughout pregnancy had a positive correlation with mammary growth and development at parturition; being greater during the fetal stage of pregnancy. In contrast, maternal serum estradiol concentration had positive correlations with mammary gland growth and development during the fetal stage of pregnancy. The results suggested that increasing maternal serum progesterone and estradiol during pregnancy, either by

exogenous administration or by increasing their endogenous secretion by superovulation, could be used to improve mammary gland growth and development at the beginning of lactation and milk production during lactation.

ACKNOWLEDGEMENT

This experiment was funded by grant provided by The Directorate General of Higher Education-Inter University Center (Contract # 007/P4M/DPPM/L.3311/PAU/ 1993. World Bank Loan XXI (Loan Agreement No.:3311-IND).

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THE EFFECT OF LIGNIN COMPOSITION ON DELIGNIFICATION RATE OF SOME TROPICAL HARDWOODS

W. Syafii*

ABSTRACT

The effect of lignin composition on delignification rate and pulp properties of albizia (*Paraserianthes falcataria* L. Nielsen), gmelina (*Gmelina arborea* Linn.), yellow meranti (*Shorea acuminatissima* Sym.), and kapur (*Dryobalanops aromatica* Gaertn.) woods was determined. The lignin characterization showed that the syringyl-guaiacyl ratio of albizia, gmelina, kapur, and yellow meranti woods are 2.03, 2.02, 1.87, and 1.30, respectively. It means that the lignin structure of the above mentioned woods are predominated by syringyl units. To assess the influence of lignin structure on delignification rate and pulp properties, these wood samples were then subjected to the kraft pulping process under the following conditions: wood-to-liquor ration = 1:4, cooking temperature = 170 °C, time to cooking temperature = 90 minutes, cooking time = 90 minutes, total active alkali = 16 %, sulfidity = 22.5 %. From the present investigation it can be explained that the delignification rate of hardwood containing syringyl-rich lignin was higher than that of hardwood containing syringyl-poor lignin. The physical properties of pulp from hardwood containing syringyl-rich lignin are also higher than that of hardwood containing syringyl-poor lignin.

Keywords : tropical hardwoods, lignin composition, syringyl-guaiacyl ratio, delignification rate, pulp properties

The process of delignification followed two pseudo first order rate kinetics in terms of lignin concentration. A faster rate of delignification was ollowed by a slower process. The rate of delignification of the initial faster process was directly proportional to the molar ratio of syringyl to guaiacyl units of lignin (Singh *et al*., 1982). Syafii & Yoshimoto (1991) reported that in one-hour chlorite treatment the rate delignification of hardwoods species such as ulin (*Eusideroxylon zwageri*), bangkirai (*Shorea laevis*), merawan (*Hopea pierrei*), and tekaliu (*Homalium foetida*) which containing guaiacyl-rich lignin was much slower than that of hardwoods containing syringyl-rich lignin.

Although the tropical hardwoods constitute a vast source of cellulose fibers, their limited use in pulp and paper industry on the one hand, could be described as due to their heterogenity. On the other hand, it may be due to the fact that practically little is known about the chemical composition of lignins of domestic tropical hardwoods and their reactions during pulping processes. Lignin is considered to be a polymeric natural products arising from an enzyme initiated dehydrogenative polymerisation of three types of primary precursors, namely (i) guaiacyl alcohol (ii) syringyl alcohol (iii) *p*-coumaryl alcohol. The constitutional model of lignin is composed of many reactive groups such as ether of various types, primary and secondary alcoholic hydroxyl groups, phenolic hydroxyl groups, carbonyl groups, methoxyl groups, aromatic sites of phenyl propanoid structure (Lewis & Sarkanen, 1998).

It is well known that chemically the lignins vary from species to species even in the same genus and their composition is also highly influenced by the climatic conditions of the place where the tree is growing. As stated by Higuchi (1985) that the softwood lignin is predominated by monomeric guaiacyl units which are connected by both ether and carbon-carbon linkages, while the hardwood lignin is composed of approximately equal amount of guaiacyl and syringyl units and connected by linkages similar to those of softwood lignin. As a result of this inherent variation in lignin composition, the pulping characteristics are bound to be highly species dependent. Therefore, in order to increase or rationalize the use of tropical hardwoods fiber in pulp and papermaking, a detailed investigation on the chemical composition of lignin and their influences during kraft pulping and their pulp properties was undertaken.

METHODS

Material

Four samples of wood species, namely albizia (*Paraserianthes falcataria* L. Nielsen), gmelina (*Gmelina arborea* Linn.), kapur (*Dryobalanops aromatica* Gaertn..), and yellow meranti (*Shorea acuminatissima* Sym.) woods were used in this experiment. The albizia and gmelina woods were taken from Bogor and Sumedang, respectively, while the kapur and yellow meranti woods from Berau (East Kalimantan). The result of pre-investigation showed that the wood samples used in this experiment having a basic density of 310 kg/m^3, 480 kg/m^3, 570 kg/m^3, and 620 kg/m^3 respectively.

Methods

Sample Preparation

A Willey mill has been used to prepare woodmeal that pass through a 40-mesh screen and retain on a 60-mesh screen. The woodmeal was then air-dried to about 15% of moisture content. Extraction of the woodmeal with alcohol-benzene (1:2 v/v) has been

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carried out in order to prepare an extractive-free woodmeal sample, which is then used to determine the Klason lignin content and lignin composition by nitrobenzene oxidation procedure.

After bark removal the wood sample was converted to chips at the size of about 2.5 cm x 2.5 cm x 0.25 cm, followed by air drying to about 15% of moisture content. The air-dried wood chips is then used for determination of delignification rate in the pulping process. Prior to pulping, the moisture content of wood chips should be determined exactly to calculate the need of cooking liquor in the pulping process.

Characterization of Lignin

The most common method for the quantitative determination of lignin in wood is based on gravimetry (Klason lignin). In this study the Klason lignin content was determined according to the procedure described by Dence (1992). The wood sample is first treated with 72 % sulfuric acid and subsequently heated with dilute acid to hydrolyze the polysaccharides to soluble fragments, after which the solid residue (Klason lignin) is washed, collected, dried, and weighed. To determine the lignin composition, the procedure of alkaline nitrobenzene oxidation reported by Syafii & Yoshimoto (1991) was applied. Approximately 50 mg of alcohol-benzene extracted wood meals was oxidized with 0.24 ml of nitrobenzene and 4 ml of 2N KOH in a stainless steel tube for 2 hours at 160^0 C. At the end of oxidation period, the stainless steel tube was immediately cooled down in running water to stop the reaction, and the solution was filtered. The oxidation products which still remaining in the residue was washed with a small amount of 0.1N KOH solution. After removing nitrogenous compounds from alkaline solution with chloroform, the pH of this alkaline solution was adjusted to 2.5 with 1N HCl solution. Finally, the solution was extracted with 30 ml of chloroform. This extraction was repeated four times. The extract was then quantitatively analyzed by gas chromatography under the following conditions: BPX5 0.5 column, N_2 carrier gas, 0.4 ml per minute flow rate, temperature of 200 ^{0}C, FID detector, 300 ^{0}C temperature injection, detector temperature of 350 ^{0}C.

Lignin composition was quantitatively determined by calculating the peak area of vanillin (guaiacaldehyde) and syringaldehyde from gas chromatogram. The total aldehyde as well as the syringyl-guaiacyl ratio were also determined. All of this parameters were calculated based on the Klason lignin content of wood samples.

Pulping Process

To study delignification rate, wood chips was pulped by using conventional kraft process in the laboratory digester. The kraft pulping process are applied because it is not only the dominant alkaline pulping technique for wood raw material but also the most important pulping process altogether. In this c[illegible] the kraft pulping process is applied according to the methods conducted by Gomide *et al.* (1997) with some modifications. The pulping was carried out in a rotating digester and electrically heated. In applying this technique, 3 different cooks were carried out using different cooking times. Each cook was 30 minutes longer than the previous one (cooking times = 0, 30, 60, and 90 minutes). The cooking conditions were: chips = 150 grams, wood-to-liquor ratio = 1 : 4, maximum cooking temperature = 170 ^{0}C, time to maximum temperature = 90 minutes, total active alkali = 16 %, based on oven-dry chips, sulfidity = 22.5 %. All cooks were carried out in duplicates.

Determination of Delignification Rate

The pulp yield and the kappa number of pulp are parameters that can be used to determine the delignification rate during pulping process. The determination of pulp yield and kappa number were applied according to the Tappi T 236 cm-85 (TAPPI Testing Methods, 1989).

Determination of Physical Properties

This procedure describes the testing of pulp handsheet for their strength and other physical properties. Information derived from handsheet testing indicates pulp quality and is a measure of the potential contribution of the pulp to the strength of the finished paper products. The physical properties of pulp was carried out according to the Tappi T 220 om-88 (TAPPI Testing Methods, 1989).

RESULTS AND DISCUSSION

Extractives Content

To asses the normality of extractives content in the wood samples, ethanol-benzene extract yield was determined (Table 1). The total amount of ethanol-benzene extractives obtained from the wood of albizia, gmelina, yellow meranti and kapur are 8.14 , 9.15 , 13.6, and 15.65 % based on oven-dry wood sample respectively. The acetone extracts obtained from this experiment is relatively higher than those of the average of extractives content in the tropical hardwoods. Tsoumis (1991) stated that the content of extractives varies from less than one percent to more than ten percent depends on the family, species, and tissue. The acetone extract obtained from the heartwood of gonzalo alves (*Astronium fraxinifolium*), rasamala (*Altingia excelsa*), ulin (*Eusideroxylon zwageri*), and sonokeling (*Dalbergia latifolia*) was reported reach to 3.34 , 2.64 , 8.18 , and 8.23 % respectively (Syafii *et al.*, 1985; Syafii & Yoshimoto, 1993; Syafii, 2000).

Table 1. **The ethanol-benzene extractives content obatined from four species of tropical hardwoods**

No.	Wood samples	Content (% based on oven-dry wood)
1.	Albizia	8.14
2.	Gmelina	9.15
3.	Yellow meranti	13.60
4.	Kapur	15.65

It is interesting to note that the ethanol-benzene extractives content has a correlation with the specific gravity of wood samples in which the higher specific gravity show the higher of extractives content. It could be understood since the extractives content is one of the factors which influence the specific gravity of wood. Tsoumis (1991) stated that extractives are compounds of varying chemical composition that are not part of the wood substances, but are deposited within cell walls and cavities. Higher amount of extractives is a cause for the higher density of wood and the removal of extractives results in reduction of density.

Lignin Characteristics

The Klason lignin content of four wood species is resumed in Table 2. The lignin content of the tropical hardwoods tested in this experiment is in the range of 22.40 to 30.72 %. The Klason lignin content of albizia wood is the lowest whereas the lignin content of kapur wood is the highest.

Table 2. **The lignin composition of wood samples (indicated by S/G ratio) calculated from nitrobenzene oxidation products *)**

Wood samples	Klason lignin (%)	Guaiacyl (G) (%)	Syringyl (S) (%)	Total aldehyde (%)	S/G Ratio (molar)
Albizia	22.40	7.00	14.22	21.22	2.03
Gmelina	25.50	8.07	16.30	24.37	2.02
Yellow meranti	30.00	9.56	17.87	27.43	1.87
kapur	30.72	11.94	15.51	27.45	1.30

*) Based on the Klason lignin content.

In order to get more detailed information on the lignin characteristics, the structural composition of lignin of all the four species was determined by analysing the alkaline nitrobenzene oxidation products on a gas chromatogram. Table 2 represented the oxidation products of lignin of the four species. The total aldehyde yields of four wood samples are in the range of 21.22 to 27.45 % on the basis of Klason lignin content. Table 2 also showed that the S/G ratio all the four wood samples are greater than one. It means that the amount of syringaldehyde content of the wood samples are higher than that of guaiacyl content. The values of S/G ratio both in yellow meranti and kapur woods are relatively lower than those of albizia and gmelina woods. It is indicating thereby that the lignin of yellow meranti and kapur woods contain a large amount of guaiacyl units, suggesting that the lignin macromolecules of these woods have more sites for internal condensation.

These results show that the amount of syringyl units in the lignin of tropical hardwoods investigated follow the order of albizia wood > gmelina wood > yellow meranti wood > kapur wood. These results are of great importance in contemplating and assessing the delignification characteristics of these woods, since S/G ratio should have a correlation with delignification rate. In other words, lignin containing higer amount of syringyl units should have less number of site available for possible internal condensation during delignification process. As stated by Fengel and Wegener (1984) that as in the case of sulfite pulping, the alkaline pulping reactions with lignin are also nucleophilic reactions which are contributing to the degradation and dissolution of lignin, and condensations of lignin units to fragments with increased molecular weight and reduced solubility.

Rate of Delignification

To assess the influence of lignin structure on delignification rate and pulp properties, these wood samples were then subjected to the kraft pulping process. The kappa number of pulp is usually used to determine the delignification rate during pulping process. The yield and the kappa number of pulp produced from four species of tropical hardwoods were presented in Table 3.

Table 3 showed that at 60 and 90 minutes cooking times the pulp yield of albizia and gmelina woods are much higher than that of yellow meranti and kapur woods. The results also showed that at the same cooking times the kappa number of pulp of albizia and gmelina are lower than that of yellow meranti and kapur woods. The low kappa number means that the delignification rate of these woods are faster than that of yellow meranti and kapur woods. The slow rate of delignification of yellow meranti and kapur woods might be due to the physical and chemical factors.

The physical factor is usually related to the wood density. Determination of specific gravity of the wood samples showed that the specific gravity of albizia, gmelina, yellow meranti and kapur woods are 0.31, 0.48, 0.57, and 0.62, respectively. Yellow meranti and kapur woods having higher specific gravity are more difficult to be delignified compared to the albizia and gmelina woods. From this results, it can be stated that the delignification rate of wood samples might be correlated with the specific gravity. Specific gravity is a measure of the weight of wood substances contained in a unit of volume of wood. Cell walls of all wood species should have the same specific gravity. Therefore, variation in specific gravity of wood species reflects differences in

Table 3. Yield and kappa number of pulp produced from four species of tropical hardwoods

Wood Samples	Cooking time at maximum temperature (minutes)							
	0		30		60		90	
	Yield (%)	Kappa Number	Yield (%)	Kappa Number	Yield (%)	Kappa Number	Yield (%)	Kappa Number
Albizia	42.75	33.79	48.55	31.65	49.15	28.05	49.98	22.71
Gmelina	49.25	33.35	44.40	30.85	53.90	25.85	49.15	20.70
Yellow meranti	43.60	42.85	39.80	35.75	34.48	31.33	31.55	30.75
Kapur	44.65	40.85	42.07	36.85	39.70	32.39	40.20	30.60

thickness of cell wall. A wood species with a high specific gravity posseses a thick cell wall, and consequently the cell lumen of this wood is small (Tsoumis, 1991). In this experiment, the wood samples of yellow meranti and kapur woods which have high specific gravity are difficult to be delignified. These results might be explained by the limited impregnation of cooking chemicals into the cell wall.

The chemical factor which affects the slow rate of delignification of yellow meranti and kapur woods is usually related to their lignin chemical structure. The results of this experiment showed that the rate of delignification increases with the increase in S/G of lignin of the wood samples. The same results were also found by previous investigators (Singh *et al.*, 1982; Syafii & Yoshimoto, 1991). These results means that the delignification of hardwood containing syringyl-rich lignin faster than that of hardwood containing syringyl-poor lignin. As previously described, the syringyl content in the lignin of yellow meranti and kapur woods are low, and consequently these two wood samples gave low rate of delignification. Therefore from this experiment, it can be suggested that the lignin structure of the wood samples is correlated with the rate of delignification by kraft pulping process.

Pulp Properties

The following data describes the strength and other physical properties of un-bleached pulp handsheets. The pulp handsheets was prepared by beating pulp at 45° SR with the grammature of 60 gram/m^2. The physical properties examined in this experiment are density, tear index, tensile index, burst index, and breaking length. This physical properties is listed in Table 4.

The physical characterization of unbleached pulp showed that the physical properties of yellow meranti wood is comparable to the kapur wood. The pulp properties of albizia has also showed the same phenomena in which it is comparable to the gmelina wood. However, the pulp properties of yellow meranti and kapur woods are much lower than those of albizia and gmelina woods. This low properties might be due to the low S/G ratio of these woods. As described before that the hardwood containing syringyl-rich lignin (high S/G ratio) showed the higher rate of delignification compared to those of hardwood containing syringyl-poor lignin (low S/G ratio), and as the consequence, it produces higher kappa number pulp. Casey (1980) stated that the high kappa number pulp correlates with high residual lignin. Therefore the physical properties of this pulp is lower than that of low kappa number pulp which contain low residual lignin.

Table 4. The physical properties of unbleached kraft pulp produced from four species of tropical hardwoods

Properties	Pulp-sheet samples			
	Albizia	Gmelina	Yellow Meranti	Kapur
Density, g/cm^3	0.68	0.57	0.28	0.28
Tear index, mN.m^2/g	5.09	4.29	0.33	0.24
Tensile index, N.m/g	102.7	90.9	65.6	52.5
Burst index, kN/g	6.37	5.75	3.02	2.23
Breaking length, km	5.55	4.67	0.36	0.26

CONCLUSION

The syringyl-guaiacyl ratio of albizia, gmelina, yellow meranti, and kapur woods were 2.03, 2.02, 1.87, and 1.30 respectively. It means that the lignin structure of the above mentioned wood samples are predominated by syringyl units.

The delignification rate of hardwood containing syringyl-rich lignin was higher than that of hardwood containing syringyl-poor lignin. The physical properties of pulp from hardwood containing syringyl-rich lignin were also higher than that of hardwood containing syringyl-poor lignin.

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WOOD HARVESTING DAMAGES, REGENERATION AND GROWTH IN THE RESIDUAL STAND OF DIPTEROCARP FORESTS (A Case Study in the Forest Concession Area of PT. Narkata Rimba, East Kalimantan, Indonesia)

Elias*

ABSTRACT

This research was conducted in the concession area of PT. Narkata Rimba (NR), Muara Wahau, Kabupaten Kutai, Kalimantan Timur, Indonesia. The result obtained indicated that the levels of residual stand damages positively correlated to the wood harvesting intensity, the incident of damages on small trees was greater and most of the damaged trees were heavily injured. The tree's mortality occurred during the wood harvesting year was 6.0-26.6% and one year after wood harvesting was 2.0-13.6%. After than the tree's mortality was 0.5-3.6%, meanwhile the mortality of trees in a virgin forest was 0.9-3.4% per year. It seems that higher damage occurred in residual stands causes the higher tree mortality. The seedling density of commercial dipterocarp species, in both the logged-over forests and a virgin forest were more then the recommended minimum amount (1,000 seedlings per ha) as stated in the regulation of the Indonesian Selective Cutting and Planting (*Tebang Pilih Tanam Indonesia/TPTI*) System. The average growth of diameter and volume of 25 nucleus trees hectare per year during the first five years after wood harvesting was 0.70 cm/year and 1.4235 m^3/ha/year, respectively. To anticipate the second cutting cycle, it is recommended to control the residual stand damages with an optinal wood harvesting intensity and to improve the technique of silviculture of dipterocarp forest, with the purpose to increase the diameter growth of nucleus trees.

Keywords : wood harvesting damage, residual stand, dipterocarp forest, regeneration, growth

For the management of a natural tropical rain forest, almost all of forest concession holders in Indonesia use the Indonesian Selective Cutting and Planting (*Tebang Pilih Tanam Indonesia*) System.

The cutting cycle of TPTI-System is 35 years. The TPTI-System is a system in which commercial trees with a diameter above 50 cm in a Permanent Production Forest and diameter above 60 cm in a Limited Production Forest are removed from a site and leaving a minimal of 25 young commercial and healthy trees per hectare distributed uniformly in the area.

An important criterion for the judgement of TPTI-System is the condition of the residual stand after wood harvesting, i.e. damage on the residual stand, tree's mortality, regeneration and growth of the residual stand. The objective of this research is to describe the actual situation of the residual stand, regeneration and growth of Dipterocarp forest in the forest concession area of the PT. Narkata Rimba (NR) East Kalimantan, after wood harvesting with TPTI-System.

The stages of the TPTI-System are as follows:

Table 1. The Stages of the TPTI-System

No.	Stages of Activities	Year of Implementation (-) : Before (+) : After Wood Harvesting
1.	Working area ordering	Et-3
2.	Inventory of stand before wood harvesting	Et-2
3.	Opening up forest	Et-1
4.	Wood harvesting	Et-0
5.	Horizontal clearance	Et+1
6.	Inventory of residual stands	Et+2
7.	Release I	Et+2
8.	Seed supply	Et+2
9.	Enrichment planting	Et+3
10.	Maintenance	Et+3, 4, 5
11.	Release II and III	Et+4, 6
12.	Spacing of residual stands	Et+10, 15, 20

Source : Direktorat Jenderal Pengusahaan Hutan. 1993. Departemen Kehutanan, Jakarta, Indonesia

METHODS

Research Location

This research was conducted in the forest concession area of PT. NR. Located in the district of Kutai in East Kalimantan, Indonesia. The forest concession of PT. NR covers an area of 68,000 ha which consists entirely of Limited Production Forest.

Vegetation type in PT. NR is highland natural forest, with the soil type consist of podzolic (68%), latosol (27%) and litosol (15%). The topography is undulating until hilly (70%) and steep slope (30%). The type of rainfall, based on Schmidt and Ferguson classification, is rainfall type A, with a mean annual rainfall amounted to 2.290 mm.

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Plots Design and Measurements

The research plots consists of 4 permanent plots, namely:

Plot I : plot before and after wood harvesting with slope 0-15%

Plot II : plot before and after wood harvesting with slope 16-25%

Plot III : plot before and after wood harvesting with slope > 25%

Plot IV : plot control or virgin forest

The size of each plot is 100x100 m. Figure 1 shows the plot design and Figure 2 shows the design of strip 2 and 4 for vegetation analysis.

The degree of residual stand damages was measured in 1992 and repeated measurements were conducted annually until 1996 on mortality of trees, regeneration and growth.

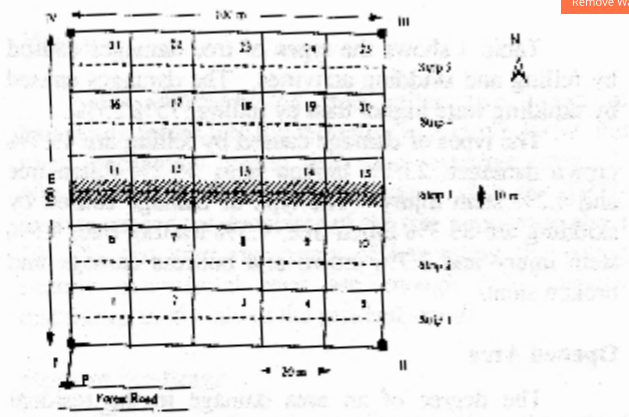
RESULTS

The Degree of Residual Stand Damages

The degree of residual stand damages based on tree population and the stages of vegetation development were 30.0% seedlings, 27.2% saplings and 24.6% poles and trees. Table 2 presents the data on the degree of tree (diameter of 10 cm and above) damages based on the wood harvesting intensity. The residual indicated that the higher intensity of wood harvesting causes heavier damages to the residual stands.

Table 2. Degree of Residual Stand Damages Based on Wood Harvesting Intensity

Permanent Plot	Number of Tree Before Wood harvesting ($\phi \geq$ 10cm)	Wood Harvesting Intensity (trees/ha)	Tree Damages ($\phi \geq$ 10cm) (trees/ha)	Degree of Damages (%)
I	620	2	58	9.4
II	697	6	146	21.1
III	748	17	259	35.4



: Sub plot (1-25), where trees with a dbh of 10 cm and above is measured

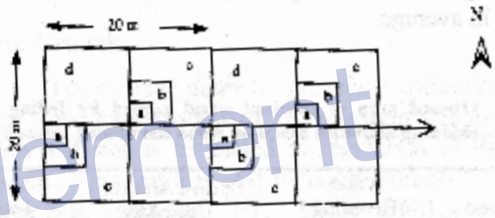
: Strip (1-5), strip 2 and 4 for vegetation analysis; strip 3 for stand profile analysis

: Strip (10x100 m), for stand profile analysis

: General line, for tree location mapping

$P_{e\ I,II,III,IV}$: Polygons

Figure 1. Constructed permanent plots design in the research site



a : Seedlings (hight <1.5 m) are measured on subplots 2x2 m

b : Saplings (hight ≥ 1.5 dbh < 10 cm) are measured on subplots 5x5 m

c : Poles (dbh ≥ 10-19 cm) are measured on subplots 10x10 m

d : Trees over 20 cm dbh are measured on subplots 20x20 m

Figure 2. Design of nested sampling for trees and regeneration

Most of the damaged trees were small sizes, namely 65.2% damaged trees with diameter of 10-19 cm and 21.2% with a diameter of 20-29 cm. Based on the size injury of each individual trees, the degrees of the damages caused by wood harvesting were 82.1% trees with heavy injury, 13.2% trees with medium injury and 4.6% trees with light injury. An analysis of the effects of diameter and height of trees and slopes on residual stand damages shows, that the effects on the residual stand damages were not significant.

Table 3. Types of tree damages caused by felling and skidding activities

Permanent Plot	Wood Harvesting Intensity (trees/ha)	Types of Tree Damage (%)					Total (%)
		Fallen Tree	Broken Stem	Crown Damages	Bark & Stem Injury	Root & Buttress Injury	
I	2	5.5	1.0	2.6	-	0.3	9.4
II	6	17.8	0.9	1.6	0.6	0.3	21.1
III	17	21.9	3.2	8.1	1.6	-	35.4

Table 3 shows the types of tree damages caused by felling and skidding activities. The damages caused by skidding were higher than by felling (75%:25%).

The types of damage caused by felling are 49.5% crown damages, 23.1% broken stem, 19.2% fallen tree and 8.2% stem injury. The type of damage caused by skidding are 83.3% fallen tree, 4.5% leaning tree, 9.5% stem injury and 2.7% crown and buttress damage and broken stem.

Opened Area

The degree of an area damage in the residual stand caused by felling and skidding depends largely on the wood harvesting intensity, mainly the opened area caused by felling. The higher intensity of wood harvesting causes a larger opened area of the residual stand. Table 4 shows the size of opened area caused by felling and skidding activities based on wood harvesting intensity. The size of the opened area caused by harvesting of one tree ranged between 285 and 512 m^2 to 396 m^2 in average.

Table 4. Opened area of residual stand caused by felling and skidding activities based on wood harvesting intensity

Permanent Plot	Wood Harvesting Intensity (trees/ha)	Opened Area (m^2) Caused by		Total (m^2)
		Felling	Skidding	
I	2	92	596	688
II	6	808	2,008	2,816
III	17	2,512	2,324	4,836

Mortality of Trees

Table 5 shows the mortality of trees with a diameter ≥ 10 cm. in the residual stand of Et-0, Et+1, Et+2, Et+3 and in the virgin forest. In general, the dead trees consisted of small trees with diameter of 10-39 cm. The mortality occurred in the current wood harvesting was 6.0-26.0 % and one year after wood harvesting was 2.0-13.6%. These figures decreased drastically after two years of wood harvesting (0.7-3.6%) and three years of wood harvesting (0.5-3.6%). It seems that higher damage occurred in residual stands causes the higher tree mortality. The mortality of trees in a virgin forest was 0.9-3.4% per year.

The Stock of Seedling

The stock of seedling of commercial Dipterocarp species before wood harvesting, one and two years after wood harvesting and in a virgin forest are as presented in Table 6.

Table 5. Mortality of trees with a diameter of 10 cm and above in the residual stand and virgin forest

Permanent Plot	Diameter Class (cm)	Mortality (%) Et-0	Et+1	Et+2	Et+3
I	10-19	7.0	2.9	0.6	0.8
	20-29	4.5	1.0	0	0
	30-39	2.5	1.3	1.3	0
	40-49	4.2	0	4.3	0
	50-59	15.4	0	0	0
	≥ 60	0	0	0	0
	Total	6.0	2.0	0.7	0.5
II	10-19	20.2	16.1	3.9	0.8
	20-29	16.1	9.9	3.9	3.7
	30-39	7.8	13.6	2.0	2.7
	40-49	9.1	5.0	5.3	0
	50-59	0	12.5	0	0
	≥ 60	0	5.6	0	8.3
	Total	17.0	13.6	3.6	2.3
III	10-19	22.3	14.6	0.6	4.0
	20-29	19.6	10.8	2.0	3.4
	30-39	22.5	9.1	0	0
	40-49	14.8	8.7	0	0
	50-59	7.1	0	0	0
	≥ 60	15.4	4.5	0	0
	Total	26.6	12.5	0.8	3.6
IV (Virgin Forest)	10-19	1.2	3.2	1.2	1.9
	20-29	1.9	0	0	1.8
	30-39	0	4.8	2.5	4.6
	40-49	9.5	0	0	8.7
	50-59	7.1	0	0	0
	≥ 60	0	0	0	0
	Total	1.6	2.4	0.9	3.4

Table 6. The Seedling Density of Commercial Dipterocarp Species in the Research Site

Permanent Plot	Et-0 (Seedlings/ha)	Et+1 (Seedlings/ha)	Et+2 (Seedlings/ha)
I	28,000	21,000	12,875
II	4,250	11,625	25,875
III	13,625	13,500	11,1225
IV (Virgin forest)	-	22,750	25,500

Table 6 shows that the total amount of seedlings of commercial Dipterocarp species, both in the logged over forests and the virgin forest were more than the minimum amount of 1,000 per hectare, as required by the regulation contained in the TPTI-System.

Growth of Nucleus Trees

Diameter measurements of nucleus trees are recorded for five consecutive years within the permanent plots in the logged-over forests and virgin forest. Table 7 shows the growth of diameter and volume of 25 nucleus trees per hectare. The average growth of diameter and volume of 25 nucleus trees after wood harvesting were 0.70 cm/year and 1.4235 m^3/ha/year.

Table 7. The growth of diameter and volume of 25 nucleus trees after wood harvesting in the concession areas of PT. NR

Permanent Plot	Period of growth	Growth of	
		Diameter (cm/year)	Volume (m^3/ha/year)
I	1st year	0.94	2.1120
	2nd year	0.50	1.1990
	3rd year	0.63	1.1050
	4th year	0.59	1.1116
	Average	0.67	1.5320
II	1st year	0.96	1.7679
	2nd year	0.40	0.6853
	3rd year	0.54	0.9582
	4th year	0.69	1.2765
	Average	0.65	1.1720
III	1st year	1.10	1.9866
	2nd year	0.53	1.0581
	3rd year	0.60	1.2926
	4th year	0.93	1.9285
	Average	0.79	1.5665
I + II + III	Average	0.70	1.4235

DISCUSSION

Wood Harvesting Intensity

The degree of residual stand damages was found correlated to the intensity of wood harvesting. The higher wood harvesting intensity causes the heavier of residual stand damages. This result has been shown also by Abdulhadi *et al.* (1981), Butar Butar (1991), Yanuar (1992) and Bertault & Sist (1995). For the implementation of this result, it should be possible to control the degree of residual stand damage by regulating the optimal wood harvesting intensity in TPTI. If level of residual stand damages is under control, an adequate number of seedlings, saplings and young commercial and healthy trees will be secured due to the fact that stocking of seedlings, saplings, poles and young trees of commercial species before wood harvesting with TPTI-System in the dipterocarp forest in East Kalimantan, were more than adequate.

Mortality

The mortality of trees after two years of harvesting seems like the mortality in a virgin forest and trees' population during that time increased about 2-10%. Although the wood harvesting with the TPTI-System caused the decreases of the tree population about 10-37%, but after two years of harvesting the young healthy commercial trees are enough (>25 trees/ha distributed uniformly in the residual stand).

Stock of Seedlings

The amount of seedlings of commercial Dipterocarp species in one and two years after wood harvesting can be considered enough. There were about 11,000-25,000 seedlings/ha. It was 11-25 times of the minimum amount of 1,000 seedlings per ha as required by the regulation of the TPTI-System. Therefore, it is not necessary to plant seedlings in the residual stand after wood harvesting.

Tree Growth

The average diameter growth of commercial trees after wood harvesting with TPTI-System was lower than 1 cm/year not as estimated in regulation of the TPTI-System. This result is also found by Sutisna (1990) in a concession area of PT. ITCI, Balikpapan, East Kalimantan. The average diameter growth of meranti group was about 0.7-0.8 cm/year and commercial trees of non Dipterocarp group were 0.6-0.8 cm/year. According to Continuos Forest Inventory (CFI) in East Mindanao, Philippines (Weidelt & Banaag, 1982), the average diameter growth of some commercial tree species is as follows:

- white lauan (*Pentacme contorta*) : 0.8-1.2 cm/year
- mayapis (*Shorea squamata*) : 0.7-1.0 cm/year
- red lauan (*Shorea negrosensis*) : 0.6-0.8 cm/year
- almond (*Shorea almon*) : 0.6-0.7 cm/year
- bagtikan (*Parashorea plicata*) : 0.4-0.6 cm/year
- apitong (*Dipterocarpus grandiflorus*) : 0.3-0.5 cm/year
- yakal (*Shorea gisok*) : 0.3-0.4 cm/year

The volume growth of 25 nucleus trees hectare during the first five years after wood harvesting with TPTI-System were 1.1720-1.5665 m^3/ha/year or 1.4235 m^3/ha/year in average. Compared to the result of CFI in the Philippines, the periodic annual increment (PAI) in dipterocarp forest in East Mindanao was 1.7 m^3/ha/year. Those results a similar one to another.

Diameter growth of 25 nucleus trees during the first five years after wood harvesting were about 0.65-0.79 cm/year or 0.70 cm/year in average. It seems after the first cycle of wood harvesting with the TPTI-System (35 years), most of the nucleus trees with diameter between 20-35 cm, still not achieve a diameter bigger than 60 cm (diameter limit in Limited Production Forest). So, those trees can not be cut in the second

cycle according to TPTI-regulation, unless the government (c.q. Department of Forestry) allows the reduction of the cutting diameter limit. To avoid this, we should improve the technique of silviculture of dipterocarp forest with the purpose to increase the diameter growth of nucleus trees.

CONCLUSION & RECOMMENDATIONS

The degree of residual stand damages ranged from 28 to 45% and the incidence of damages to small trees greater (ca. 87%). Most of the damaged trees were heavily injured (ca. 82%) and had little chance to recover since their growth would be affected by the damage.

The degree of residual stand damage was found correlated to the intensity of wood harvesting. The mortality rate of young trees left after wood harvesting was between 6.0-26.6%. During the fist year 2.0-13.6% and then the mortality decreased drastically to 0.5-3.6%. The mortality of trees in virgin forest was 0.9-3.4% per year.

It is not necessary to plant seedling in the residual stand after wood harvesting in dipterocarp forests. The natural regeneration is sufficient. The average growth of diameter and volume of 25 nucleus trees per hectare during the first 5 years after wood harvesting with TPTI-System were 0.70 cm/year and 1.4235 m3/ha/year in average.

For the implementation of this research result, it is recommended to TPTI regulation as follows:

1. To control the residual stand damages caused by wood harvesting with optimal logging intensity.
2. The diameter growth of nucleus trees after wood harvesting with TPTI-System is smaller than 1 cm per year. To anticipate the next cycle, it is necessary to improve the technique of silviculture of dipterocarp forest, with the purpose to increase the diameter growth of nucleus trees.

ACKNOWLEDGMENTS

Appreciation and thanks are due to the Directorate of Research and Community Service, Directorate General of Higher Education, Department of Education and Culture Republic of Indonesia, for providing funds for this research through "Hibah Bersaing" program.

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