The Effect of Pollen Substitutes on the Productivity of *Apis cerana* in Indonesia

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The aims of this study were to examine the preferences and productivity of *A. cerana* colonies when fed on a variety of pollen substitutes.

Introduction

Beekeeping with *Apis cerana* Fab. has been traditionally adopted and is a part of natural heritage of some communities in West Java, Indonesia. Although *A. cerana* are poor honey yielders, they require low cost management and technology; they are adapted to cope with pests, diseases, and predators (Verma, 1998; Joshi *et al.*, 2002; Pokhrel *et al.*, 2006; Hishashi, 2011), and require no medication (Verma, 1998; Hishashi, 2011). Small scale beekeeping operations benefit from *A. cerana* (Joshi *et al.*, 2002). Substitutes for pollen are necessary during periods of pollen dearth to provide the required nutrients thereby preventing colonies from absconding as well as maintaining healthy and productive colonies (Standifer *et al.*, 1977; De Jong *et al.*, 2009; Saffari *et al.*, 2010). Providing pollen substitute for honey bee colonies also resulted in greater comb building, greater brood rearing, stronger colonies and greater hive storage (Pokhrel *et al.*, 2006).

Apis cerana colonies are kept traditionally in the apiary in Bandung, West Java using hives without movable frames. *Apis cerana* build combs parallel to each other. Productivity in *A. cerana* colonies can be measured by counting the number and circumference of the combs before and after experiment. The measurement of combs could only be done at night, in order not to interfere with the activities of the honeybees.

Pollen substitute is a protein rich mixture of honeybee diets with no added pollen. The most popular formula for a substitute is soy flour, dry brewers' yeast and dry skimmed milk (Haydak, 1967; Akratanatul, 1990; Somerville, 2000; Prakash *et al.*, 2007; Brodschneider and Crailsheim, 2010) with honey or sugar syrup added to form it into pellets or patties (Akratanatul, 1990). Yeast in pollen substitute provides vitamin B complex (Somerville, 2005) and yeast protein levels are around 50% (Somerville, 2000; Somerville, 2005). Yeast plays an important role in honeybee nutrition. Gilliam (1979) reported that yeast associated with honeybees could provide enzymes, amino acids, vitamins, and minerals to change pollen to bee bread biochemically.

Fig. 1. Map of Indonesia, Java, and the location of apiary in Bandung, West Java.

Sjamsuridzal *et al.* (2010) isolated yeasts associated with *A. cerana* in West Java. A yeast was identified as *Candida hawaiiana,* CR 015, from the stamens of kecubung gunung (*Brugmansia suaveolens*) flowers visited by *A. cerana* worker bees. As yet there is no report on the use of local yeast isolated from honey bees for pollen substitute. All this time brewers' yeast or bakers' yeast was widely used in pollen substitutes.

Materials and Methods

The basic ingredients of the pollen substitute were soy flour (hexane extracted and vacuum heated afterwards), skimmed milk powder and yeast. The yeast *C. hawaiiana* CR015 was deposited in the University of Indonesia Culture Collection (UICC). Yeast cells on Yeast-Extract Malt-Extract Agar (YMA) inoculated in to Yeast-Extract Malt-Extract Broth (YMB) prepared for production of biomass. Lyophilisation technique was used for dry yeast biomass. A brand of bakers' yeast containing *Saccharomyces cerevisiae* was used as commercial yeast for comparative purposes in pollen substitute. To make pollen substitute patties *A. cerana* honey from the apiary, water or sugar syrup 50% (w/v) were added. Four local pollen substitutes i.e. PS-A, PS-B, PS-C, PS-D and an imported pollen substitute (PS-E) were prepared for the honeybee colonies and the ingredients are presented in Table 1. The nutritional values of pollen substitutes powder i.e. protein, carbohydrate, fat, and ash were analyzed. The nutritional information of the imported pollen substitute was taken from the label on its packaging.

The experiment was carried out in an apiary at Ciburial Village, Bandung, West Java, Indonesia (Fig. 1) in January - March 2012 over a period of seven weeks. Eighteen *A. cerana* colonies, were used in this experiment. Each type of pollen substitute was fed to groups of three colonies, and the final three colonies were used as a control. The colonies were not given sugar syrup, and were allowed to forage on flowers around the apiary during the period of observation.

Every week, 40 g patties of pollen substitute were given to each colony. The consumption of pollen substitute was measured by weighing once a week. The patties were placed just below the comb on yellow, thick, plastic cartons measuring 20 x 20 cm and were easily accessible (Fig. 2). The consumption data per week, per colony were used to determine the preference of the bees for a particular substitute. The comb circumferences were measured, using a tape measure, before and after treatment. Another measure of productivity was by the colony's weight showing the amount of honey production. The honey was harvested by cutting the honeycombs, then squeezing and filtering to separate the honey from the wax before weighing.

Results

Table 2 presents data on the nutritional value of all the pollen substitutes. Four local pollen substitutes contained high protein, higher than PS-E, the imported pollen substitute. All four local pollen substitutes in general contained lower carbohydrate and fat content compared to PS-E. Ash values of pollen substitutes were in the range of 2.69%-3.11%.

Table 3 presents data on the percentage consumption of four local and imported pollen substitutes per colony, per week, for seven weeks. The mean percentage consumption of pollen substitute PS-A and PS-D were significantly higher ($p < 0.05$) than PS-C. The total mean percentage consumption of pollen substitutes for PS-A, PS-B, PS-D and PS-E were not significantly different ($P < 0.05$).

Table 1. Composition of variety of local pollen substitutes in patty form fed to honeybee colonies.

Pollen Substitute code	Composition (ratio in weight)
PS-A	Soy flour, skimmed milk, yeast *C. hawaiiana* CR015, honey, sugar syrup (3:1:1:5:0)
PS-B	Soy flour, skimmed milk, baker yeast, honey, sugar syrup (3:1:1:5:0)
PS-C	Soy flour, skimmed milk, yeast, honey, sugar syrup (3:1:0:0:5)
PS-D	Soy flour, skimmed milk, yeast, honey, sugar syrup (3:1:0:5:0)
PS-E	Imported pollen substitute, sugar syrup (3:1)

Table 2. Gross nutritional analyses of pollen substitute powder fed to honeybee colonies.

Pollen substitute code	Protein (%)	Fat (%)	Carbohydrates (%)	Ash (%)
PS-A	45.45	1.78	44.38	3.00
PS-B	44.31	1.93	44.30	3.11
PS-C	51.31	1.79	38.49	2.87
PS-D	51.31	1.79	38.49	2.87
PS-E*	36.40	3.90	51.80	3.10

*The nutritional information was taken from the packaging of imported pollen substitute.

Table 3. The consumption of *A. cerana* colonies to a variety of pollen substitutes for seven weeks.

Pollen substitute code	Consumption (%)/colony/week	
	Mean	SD
PS-A	97.89	6.21
PS-B	92.06	9.65
PS-C	83.32	15.99
PS-D	95.38	11.98
PS-E	90.89[a]	10.51
Treatment effect F=4.385		

Table 4. The increase of comb circumferences, colonies weight and honey yields of *A. cerana* colonies fed on pollen substitutes for seven weeks.

Pollen substitute code	Mean increase of comb circumferences (%)	Mean increaseof colonies weight (%)	Mean honey yield weigh (g)
PS-A	98.04	55.35	1580
PS-B	35.57	14.24	320
PS-C	72.64	28.18	370
PS-D	106.15	46.07	1200
PS-E	116.67	42.56	800
Control	30.26	21.22	330

Table 4 presents data on the percentage comb circumference increase for colonies, the colony's weight-increase percentage, and honey yield of *A. cerana* colonies fed on pollen substitute for seven weeks. All of the total increase of comb circumference; increase in colony weight; and total mean honey yield were not significantly different ($P < 0.05$).

The mean increase in colony weight, comb circumference and honey yields for colonies fed on PS-A, PS-D, and PS-E were higher than those of PS-B and PS-C. The increase of colony weight and honey yield was highest in colonies fed on pollen substitute PS-A.

Discussion

The need to develop a local pollen substitute is important for *A. cerana* in Indonesia where a dearth of pollen sometimes occurs during the long dry season or the rainy season. Artificial feeding during this period is necessary. Pollen substitutes with a low cost, good nutritive value, acceptable for the honey bees and easy to prepare by beekeepers themselves are essential in the local *A. cerana* static beekeeping system.

In this study we processed soy flour to decrease fat and deleted the anti-nutritional factor in soy flour. The nutritional values of defatted soy flour were 58.88% protein; 30.16% carbohydrate; 1.74% fat; and 3.25% ash. To increase the nutritional value of the substitute we added dried skimmed milk and dried yeast. Skimmed milk was reported to fortify the amino acids in pollen substitute. There have been a few reports on the use of soy flour as an ingredient in pollen substitute. (Haydak, 1967; Standifer *et al*., 1978; Somerville, 2000; Manning, 2008; Pokhrel *et al*., 2006; Prakash *et al*., 2007; Sihag and Gupta, 2011). Our reports showed good results and soy flour was recommended for its protein content and low cost.

Yeast has been reported to play an important role in the nutrition and health of honeybees. We also expected that local yeast would process the pollen substitute into bee bread. Pollen substitutes PS-A with added *C. hawaiiana* CR015- a local yeast associated with *A. cerana* were expected to be palatable to the colonies. *Candida hawaiiana* CR015 was isolated from *Br. suaveolens* stamens, and this species was also found on pistil of *Br. suaveolens*, the pistil of *Caliandra calothyrsus*, and in the pollen of *Mimosa pudica* around the apiary and pupae of *A. cerana.* All flowers were frequently visited by *A. cerana* during foraging. Gilliam (1979) stated that the shift in the quality of bee bread is attributed to a micro-organism associated with the honey bee.

Akratanatul (1990) stated that there was no mixture or substitute that can totally replace fresh pollen which has various trace elements important for the honeybee. Our study showed that protein, fat, carbohydrate and ash content of local pollen substitutes were in the range of those of various natural pollens. Our analysis showed that the protein content of all pollen substitutes was high (44.31%-51.31%) and so satisfied the honey bee requirement for protein. Some natural pollens with less than 20% crude protein cannot satisfy a colony's requirements for optimum production (Somerville, 2000). Pollen quality and quantity affects bee longevity and the development of the hypo-pharyngeal gland (Manning, 2008). Many studies have investigated worker bee preference for protein diets by measuring consumption. The reported consumption of protein in the diets varies widely (Brodschneider and Crailsheim, 2010). According to these studies, the protein content of pollen from different species and regions also varies widely from 2.5%-61% (Roulston *et al*., 2000)

All of our local pollen substitutes contained low fat - less than 2%. Imported pollen substitute contained 3.90% fat. Several references suggest that the fat content in pollen substitutes should be 5%-7% or lower (Haydak, 1967; Black, 2006). Manning (2008) however stated that the soy flour protein concentrate with low fat content (0.6% lipid) gave honeybees a greater life-span. In various flower species the lipid content of pollen was in the range of 0.8%-8.9% (Roulston *et al*., 2000).

Ash revealed inorganic elements and important minerals for the honeybee. Ash content in our local substitute powder was in the range of 2.69 %-3.11%. Herbert and Shimanuki (1978) reported the ash of pollen samples in nature was in the range of 2.4%-3.4%.

Carbohydrate content in substitute powder was in the range of 38.49%-48.04%. We did not examine the kind of sugar and other carbohydrate's component of the pollen substitutes.

Sihag and Gupta (2011) reported the use of soy flour with a composition of 42.0% protein, 3.5% fat, 6.5% ash, sugar 37%. They stated that such substitutes and supplements could induce the *A. mellifera* colonies to continuously produce and rear more brood and forage for more pollen and nectar.

Consumption is a good first indication of the acceptance of supplementary diets (Brodschneider and Crailsheim, 2010). In our study, the consumption of local pollen substitute (PS-A, PS-B, PS-D) and imported pollen substitute (PS-E) were not significantly different (Table 3). The scent, taste, and particle size of the local pollen substitute (except PS-C) were attractive and readily acceptable to the honey bees in the colonies of our study.

Pollen substitute PS-C was consumed the least during the seven week period (Table 3) which showed that PS-C was not as attractive to *A. cerana*. PS-C contained the basic ingredients, soy flour, dried skimmed milk and sugar syrup only. Consumption of local pollen substitutes mixed with honey from the apiary were higher than PS-C. Palatability can be inferred from acceptability. Acceptability was measured by the amount of material taken from the feeders. Honeybees are not expected to take non-palatable materials back to the hive unless they are starving (Saffari *et al*., 2010).

An indicator for the productivity of an *A. cerana* colony in a traditional hive was comb circumference. All *A. cerana* colonies fed on pollen substitute during the experiments built new cells in old combs or built new combs. Comb can be either brood comb or honeycomb which stores honey and pollen. A lot of protein is needed by honeybeess for heavy wax production. All of the local pollen substitute in our study containing high protein gave high wax production. The bees also re-used less comb, tearing down old and building new wax regularly (cerana means 'wax maker'). Although the increase total mean of comb circumferences was not significantly different, the mean increase of comb circumferences of colonies fed on pollen substitute PS-A, PS-D, and PS-E were three to four times higher than in the

Fig. 2. The same hive photographed on 30 January 2012 (left) showing small circumference comb and pollen substitute on plastic tray. The photo (right) was taken on 15 March 2012 showing increased circumference comb and stronger colony.

control colonies (Table 4). Haydak (1967) stated that a suitable nutrient can be observed by the building activity of the colonies. Somerville (2000) reported that wax glands use a lot of protein. Pokhrel *et al*. (2006) reported that comb building was highly correlated with brood rearing, honey storage, and pollen storage. Hishashi (2011) stated that *A. cerana* queens lay eggs only in newly built cells.

Although the total mean percentage of increase in the colony's weight was not significantly different (P < 0.05), the highest mean increase in weight was in colonies fed on PS-A was more than two times that in the control colonies (Table 4).

Although the total mean honey yield was not significantly different (P < 0.05), the highest mean honey yield was in colonies fed on PS-A, four times than in the control colonies (Table 4). A colony's weight was total weight of combs, brood, honey, bee bread and adult honeybees. Honey production was affected by a number of worker honeybee which foranging for nectar.

The substitute consumption data (Table 4) showed that the substitute containing baker's yeast (PS-B) were favoured by *A. cerana* colonies. The nutritional value of PS-B was similar to other substitutes. PS-B contained *S. cerevisiae* which could lead to the fermentation of the honey in pollen substitute patty. Pereira *et al.* (2013) reported that fermentation of honey contains ethanol and volatile compounds *esters isoamyl acetate, ethyl octanoate* dan *ethyl hexanoate.* In our study, *A. cerana* workers preferred PS-B, because it smelled of volatile compounds. The honeybee consumed PS-B, but the ethanol it contained was toxic. Abramson *et al*. (2000) reported that some bees that consume ethanol become too inebriated to find their way back to the hive, and will die as a result.

The highest mean increase weight was in colonies fed on PS-A and it was similar to the highest mean increase of honey yield. Although the mean increase of comb circumferences of colonies fed on PS-A was not the highest.

Conclusion

This study revealed that the substitute containing *Candida hawaiiana* CR015 (PS-A) was much preferred by the *A. cerana* colonies and gave the highest productivity. It could be considered as good as imported pollen substitute.

Acknowledgements

The authors gratefully acknowledge the financial supports recieved from Ministry of Education Republic of Indonesia (Hibah Program Kompetitif Nasional / Hibah Penelitian Strategis NasionalTahun 2010-2011), and from University of Indonesia (Hibah Riset Pasca sarjana UI Tahun 2011).

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