



Evaluation of the Viscosity of Different Molasses and Binders in Wafer Supplements Containing Prill Fat and their Effect on Fermentability and Digestibility of Dairy Cows in Vitro

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Abstract | This study aimed to evaluate the effects of different molasses viscosities and various binder options on the physicochemical quality of wafer supplements containing prill fat, as well as their impact on fermentation and digestibility in dairy cows in vitro. Molasses with viscosities of 320 mPas⁻¹ and 6400 mPas⁻¹ were utilized alongside pollard binders, cassava meal, and cassava waste. The experimental design employed a 2x3 factorial pattern CRD with 5 replicates. Analysis of Variance and DMRT were employed to assess treatment differences, focusing on moisture content, water activity, specific gravity, and wafer durability index. The best treatments were further evaluated in in vitro fermentability and digestibility tests using RBD with 6 treatments and 4 blocks, examining pH, NH₃, Total VFA, DMD, and OMD. Polynomial Orthogonal analysis was used to determine optimal levels. Results indicated significant ($p < 0.05$) effects of molasses viscosity and binders on wafer supplement quality. Molasses with 6400 mPas⁻¹. Viscosity and pollard binders exhibited superior WDI (80.14%), MC, and aw values. In vitro tests demonstrated significant ($p < 0.05$) impacts on fermentability and digestibility in dairy cows, showing potential for increased values by 2.77% initially, followed by a decline due to fat interference with rumen microbes. In conclusion, viscous molasses combined with pollard binder provided optimal physical qualities and did not compromise rumen fermentability and digestibility when supplemented in rations.

Keywords | Binder, Biohydrogenation, Dairy cattle, In vitro, Precision, Wafer supplements

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INTRODUCTION

Dairy cows in tropical climates face the challenge of low milk production and poor quality due to heat stress

(Singh *et al.*, 2021) and negative energy balance (Sharma *et al.*, 2016). One of the factors contributing to this problem is the limited energy in dairy feed. Fat supplementation can be one solution to increase energy in feed which can

increase milk fat (Pérez *et al.*, 2022) and milk production (Ghasemi *et al.*, 2021). One of them is fat in the form of prill fat.

Prill fat is a protected fat by-product of crude palm oil processing into granules form through the spray drying method (Riestanti *et al.*, 2021). Prill fat in the form of granules is physically less efficient when given to livestock because a lot will be scattered and wasted, so processing technology is needed, one of which is in the form of wafer supplements (Retnani *et al.*, 2014). Wafer supplements are high-energy supplements used to improve the productivity and quality of dairy cow's milk in the tropics, supplement wafer processing can affect the physical characteristics and palatability of livestock through the gelatinization process (a gel formation process that begins with the swelling of starch granules due to water absorption during heating), as well as facilitating packaging and transportation (Retnani *et al.*, 2019). The wafer manufacturing process undergoes heating, pressing, shaping, and colling so that it can affect the physicochemical characteristics of wafer products (Sandi *et al.*, 2016) in wafer manufacturing requires binders such as feed materials containing starch, which when heated at a certain temperature and pressure will undergo gelatinization so as to strengthen the bonds of raw materials with each other and ultimately have an impact on the structure of the wafer.

Prill fats used in the manufacture of supplement wafers high in palmitic fatty acids (Sanidita *et al.*, 2021). Palmitic acid may improve milk production and quality (Souza *et al.*, 2019). However, the addition of fat in feed according to (NRC, 2001) is only about 6-7%, more than that it can interfere with rumen microbial activity. Some important things should be considered when adding fat to feed to predict the effects. Such as how strongly fat survives rumen bi-hydrogenation, as well as the effect of milk synthesis from rumen are important elements that affect milk production and quality.

Supplementation by skipping fat in an effort to increase energy consumption is known to have a significant effect on increasing milk production. Until now, studies on prill fat supplementation in dairy cows in the tropics have not been carried out much, especially its impact on the fermentability and digestibility of feed in the rumen. Previous research has been conducted by (Riestanti *et al.*, 2020) which showed that prill CPO fat with a content of 2% provides the best results in terms of fermentability and performance of dairy cows, but the processing of prill fat into a wafer supplement form has not been researched. Evaluation of the supplementation of supplement wafers containing prill fat into dairy cow feed needs to be carried out by considering the gelation process in the manufacturing process, in order to achieve optimal dairy cow productivity. Based on the above background, this study aims to evaluate the effect of molas-

ses viscosity and binder selection on the physicochemical quality of wafer supplements with prill fat, as well as its effect on the fermentation and digestibility of dairy cows in vitro. It is expected that molasses with higher viscosity and certain binders will improve the physicochemical quality, fermentability, and digestibility of wafer supplements.

MATERIALS AND METHODS

The study was conducted in two stages at the Faculty of Animal Husbandry, IPB University. The first stage involved physicochemical testing of wafer supplements at the Feed Industry Laboratory. The best-performing wafers from this stage were then used for in vitro digestibility analysis in the Dairy Laboratory from November 2023 to January 2024.

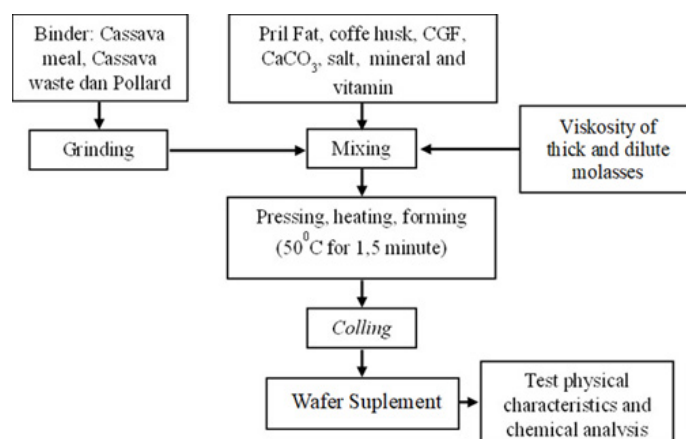


Figure 1: The stage of wafer supplement production.

WAFER SUPPLEMENT MAKING

Additive wafer manufacturing diagram Figure 1. The production process of supplement wafers begins by using raw materials such as cassava meal, cassava waste, and pollard as binders, which are then mixed with prill fat, corn gluten feed, molasses, salt, as well as minerals and vitamins. All these ingredients are mixed using a WLH200 mixer with a capacity of 100 kg for 15 minutes. After mixing evenly, the mixture is pressed and heated to a temperature of 50°C for 1.5 minutes to form a supplement wafer. The wafers that have been formed are then cooled at room temperature. Pollard, cassava flour, and cassava waste were chosen as adhesives because they contain amylose and amylopectin that function in the gelatinization process. The fat content in this formulation follows the (NRC, 2001) guidelines, which is 6-7%, in accordance with the use of 300 g of prill fat in dairy cows based on research by (Riestanti *et al.*, 2021).

The research was carried out in two stages. A two-stage approach is necessary because it makes it possible to separate the physicochemical analysis stage from the wafer supplement formulation and testing stage. The first stage makes it possible to determine the physical and chemical characteristics of the wafer supplement, such as viscosity, sugar

content and nutrient content. The results of this analysis are then used as a reference for fermentability and digestibility testing in the second stage. Thus, it can ensure that the wafer supplements produced are of good quality and in accordance with the needs of dairy cows.

PHYSICOCHEMICAL ANALYSIS OF WAFER SUPPLEMENT

Phase I of the physical characteristics test for wafer supplements began by measuring viscosity using a viscometer, where the sample was inserted into a test glass with a spindle or rotor attached. The device spun the rotor at 30 rpm for one minute. Simultaneously, molasses sugar content was measured using a pocket refractometer, where molasses was applied to the sensor and its refractive index observed to determine sugar content.

Table 1: Wafer supplement formulation (100% DM).

Ingredients	Proportion of Use (%)
Prill fat	60
CGF	15
Cassava Waste/Cassava Meal/Pollard	15
Molasses	5
Coffe Husk	2
Vitamins dan Minerals	1,6
CaCO ₃	0,8
Salt	0,6
Total	100

Description: CGF: Corn glutan meal, CaCO3: Calcium carbonate Carbonate

Table 2: Nutrient content of treated feed (100% DM).

Ingredients	Composition (%)					
	DM	EE	CP	CF	NFE	TDN ¹
Elephant Grass*	21,16	1,6	8,29	29,61	44,82	55,98
Concentrates*	91,28	1,18	7,79	33,83	37,76	51,53
Pellet*	89,85	2,3	18,5	12,7	61,14	78,5
Tofu Waste*	17,29	7,15	26,1	16,8	45,02	76,3

* Results of Biotech IPB University laboratory analysis ;¹TDN (Sutardi 1980).

Physical tests of moisture content and water activity were carried out to see the quality of the supplement wafer from resistance to microorganisms and to impact and storage related to specific gravity and wafer durability index. The moisture content was measured using the PM-650 Grain Moisture Tester; water activity was measured with the Aw-Wert-Messer Lufft hygrometer, model 5803.00, following the method of (Leistner and Rodel, 1976). Specific gravity was measured following the method of (Lopez et al., 1996) by dividing the weight of feed (in grams) by the change in water volume (in milliliters The wafer durability index was measured by the (Pakpahan et al., 2023) method, namely by

rotating the wafer at 55 rpm for 5 minutes, then calculating the percentage by dividing the weight of the wafer after spinning by the initial weight of the wafer. In addition, chemical tests to determine the nutritional content of feed are carried out in accordance with the standard procedures set by (AOAC, 2005).

FEED PREPARATION

Feed consisting of forage grass *Pennisetum purpureum* and concentrate, is used. Grass and concentrate are obtained from Boyolali Regency with an altitude of 400–1500 meters above sea level. (Table 1). *Pannisetum purpureum* grass is widely developed in the area because it has high biomass and nutrient resistance. The grass is harvested at the age of 1 month of cutting. Fresh grass is dried in the sun for 2-3 days. Each grass and dry concentrate is finely ground and then mixed into rations with a ratio similar to that of a small dairy farmer in Boyolali. All analyses were carried out in accordance with (AOAC, 2005) Table 2 and 3.

Table 3: Composition and nutrient content of experimental rations.

Description	Percentage
The composition of feed ingredients	
Elephant Grass	37,69
Pellet	4,57
Concentrates	10,35
Tofu Waste	47,39
Nutrient Content	
CP	11,7
EE	2,03
CF	22,45
ETN	51,07
TDN	63,28

IN VITRO STUDY

Wafer supplement supplement ration best stage 1 in vitro digestibility analysis with (Tilley and Terry, 1963). A 0.5 g ration is mixed with 10 mL of rumen liquid and 40 ml of artificial saliva. Rumen fluid is taken from Friestian-Holstein bulls in fistulas. Rumen fluid is collected in the morning before breastfeeding. The rumen liquid is conditioned to remain anaerobic using flowing CO₂ gas. The mixture of rumen liquid, buffer solution and feed was incubated at 39°C for 4 hours for fermentability analysis and 48 hours for digestibility analysis. pH is measured using Hanna's pH meter. The remaining incubation samples were dripped with HgCl₂ solution and centrifuged at 3500 rpm for 10 minutes to separate the residue from the supernatant.

Supernatants were used for ammonia (NH₃) and total VFA concentration analysis. Residues from 4-hour incubation

Table 4: Viscosity and sugar content of some molasses samples.

Origin of Molasses	Viscosity (mPas ⁻¹)	Price (Rp)	Sugar Content (%)	Description
Supplier A	6400	3.800,00	72	Thick
Supplier B	356	5.000,00	48	Dilute

Table 5: Physical quality wafer supplements containing different viscosity molasses and binders.

Variable	Treatment	B1	B2	B3	Means ± SD
MC (%)	M1	6,82±0,04 ^a	5,76±0,16 ^b	5,34±0,22 ^c	5,97±0,62
	M2	4,22±0,07 ^c	4,76±0,12 ^d	5,72±0,13 ^b	4,90±4,90
	Means ± SD	5,52±1,3	5,26±0,5	5,53±0,19	
aw	M1	0,74±0,01 ^a	0,73±0,00 ^a	0,70±0,01 ^b	0,73±0,002
	M2	0,66±0,00 ^d	0,68±0,02 ^b	0,67±0,00 ^{cd}	0,67±0,005
	Means ± SD	0,70±0,03	0,71±0,02	0,69±0,01	
Specific gravity (g m ⁻³)	M1	1,02±0,02 ^a	0,92±0,02 ^b	0,98±0,02 ^c	0,97±0,04
	M2	1,04±0,02 ^a	1,03±0,02 ^a	1,02±0,01 ^a	1,03±0,01
	Means ± SD	1,03±0,01	0,98±0,06	1,00±0,02	
WDI (%)	M1	39,63±7,88 ^c	83,38±11,66 ^{ab}	35,81±0,37 ^d	75,94±21,11
	M2	80,14±10,58 ^b	99,44±3,01 ^a	48,23±21,29 ^c	42,94±31,75
	Means ± SD	59,89±20,25	91,41±31,75	27,021±21,21	

Description; M1: 320 mPas⁻¹; M2: 6400 mPas⁻¹; B1: Pollard; B2: Cassava meal; B3: Cassava waste; MC: moisture content; Aw: activity water; WDI: wafer durability index; SD: Standard Deviation. Different superscripts on the same line show a noticeable difference (p<0.05).

samples were mixed with pepsin HCl solution and re-incubated for 48 hours. After 48 hours, the samples were filtered with Whatman No 40-filter paper and heated at 105°C for 24 hours to calculate dry matter digestibility (DMD), and the samples were further heated at 600°C for 4 hours to calculate organic matter digestibility (OMD). A 4-hour incubation supernatant was used to analyze NH₃ concentrations using the Conway microdilution method. Total VFA is determined using the steam distillation method. The total analysis of NH₃ and VFA was carried out in accordance with the (General Laboratory Procedure, 1966).

DATA ANALYSIS

The experimental design in phase 1 research uses a Complete Randomized Design (CRD) with a factorial pattern. Factor A is the viscosity of molasses (320 mPas⁻¹ and 6400 mPas⁻¹) and factor B is pollard, cassava waste, and cassava meal, with 5 repetitions. The experimental design of the phase 2 study uses a Block Random Design (RBD) with groups based on different rumen fluid collection times. In vitro tests were carried out at 6 levels with 4 repetitions for each treatment. The treatments used are:

- P0: Control ration + 0% wafer supplement.
- P1: P0 + 0,92% suplemen wafer
- P2: P0 + 1,84% suplemen wafer
- P3: P0 + 2,77% suplemen wafer
- P4: P0 + 3,69% suplemen wafer
- P5: P0 + 4,61% suplemen wafer

Data from phase 1 and 2 research were analyzed using analysis of variance (ANOVA) with SPSS version 25. If there is a real effect, follow-up tests are performed, Duncan Multiple Range Test for stage 1 (Steel and Torie, 1993) and Polynomial Orthogonal for stage 2 (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The viscosity value of molasses can vary depending on its sugar content. The viscosity values and sugar content obtained from suppliers A and B are 6400 mPas⁻¹ and 356 mPas⁻¹. Presented in Table 4.

The results of the analysis showed a significant influence (p<0.05) of the use of molasses with different viscosities on MC, a_w, specific gravity, and WDI. It shows that the viscosity of molasses and the type of binder affect the moisture content, water activity, specific gravity, and durability of the supplement wafer. The interaction of the two also had a significant effect (p<0.05) on all parameters except WDI, meaning that this combination affected the physical quality of the wafer except for the durability value. The average values of the effects of the use of molasses and binders of different viscosities on the physical quality of the supplement wafers are presented in Table 5.

The average MC value in the treatment showed 4.22±0.07 – 6.82±0.04 while the a_w value was 0.663±0.00 – 0.739±0.01.

MC is also closely related to a_w , the relationship will form the Moist Sorption Isotherm (ISL) curve. The results showed that there was a difference ($p<0.05$) in each treatment between the viscosity of molasses and different binders, the average value ranged from 0.663-0.739. The a_w rating is classified as safe for storage.

The DMRT test results showed a significant difference ($p<0.05$) in the specific gravity of the supplement wafer with different molasses and viscosity binders. Treatment of M1B1 (1.02 g ml^{-3}) was not significantly different from M1B2 (0.92 g ml^{-3}), M1B3 (0.98 g ml^{-3}), and M2B1 (1.04 g ml^{-3}), but was significantly different from M2B2 (1.03 g ml^{-3}) and M2B3 (1.02 g ml^{-3}). Specific gravity was followed by the WDI value, where the smallest WDI was found in M1B3 (5.81%) and the highest in M2B2 (99.44%). The best WDI score in M2B1 (80.14%). In conclusion, wafers with viscous molasses and pollard binders produce the best physical qualities, with the smallest MC and a_w . The results of the measurement of the nutrient content in supplement wafers, using molasses of different viscosities and various binders, are presented in Table 6.

Table 6: Nutrient content of wafer supplements containing different viscosity molasses and binders

Content	Variable					
	M1B1	M1B2	M1B3	M2B1	M2B2	M2B3
Dry Matter (%)	93,79	95,36	95,05	94,83	94,30	94,61
Ash (%)	3,23	2,96	2,72	3,67	4,38	4,62
Crude Protein (%)	9,70	9,73	9,51	8,78	7,83	8,05
Crude Fiber (%)	13,01	14,58	16,03	13,81	13,04	11,59
Extract Eter (%)	25,05	29,16	25,87	24,83	20,50	23,79

Description: M1= 320 mPas^{-1} , M2 = 6400 mPas^{-1} , B1 = Pollard, B2 = Cassava meal, B3 = Cassava waste

Wafer supplementation supplements have no noticeable effect ($p>0.05$) on pH values. However, wafer supplementation supplements have a very pronounced effect ($p<0.01$) on NH_3 , Total VFA, DMD and OMD. In the follow-up test of orthogonal polynomial wafer supplementation, it showed the similarity of regression with the quintessential trend. Average pH value. NH_3 , total VFA, DMD and OMD can be seen in Table 7.

The regression equation was used to see the effect of the addition of supplement wafers on the fermentability of the rumen (Figure 2). Prediction of NH_3 concentration: $y = 0.0914x^5 - 0.9949x^4 + 3.6363x^3 - 5.2568x^2 + 3.2405x + 8.3486$, with an optimal value of 10.13 mM at 2.18% supplementation. Total VFA prediction: $y = 1.3794x^5 - 15.512x^4 + 60.583x^3 - 105.12x^2 + 97.554x + 74.499$, with an optimal value of 133.88 mM at 2.25% supplementation. The results of the polynomial test showed that the optimal

digestibility level of the ration with wafer supplements was 2.77% (Figure 3). The dry matter digestibility prediction equation (DMD) is $y = 0.3782x^5 - 4.3645x^4 + 17.186x^3 - 27.17x^2 + 16.983x + 55.596$, with an optimal value of 62.28% at 2.30% supplementation. The organic matter digestibility prediction equation (OMD) is $y = 0.2875x^5 - 3.2852x^4 + 12.659x^3 - 19.381x^2 + 12.468x + 55.41$, with an optimal value of 62.09% at 2.28% supplementation. Supplementation of 2.28% resulted in an OMD of 62.09%, close to the result at the level of 2.77%.

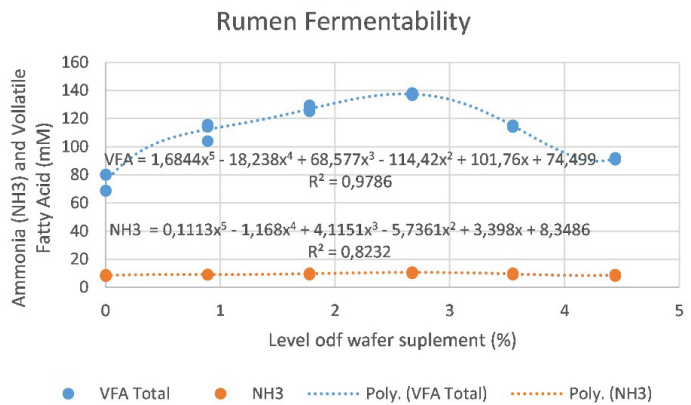


Figure 2: Effect of water supplementmation on rumen femententability.

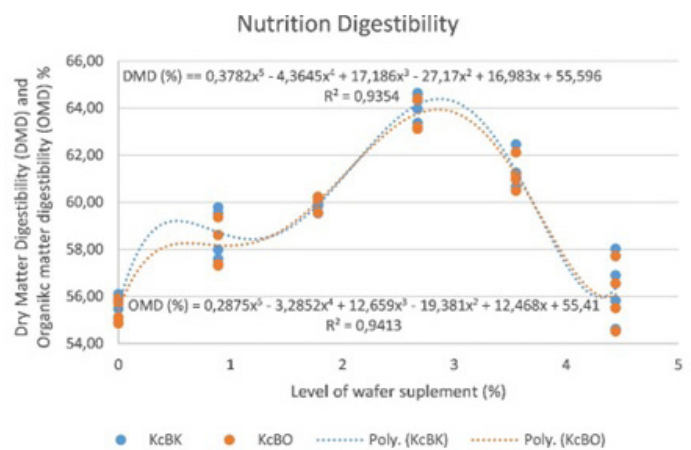


Figure 3: Effets of water supplement supelemntation on nutrition degstibility.

In terms of NH_3 , VFA, DMD and OMD trends, the same pattern where the supplementation of supplement wafers up to the level of 2.77% showed an increase and decreased at 3.69% and 4.61% supplementation. The decrease showed that supplementation above 2.77% began to interfere with the fermentability and digestibility process by microbes.

PHYSICAL CHARACTERISTIC OF WAFER SUPPLEMENT
VISCOSITY MOLASSES: The results of the viscosity test showed that supplier A's molasses was higher than B's, which was 6400 and 356 mPas^{-1} . The sugar content expressed in brix at suppliers A and B is 72% and 48%. The effectiveness of using molasses as an adhesive is influenced

Table 7: Average values of fermentability and digestibility of rumen effect of wafer supplementation supplement containing prill fat.

Perlakuan	Variable				
	pH	NH3 (mM)	VFA Total (mM)	KcBK (%)	KcBO (%)
P0	6,93±0,05 ^c	8,35±0,43	74,50±6,51 ^c	55,60±0,45 ^d	55,41±0,44 ^d
P1	6,92±0,03 ^{cd}	9,06±0,05	112,28±5,47 ^c	58,73±0,95 ^c	58,17±0,87 ^c
P2	6,93±0,03 ^b	9,70±0,040	126,87±1,96 ^b	59,89±0,25 ^c	60,02±0,27 ^b
P3	6,95±0,04 ^a	10,52±0,44	137,48±0,79 ^a	64,14±0,51 ^a	63,76±0,60 ^a
P4	6,90±0,06 ^{bc}	9,50±0,49	114,88±0,58 ^c	61,34±0,68 ^b	61,18±0,59 ^b
P5	6,91±0,07 ^{de}	8,75±0,40	91,74±0,67 ^d	56,35±1,26 ^d	56,08±1,19 ^d

Description: Different superscripts in the same column show a real difference (P<0.05).

by viscosity and sugar content (Rahman *et al.*, 2020). Meanwhile, the viscosity content of a molasses will be affected by the content of dry matter and polysaccharides as well as the environmental temperature. The addition of molasses with different viscosity and sugar content will affect the durability of the wafer (Kurniawan, 2019). The addition of molasses is effectively used in the process of making pellet feed (Wang *et al.*, 2019). So it is important to measure the degree of viscosity before carrying out the process of making supplement wafers.

WATER CONTENT: The results showed that molasses with a viscosity of 320 mPas-1 experienced a decrease in moisture content when interacting with pollard, cassava meal, and cassava waste binder by 6.82%, 5.76%, and 5.34%, respectively. In contrast, molasses with a viscosity of 6400 mPas⁻¹ increased its moisture content by 4.22%, 4.76%, and 5.72%. This difference is due to the viscosity of molasses and the moisture content of the binder used. Hydrolysis, the breakdown of water molecules, is an important reaction in the depolymerization process. The increase in moisture content at 6400 mPas⁻¹ viscosity molasses is likely due to the innate moisture content of each binder: pollard 10.34% (Despal *et al.*, 2008), cassava meal 13.48% (Hartanti *et al.*, 2017), and cassava waste 14.88% (Isnandar, 2011). Dilute molasses has high hygroscopic properties so that it is easier to be absorbed into starch grains, while viscous molasses with a denser structure inhibits its hygroscopicity (Trisyulianti, 2003). Binder starch concentrations: pollard 42.95%, cassava meal 87.45% (Hartanti *et al.*, 2017), and cassava waste 72.43% (Abdullah *et al.*, 2019) also affect water absorption, with high starch concentrations having a tighter granular structure and difficulty absorbing water (Wariyah *et al.*, 2007), causing differences in water content in supplement wafers.

ACTIVITY WATER: The value of water activity in supplement wafers is very important to know. The results showed that there was a difference (p<0.05) between the viscosity of molasses and different binders, the average value ranged from 0.663-0.739. The a_w rating is classified as safe for storage. The minimum a_w value for bacteria is usually

around 0.9 (Rousseau and Donèche, 2001), while yeast has a minimum a_w requirement between 0.8-0.9 and fungi have a minimum a_w requirement of 0.6-0.7 (Simbolon *et al.*, 2019). The data showed that molasses with a sugar content of 72% produced a greater a_w value. Sugar is the main component in molasses, a_w will definitely increase after molasses is added (Wang *et al.*, 2019). In addition, the data shows that the higher the water activity of the supplement wafer, the higher the pressure and the rate of water migration in the supplement wafer is also high. This suggests that the addition of molasses improves the hygroscopic behavior of a product.

SPECIFIC GRAVITY: The difference in average specific gravity values is caused by the moisture content of the binder (Syamsu, 2007), as well as the same environment (Bira *et al.*, 2019). The wafer formulation uses uniform material particles in each treatment, this is suspected to be the reason why some treatments of M1B1, M1B2, M1B3, and M2B1 do not show any difference (p<0.05), as well as the same environment in the manufacture of supplement wafers (Bira *et al.*, 2019). According to (Milawarni *et al.*, 2020), the use of coffee waste as a component of wafers, affects the specific gravity of the wafer, the use of the same coffee husk waste in each treatment will cause an unreal difference. In terms of average values, it can be seen that the use of molasses with dilute viscosity tends to have a lower specific gravity value (0.92-1.02 g ml⁻³) than thick molasses (1.02-1.04 g ml⁻³), this is because viscous molasses has a more effective binding force between material parts and also viscous molasses has a higher chemical content (Br Pakpahan *et al.*, 2023).

WAFER DURABILITY INDEX: The wafer durability index (WDI) is an indicator of the success of wafer production (Kurniawan *et al.*, 2019). The results showed that the difference in treatment was caused by the gelatinization process of the binders used. Gelatinization occurs when starch is heated with water, causing starch grains to expand and release amylose (Chakraborty *et al.*, 2022). Pollard contains 25% amylose (Arnyke *et al.*, 2001), cassava waste 16%, and cassava meal 49,15% (Anugrahati and Carista,

2020). The high amylose content increases WDI due to a more effective gelatinization process (Faridah, 2011).

EFFECTS OF WAFER SUPPLEMENT ON FERMENTABILITY AND DEGRATIBILITY PARAMETERS

The in vitro fermentation process can be optimal if the rumen liquid is in a pH condition that is suitable for the rumen microbial environment. The pH value in all treatments was in the range of 6.90-6.95 which indicates that this value is under normal conditions to support fermentation activity in the rumen (McDonald *et al.*, 2010).

The average ammonia concentration of rumen liquid with wafer supplementation supplements in this study ranged from 8.35-10.52 mM. (McDonald *et al.*, 2010) stated that the concentration of NH_3 ammonia in the rumen is about 5-17.65 mM. In this study, wafer supplementation supplements had an effect on NH_3 concentration. The supply of fat in the rumen can reduce the value of NH_3 , with fat as an energy source for microbes, the rumen is more likely to use fat as an energy source rather than breaking down proteins, besides that fat can act as microbial inhibition thereby reducing the ability of microbes to break down proteins into NH_3 .

The role of VFA as a source of livestock energy and a source of carbon for microbial protein synthesis. The average value of total VFA concentration with wafer supplementation supplements ranged from 107.78-127.14 mM. This value is still within the range of normal VFA concentrations found in ruminant livestock. According to (McDonald *et al.*, 2010), the concentration of VFA that supports microbial growth ranges from 80-160 mM. The supplement wafers used are able to survive the biohydrogenation process in the rumen, and are absorbed in the small intestine. According to (Riestanti *et al.*, 2021), one of the drawbacks of in vitro techniques is that they do not measure post-rumen digestibility so that they cannot describe the fat absorbed by the intestines. However, the decrease in the total VFA value can be attributed to not all protected fats being able to escape the rumen. Fat left inside the rumen can interfere with fiber digestibility, which in turn reduces the production of VFA from fiber fermentation.

The digestibility of ration nutrients supplemented with wafer supplements in this study was above 60% normal digestibility (Lestari and Abdullah, 2015) for P2, P3, and P4 treatments. Although there is a depression in the digestibility of dry and organic materials as wafer levels increase, this is due to the content of prill fat CPO (Riestanti *et al.*, 2021). The percentage of digestibility of the ration was in the range of 55.41-63.76% for OMD and 55.60-64.14% for DMD, close to the values reported by Despal *et al.* (2022) Canola produced 56.40% DMD and 54.39% OMD, soybean oil produced 59.64% DMD and 57.3% OMD, while palm oil produced 58.59% DMD and

58.95% OMD and (Riestanti *et al.*, 2021). The difference in digestibility can be caused by the difference in the rumen inoculum used. Fat supplementation generally reduces the digestibility of dry and organic materials, but processing fat in wafer form can improve digestibility through the gelatinization process (Han *et al.*, 2022).

CONCLUSIONS AND RECOMMENDATIONS

The best quality supplement wafers are shown in the use of viscosities of viscous molasses that interact with pollard binders, and the supplementation of supplement wafers in feed does not interfere with the fermentability and digestibility values in the rumen.

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ETHICAL APPROVAL AND INFORMED CONSENT

The study was conducted according to the principles of the animal welfare. The protocol was approved by the animal ethics committee, School of Veterinary Medicine and Biomedical Science, IPB University. The ethical approval number is: 047/KEH/SKE/XI/2021.

NOVELTY STATEMENT

This research pioneered the development of wafer supplements with optimized physical properties, which was achieved through comprehensive characterization of physical test properties containing prill fat so as to produce wafer supplements with better physical quality. This research contributes to the advancement of food technology by presenting a new paradigm for the design and development of functional food products with customized physical properties.

AUTHOR'S CONTRIBUTION

Muhammad Ambar Islahuddin acted as the main author of the manuscript and conducted research related to physical testing and analysis of fermentability and digestibility in vitro. Yuli Retnani contributed to editing the original manuscript of the journal. Despal contributes to the analysis of physical quality and fermentability and digestibility data in vitro.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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