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Preference, Feeding Behavior, and *in Vitro* Fermentation Characteristics of Pelleted Feeds Containing Alkaline Aqueous Lignin By-Product from Paper Processing in Holstein Heifers

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Abstract: The objectives of this study were to evaluate the effect of using multiple dietary inclusion rates of alkaline aqueous lignin by-product (AALB), in replacing a commercial binder, on the preference and feeding behavior of dairy heifers, and *in vitro* digestibility. In the experiment I, five pelleted diets including negative control (no-binder), commercial lignosulfonate binder, and 1.6%, 3.2%, and 4.8% of AALB were tested. In the experiment II, five pelleted diets including negative control, positive control (2% molasses), and 1%, 2.4%, and 3.8% of AALB (all combined with 2% of molasses) were tested. Feeding behavior and preference were determined for 60 min per animal per evaluation. *In vitro* digestibility was determined for each pelleted diet for 48 h over five incubation runs. Results from Experiment I showed animals receiving 1.6% of AALB diet showed the greatest preference (dry matter (DM), intake) and a greater number of approaches with eating than other diets. The preference was lower when animals received control or 4.8% AALB diets relative to other diets. Results from Experiment II revealed animals receiving 3.8% AALB combined with molasses showed the greatest DM intake preference over 60 min, and DM intake per approach. Diets that included 2.4% and 3.8% of AALB combined with molasses presented the greatest *in vitro* DM digestibility. Overall, heifers showed a greater intake when receiving pelleted diets with 1.6% AALB. Furthermore, preference, intake over 60 min, and *in vitro* DM digestibility, were greater in the pelleted diets that contained higher rates of AALB when combined with molasses.

Key words: Dairy heifer, feeding behavior, feed binder, lignin, pellet.

1. Introduction

Pellets have been used in non-ruminant diets for more than 60 years [1-3]. Studies have shown that pelleting modifies chemical and physical composition because of pressure, heat, and moisture employed [4]. These modifications are associated with improved digestibility in non-ruminants [5]. However, the use of pelleted total mixed ration (TMR) in ruminant diets is still not highly common.

Ruminants' diets are commonly composed of grains and forages to meet the nutrient requirements. Most farms feed heifers with diets such as TMR or forage with top-dressing of grains [6]. TMR can result in a better balance of nutrient intake [7], as it may be achieved by pelleted diets as well. In addition, providing pelleted diets as a part of the total animal in take might be a key to the nutritional management of heifers because it would combine two benefits: avoiding ingredient loss (e.g., by dust) and improving

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digestibility by modification in the nutrient availability. Moreover, pelleting reduces the space required for storage [8] and increases the surface area because of the grinding process, increasing rumen microbial degradation [9].

There are several factors that improve the quality of the pellet. One of these factors is the use of binders during the process [10]. Using binders during the alfalfa pelleting process improved the durability of alfalfa pellets [11]. Among the most common components to improve the quality of pelleted diets is the use of calcium lignosulfonate, a by-product from the paper industry that is rich in salts of lignosulfonic acid [8, 12-14]. A new potential lignin by-product from the paper industry is an alkaline aqueous solution that consists of modified lignin, carbohydrates, and micro-nutrients.

Positive results have been observed when pelleted diets are used in ruminant nutrition. Grinding and pelleting ingredients with poor nutrient composition showed greater metabolizable energy for sheep [15]. In addition, fattening lamb showed an increased average daily gain, digestibility, and acetate: propionate ratio in rumen fluid when fed a pelleted diet compared to a non-pelleted diet [8]. There is however no evidence in the literature of the effect of pelleted diets containing this new lignin by-product (AALB) from paper processing industry on the feeding behavior of heifers and *in vitro* digestibility. We hypothesized that adding AALB would improve the variables related to feeding behavior and intake while supporting greater ruminal dry matter (DM) and organic matter (OM) digestibility. Therefore, our objectives were firstly to evaluate the effect of using multiple dietary inclusion rates of AALB, as an alternative source replacing a commercial binder, on the preference and feeding behavior of heifer, and secondly to test its *in vitro* DM and OM digestibility.

2. Material and Methods

2.1 Experiment I

2.1.1 Experimental Design and Animals

All animal procedures were approved by the Animal Care and Use Committee at the University of Idaho. Ten Holstein dairy heifers (452 ± 21.8 kg of body weight, BW), 18 months of age, and 120 d of pregnancy were distributed in a change-over design with five diets. The animals were previously adapted over one week with *ad libitum* intake to a basal diet (grass hay, alfalfa silage, alfalfa hay, triticale silage, rolled barley grain, ground corn grain, corn dry distiller grain, mineral and vitamin supplement, and salt), and maintained in individual stalls (3.4 × 3.7 m) during the feeding behavior evaluation or preference test. The experiment consisted of four periods of 5 d, when each animal was subjected to different treatments on each day of the period. Therefore, each animal was submitted 4 times to each treatment, with a total of 200 observations. The animals had one day off for evaluation between the periods and were kept in a free stall when feeding behavior or preference tests were not performed.

2.1.2 Experimental Diets

The experimental diets were formulated to meet the requirements of growing Holstein dairy heifers of 450 kg of BW (Table 1) and estimated target average daily gain of approximately 0.6 kg/d, according to National Research Council (NRC) [18]. The experimental diets consisted of negative control (NCTL; no addition of any commercial binder or AALB), a positive control (PCTL; with the inclusion of Ameri-Bond 2X at 2.1%, DM basis; Borregaard Ligno Tech, Rothschild, WI); 16AALB (inclusion of AALB at 1.6%, DM basis), 32AALB (inclusion of 3.2% of AALB, DM basis), 48AALB (inclusion of 4.8% of AALB, DM basis). The ingredients were ground, homogenized, and posteriorly pelleted in ¼ inch diameter using a pellet mill (California Pellet Mill, 100-hp, Crawfordsville, IN). The inclusion rates of AALB were based on the recommendation for other commercial binders. The animals were fed twice daily with a basal diet (grass hay and alfalfa hay) from which they were removed 30 min prior to the feeding behavior or preference test.

2.1.3 Feeding Behavior

The acceptance, intake, and feeding behavior of pelleted diets were determined in rounds of 60 min per day, and individual videos were recorded each time. Approximately 10 kg of pelleted diets were weighed before offering to the animals and weighed again after each round to determine the intake. The feeding behavior was evaluated over 20 d, with a total of 200 observations for 60 min each, which consisted of 20 observations per animal.

The video recordings were evaluated to determine the behavior measurement including the absolute number of approaches to the feed bucket without eating; the absolute number of approaches to the feed bucket with eating; time that the animals spent eating (min); time spent drinking water (min); time spent ruminating (min); and time spent on non-nutritive behavior/inactivity (non-eating/non-ruminating/non-drinking, min). The intake was determined over 60 min and by eating approach. The intake over 60 min corresponded to the difference between the amount of feed offered and the remaining feed after time of evaluation, whereas intake by approach was calculated by dividing the total intake over 60 min per number of approaches that the animal ate.

Feed samples were collected from 5 different spots of the bulk bags to ensure representative sampling. Samples were stored at 4 ℃ until future processing for the 48 h *in vitro* digestibility.

2.1.4 Preference Index

During the preference test, two different diets were offered to each animal, where one was the NCTL, and the other was one of the test diets. One kg of each diet (test diets vs. NCTL) was provided in 5-gallon buckets and weighed before and after each round. Preference was evaluated over 60 min and was calculated according to the following equation:

$$\text{Preference (\%)} = \text{Test diet}_{,as\ fed}/(\text{Test diet}_{,as\ fed}+\text{NCTL}_{,as\ fed})\times 100$$

The animals with a higher preference for the test diet show a preference greater than 50%, whereas animals with a lower preference for the test diet show a preference less than 50%.

2.1.5 *In Vitro* Digestibility

Feed samples were ground to pass through a 1 mm sieve (Retsch Cutting Mill SM 200, Retsch, Haan, Germany) to determine DM, chemical composition, and *in vitro* digestibility. Ruminal content was collected from 3 non-lactating dairy cows through the ruminal cannula. The cows were fed *ad libitum* with a diet formulated to meet the maintenance requirements according to the NRC (2001) [16]. The ruminal content was collected manually prior to the morning feeding, squeezed through 4 layers of cheesecloth, and stored in pre-warmed thermal bottles. The squeezed fluid from both animals was combined and used during the incubation.

The determination of *in vitro* DM and OM digestibility was performed over 48 h, according to Goering and Van Soest [17], using serum bottles. A mixture solution was prepared for the incubation by adding 0.125 mL of micro mineral solution (13.2 g/100 mL of $CaCl_2 \cdot 2H_2O$, 10 g/100 mL of $MnCl_2 \cdot 4H_2O$, 1 g/100 mL of $CoCl_2 \cdot 6H_2O$, 8 g/100 mL of $FeCl_2 \cdot 6H_2O$), 250 mL of buffer solution (4 g/L of NH_4HCO_3, 35 g/L of $NaHCO_3$), 250 mL of macro-mineral solution (5.7 g/L of Na_2HPO_4, 6 g/L of KH_2PO_4, 0.6 g/L of $MgSO_4 \cdot 7H_2O$), 1.25 mL of 0.1% resazurin, 0.313 g of L-cysteine, and 0.313 g of sodium sulfide were mixed with a tryptone solution (2.5 g/500 mL), and exposed to CO_2 until the solution becomes grey. Approximately 0.7 g of diet at 1 mm was weighed into fiber filter bags (F57, 25-micron porosity; Ankom, Technology, Macedon, NY). Three bags were incubated for each diet per run. Each bag was placed into a 125 mL serum bottle with 48 mL pre-warmed mixture solution and added with 12 mL of ruminal fluid. The bottles were saturated with CO_2 to provide an anaerobic condition, immediately sealed, and incubated at 39 ℃ for 48 h in a rotary shaker. Three separate *in vitro* repeats were carried out, and in each round, one negative control was incubated that consisted of buffer, rumen fluid, and an empty filter bag. The bags were removed from the ruminal fluid when the incubation reached 48 h, drained, washed with water until the water remained clean, and dried at 55 ℃ for 24 h. The concentrations of DM and ash were analyzed in the residue from the incubation to determine DM and OM digestibility.

DM content was determined in an oven according to method No. 930.15 [18]. Ash was determined in a furnace by combustion at 600 ℃ for 6 h, according to method No. 924.05 [18]. The OM was obtained by the difference between DM and ash content.

The DM and OM digestibility were calculated using the following equations:

$$\text{DM digestibility (\%)} = \frac{\text{mass (g) of DM before incubation} - \text{mass (g) of DM after incubation}}{\text{mass (g)of DM before incubation}} \times 100$$

$$\text{OM digestibility (\%)} = \frac{\text{mass (g) of OM before incubation} - \text{mass (g) of OM after incubation}}{\text{mass (g) of OM before incubation}} \times 100$$

2.2 Experiment II

2.2.1 Experimental Design and Animals

All animal procedures were approved by the Animal Care and Use Committee of the University of Idaho. Eight Holstein dairy heifers (399 ± 9.0 kg of body weight, BW), 16 months of age, and 60 d pregnant were distributed in a change-over design with 5 diets. The animals were previously adapted over one week under *ad libitum* intake to a basal diet (grass hay, alfalfa silage, alfalfa hay, triticale silage, rolled barley grain, ground corn grain, corn dry distiller grain, mineral and vitamin supplement, and salt), and maintained in individual stalls (3.4 × 3.7 m) during the feeding behavior evaluation or preference test. The experiment consisted of 3 periods of 5 d each, where each animal was subjected to a different treatment on each day of the period. Therefore, each animal was submitted 3 times to each treatment, with a total of 120 observations. The animals had 1 d off between the periods, every 5 d. The animals were kept in a free stall when feeding behavior or preference tests were not performed.

2.2.2 Experimental Diets

The experimental diets were formulated to meet the requirements of growing Holstein dairy heifers of 400 kg of BW (Table 4), and estimated target average daily gain of 0.6 kg/d, according to NRC [16]. Diets were pelleted as previously described for Experiment I. The experimental diets consisted of negative control (NCTL; no inclusion of molasses or AALB), a positive control (PCTL; inclusion of liquid molasses at 2.0%, DM basis), 10AALB+M (inclusion of AALB at 1%, plus liquid molasses at 2%, DM basis), 24AALB+M (inclusion of 2.4% of AALB, plus liquid molasses at 2%, DM basis), 38AALB+M (inclusion of 3.8% of AALB, plus liquid molasses at 2%, DM basis). Liquid molasses was added to improve feed palatability. The animals were fed twice daily with a basal diet (grass hay and alfalfa hay) from which they were removed 30 min prior to the feeding behavior test.

2.2.3 Feeding Behavior, Preference Test, Sampling Processing, *in Vitro* Assay, and Chemical Composition

Feeding behavior and preference test studies were carried out as described in the respective sections of Experiment I, except that the feeding behavior consisted of 3 periods of 5 d of observation, with a total of 120 observations of 60 min each. The procedures for collecting and processing samples, *in vitro* digestibility, and determination of chemical composition were conducted as described for Experiment I.

2.2.4 Statistical Analyses

All statistical analyses for both experiments were carried out using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data from feeding behavior and preference tests were analyzed in a change-over design, and diets were used as a fixed effect in the model while animals and rounds were used as random effects. Data from *in vitro* digestibility were analyzed in a completely randomized design, and diets were used as a fixed effect in the model and incubation round as a random effect. The means were compared using the Tukey post-hoc test. Differences were declared significant at $p \leq 0.05$ and tendencies when $0.05 < p \leq 0.10$.

3. Results and Discussion

3.1 Experiment I

Animals showed a preference for diets with 1.6% and 3.2% of AALB and PCTL compared to NCTL and 4.8% of AALB ($p < 0.01$, Table 2). The lower preference index when the highest inclusion rate of AALB (4.8% of AALB) was used might be attributed to the reduction of palatability. It is known that physical and chemical characteristics are the main factors affecting diet palatability [19]. Although, we did not evaluate pellets' physical characteristics, we assume that a high inclusion rate (i.e., 4.8% of AALB) may have affected the physical and/or chemical characteristics of pellets. Ran *et al*. [20] did not observe any effect of different temperatures in the pelleting process, but they described that using higher temperatures increased the hardness of the pellet, and

Table 1 Ingredient composition of the diets (Experiment I).

	Experimental diets[a]				
Ingredients (% of DM)	NCTL	PCTL	16AALB	32AALB	48AALB
Alfalfa	17	17	17	17	17
Grass hay mix	65.4	64.3	64.8	63.2	61.6
DDGS[b]	1	1	1	1	1
Barley grain, rolled	9	9	9	9	9
Corn, ground	5.4	4.4	4.4	4.4	4.4
Canola meal	1	1	1	1	1
Salt	0.1	0.1	0.1	0.1	0.1
ADE premix	0.1	0.1	0.1	0.1	0.1
Mineral premix	1	1	1	1	1
Lignin binder	0	0	1.6	3.2	4.8
Ameri-Bond 2X	0	2.1	0	0	0

[a] NCTL: negative control; PCTL: positive control with 2.1% of Ameri-Bond 2X; 16AALB: 1.6% of alkaline aqueous lignin by-product (AALB) from paper processing; 32AALB: 3.2% of AALB; 48AALB: 4.8% of AALB.

[b] Dried distillers' grains with solubles.

ADE: vitamin A (92,500 IU/kg), vitamin D (28,500 IU/kg) and vitamin E (990 IU/kg).

Table 2 Feeding behavior of Holstein heifers fed pelleted diets with binders inclusion(Experiment I).

Items	Experimental diets*					SEM	*p*-value
	NCTL	PCTL	16AALB	32AALB	48AALB		
Preference, %	50.0[b]	66.9[a]	69.0[a]	66.1[a]	54.3[b]	2.49	<0.01
DM intake/approach, g	102.22	112.07	122.09	125.09	113.16	7.44	0.21
Approach and no eating, number	6.27	6.12	5.37	5.55	5.47	0.45	0.50
Approach and eating, number	33.40[ab]	31.13[bc]	36.35[a]	26.93[c]	30.65[bc]	1.71	<0.01
Time eating, min	28.33[ab]	25.07[bc]	32.27[a]	23.90[c]	25.69[bc]	1.51	<0.01
Time ruminating, min	1.40[ab]	1.80[a]	0.24[b]	2.55[a]	1.86[a]	0.55	0.05
Time NNB/inactivity, min	28.26[ab]	31.86[a]	26.32[b]	31.32[a]	30.77[a]	1.37	0.02
Time drinking water, min	2.59	1.64	1.77	1.94	2.07	0.34	0.33

* NCTL: negative control; PCTL: positive control with 2.1% of Ameri-Bond 2X; 16AALB: 1.6% of AALB from paper processing; 32AALB: 3.2% of AALB; 48AALB: 4.8% of AALB.

SEM: standard error of the means

min: minutes.

NNB/inactivity: non-nutritive behavior or inactivity.

[a, b, c] Different superscripts in the same row are statistically different ($p < 0.05$).

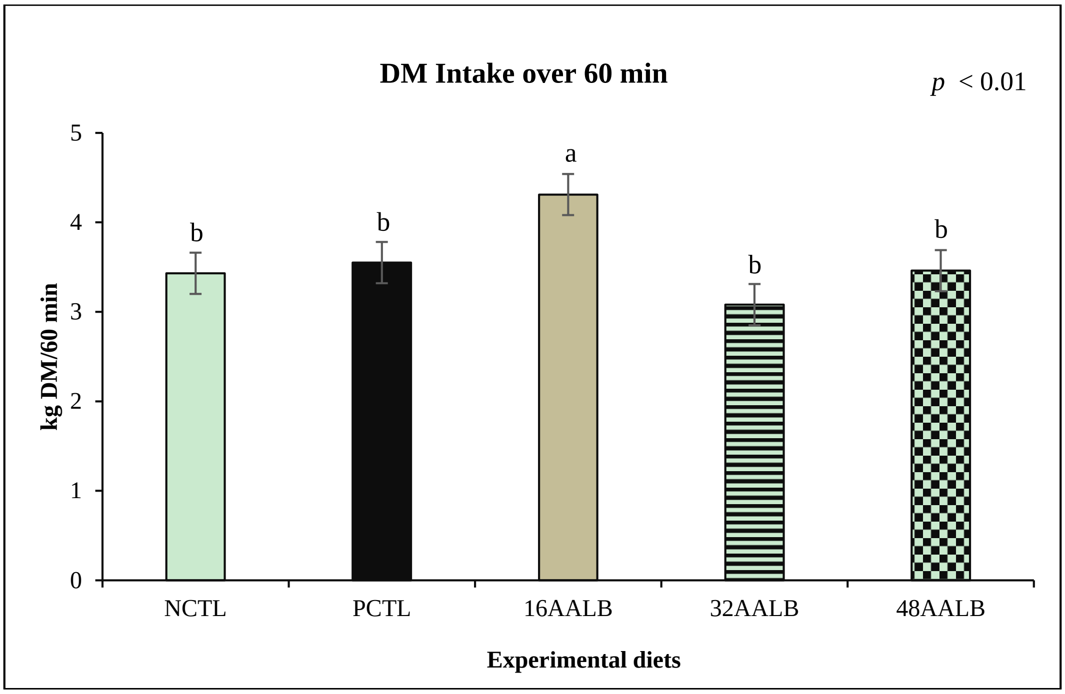

Fig. 1 Dry matter (DM) intake over 60 min for dairy heifer offered pelleted diets with binders inclusion (Experiment I).
NCTL: negative control; PCTL: positive control with 2.1% of Ameri-Bond 2X; 16AALB: 1.6% of AALB from paper processing; 32AALB: 3.2% of AALB; 48AALB: 4.8% of AALB.
[a, b] Different superscripts show statistically different means ($p < 0.05$). Error bars represent standard error of the means.

it could lead to more difficult chewing, which might explain our observation. Therefore, further investigations and pellet physical tests are needed to better understand how the high inclusion rate of AALB affects the preference by the pellet characteristics.

Animals fed 16AALB showed the greatest DM intake over 60 min than all the other diets ($p < 0.01$, Fig. 1). However, the DM intake over 60 min for other diets did not differ from each other. The modification in intake over 60 min possibly indicates that 16AALB improved pellet characteristics, but this observation is limited to low inclusion rates only because high inclusion, such as 3.2% and 4.8% of AALB did not perform the same way. The lack of significant DM intake over 60 min with high levels of AALB might be related to the palatability factor. In a previous study, Dos Santos *et al.* [21] did not observe any effect in dairy cow intake with the inclusion of 0.5% of lignosulfonates, one of the most common pellet binders, in the concentrate pelleting process. Wood *et al.* [24] also did not observe a difference in intake when heifers were fed with pellets that contained 0.5% of lignosulfonate as a part of the total diet.

The number of times that the animals approached the feed and ate, as well as the time spent eating, was greater for the diet 16AALB compared with that for PCTL, 32AALB, and 48AALB; however, it did not differ from NCTL ($p < 0.01$). The approach and eating behavior follow a similar pattern for the other variable previously described, supporting the notion that a lower inclusion rate of AALB might favor feeding behaviors.

On the other hand, the longest time spent ruminating over 60 min, as well as time spent in non-nutritive behavior or inactivity was observed when the animals received the diets PCTL, 32AALB, and 48AALB (p = 0.05; 0.02, respectively). As the variables of feeding behavior were expressed over 60 min, animals fed 16AALB spent less time ruminating and on non-nutritive behavior/inactivity than that for the other diets with the AALB.

Table 3 *In vitro* digestibility (48 h) of pelleted diets with binders inclusion (Experiment I).

Digestibility (%)	Experimental diets*					SEM	*p*-value
	NCTL	PCTL	16AALB	32AALB	48AALB		
DM	43.2	44.9	42.5	40.7	41.6	1.04	0.07
Organic matter (OM)	48.1^{ab}	49.3^{a}	46.3^{bc}	45.0^{c}	46.7^{abc}	0.93	0.02

* NCTL: negative control; PCTL: positive control with 2.1% of Ameri-Bond 2X; 16AALB: 1.6% of AALB from paper processing; 32AALB: 3.2% of AALB; 48AALB: 4.8% of AALB.

a,b,c Different superscripts in the same row are statistically different ($p < 0.05$).

Table 4 Ingredient composition of the diets (Experiment II).

	Experimental diets*				
Ingredients, % of DM	NCTL	PCTL	10AALB+M	24AALB+M	38AALB+M
Alfalfa	17	17	16.7	16.5	16.3
Grass Hay mix	65.4	64.4	63.8	62.8	61.9
DDGS	1	1	1	1	1
Barley grain, rolled	9	9	8.9	8.8	8.6
Corn, ground	5.4	4.4	4.4	4.3	4.2
Canola meal	1	1	1	1	1
Salt	0.1	0.1	0.1	0.1	0.1
ADE premix	0.1	0.1	0.1	0.1	0.1
Mineral premix	1	1	1	1	1
Molasses	0	2	2	2	2
Lignin binder	0	0	1	2.4	3.8

* NCTL: negative control; PCTL: positive control with 2% of molasses; 10AALB+M: 1% of AALB from paper processing combined with 2% of molasses; 24AALB+M: 2% of AALB combined with 2% of molasses; 38AALB+M: 3.8% of AALB combined with 2% of molasses.

Dos Santos *et al.* [23] did not observe a difference in the total-tract DM digestibility of dairy cows when the concentrate was pelleted with 0.5% of lignosulfonate. However, we observed a greater DM digestibility (48 h *in vitro*) in the diets pelleted with commercial lignosulfonate than in the diets with 3.2% and 4.8% of AALB ($p = 0.07$, Table 3). In addition, diet pelleted with commercial lignosulfonate also showed a greater OM digestibility than that with 1.6% and 3.2% of AALB ($p = 0.02$). This difference may be attributed to 3 factors. The first factor is that our study evaluated using *in vitro* rumen digestibility whereas in their study [23] total-tract digestibility was measured. The second factor is the inclusion rate that corresponded to 2.1% of commercial lignosulfonate binder in our research whereas the inclusion rate was 0.5% in that study. Lastly, in our work, the whole diet was pelleted whereas in their study only the concentrate was pelleted.

3.2 Experiment II

Unlike the first experiment, AALB was tested while molasses was added as well to improve the overall palatability because there was a reduction in the preference of heifers for the diet with high AALB inclusion rates in experiment I. Also, the AALB inclusion rates were adjusted to those for experiment I.

Animals showed a preference for diets that contained 2.4% and 3.8% of AALB combined with liquid molasses when compared with NCTL and 10AALB+M ($p < 0.01$). On the other hand, the preference for PCTL was lower than the 38AALB+M but did not differ from the other diets (Table 5). The greater preference for the diets with the inclusion of 2.4% and 3.8% of AALB + molasses vs. NCTL and 10AALB+M can possibly be related to the associative effect of molasses leading to a better palatability and adhesiveness of the pellets. It is believed that molasses played a role in improving

Table 5 Feeding behavior of Holstein heifer dairy fed pelleted diets with binders inclusion (Experiment II).

Items	Experimental diets*					SEM	*p*-value
	NCTL	PCTL	10AALB+M	24AALB+M	38AALB+M		
Preference, %	50.00[c]	50.88[bc]	50.30[c]	57.22[ab]	58.05[a]	0.88	<0.01
DM intake/approach, g	34.1[c]	127.9[b]	50.9[bc]	118.1[b]	304.8[a]	34.6	<0.01
Approach and no eating, number	8.54	9.97	8.36	11.1	11.1	1.37	0.22
Approach and eating, number	2.46[c]	6.06[b]	3.29[c]	7.53[ab]	8.80[a]	0.99	<0.01
Time eating, minutes	7.02[c]	20.0[b]	10.0[c]	24.2[b]	32.9[a]	2.84	<0.01
Time ruminating, min	24.9[a]	10.4[b]	18.1[a]	7.17[b]	0.60[c]	2.36	<0.01
Time NNB/inactivity, min	27.8	29.0	31.2	27.7	26.3	2.60	0.60
Time drinking water, min	0.30[b]	0.63[ab]	0.67[ab]	0.91[a]	0.26[b]	0.22	0.03

* NCTL: negative control; PCTL: positive control with 2% of molasses; 10AALB+M: 1% of AALB from paper processing combined with 2% of molasses; 24AALB+M: 2% of AALB combined with 2% of molasses; 38AALB+M: 3.8% of AALB combined with 2% of molasses.

min: minutes.

NNB/inactivity: non-nutritive behavior or inactivity.

[a,b,c] Different superscript letters in the same row are statistically different ($p < 0.05$).

the palatability and AALB in the pelleting properties. However, further investigation is needed to better understand how the association of molasses with high levels of AALB increased the preference.

Animals fed molasses and 24AALB+M or 38AALB+M showed an improved intake compared with that for NCT. In addition, the 38AALB+M possibly provided a positive associative effect by the molasses because the intake for this diet was greater than all the others. However, the 10AALB+M did not differ from the NCTL ($p < 0.01$). Also, the animals showed a greater DM intake per approach evaluated over 60 min when they received the 38AALB+M than all the other diets, whereas the NCTL did not differ from 10AALB+M but was lower than all the other diets ($p < 0.01$; Fig. 2). This observation regarding the intake shows that although the molasses improved DM intake over 60 min (NCTL vs. PCTL), the addition of AALB combined with molasses possibly provided a positive associative effect on the intake. The palatability might explain the greater DM intake over 60 min when molasses was used. As previously discussed, palatability is driven by physical and chemical characteristics [21].

Molasses and other liquid feed containing high sugars are known for their high palatability, which might lead to an increase in feed intake of dairy cows [25, 26]. Havekes *et al*. [25] observed that transition dairy cows fed a diet with a high straw that included molasses-based liquid feed showed a greater DM intake than cows fed diets without molasses-based liquid feed. In addition, molasses is a binder used in the pelleting process [27], which plays an important role in improving the quality of the pellet because of its capacity for solid-solid interaction [10, 28]. Therefore, molasses also has been used in ruminant diets to reduce the sorting of the diets because of its adhesiveness [26, 29, 30].

The number of times that the animals approached the feed and ate was greater for the diet 38AALB+M compared with that for NCTL, PCTL, and 10AALB+M; however, it did not differ from the 24AALB+M. Animals fed with diet 38AALB+M had the longest time eating during the evaluation period of 60 min, whereas the 10AALB+M and NCTL had the shortest time eating ($p < 0.01$). The longest time spent on eating, over 60 min, observed for 38AALB+M needs further investigation, such as the evaluation of DM intake over 24 h, to better understand this effect regarding intake and eating behavior. Animals showed the longest time drinking water over 60 min when fed 24AALB+M, and the shortest at NCTL and

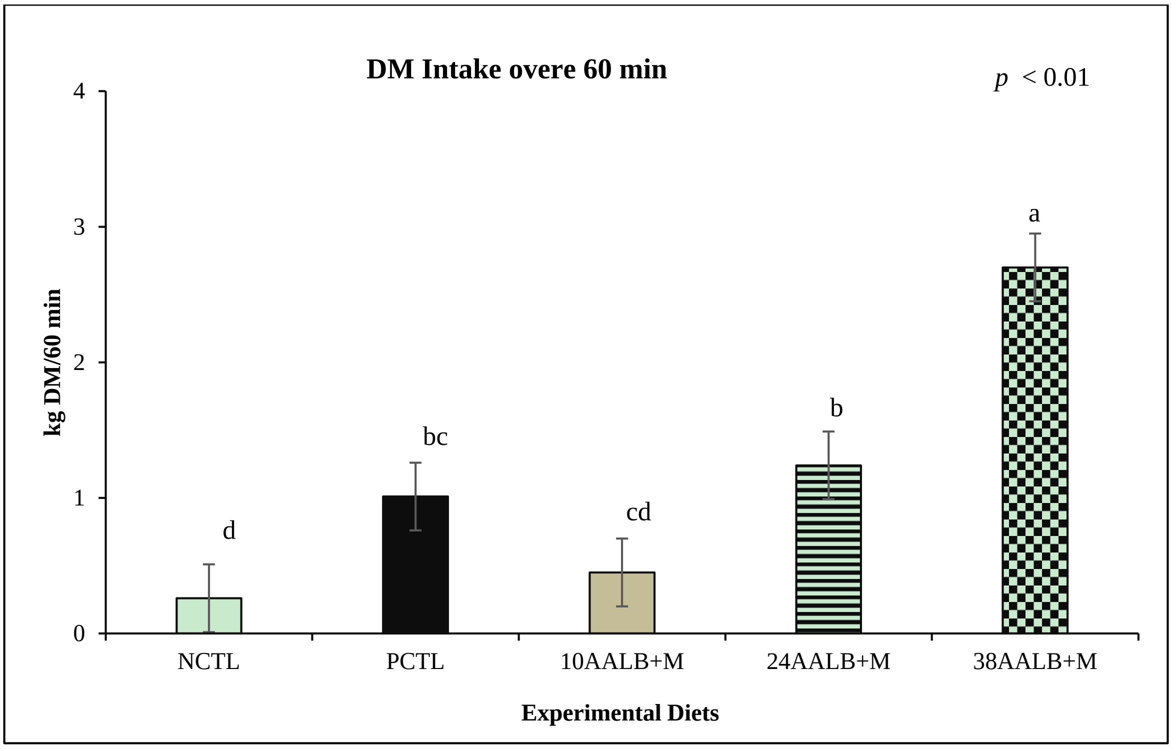

Fig. 2 DM intake over 60 min of dairy heifer offered pelleted diets with binders inclusion (Experiment II).
NCTL: negative control; PCTL: positive control with 2% of molasses; 10AALB+M: 1% of AALB from paper processing combined with 2% of molasses; 24AALB+M: 2% of AALB combined with 2% of molasses; 38AALB+M: 3.8% of AALB combined with 2% of molasses.
[a,b,c] Different superscripts show statistically different ($p < 0.05$). Means error bars represent standard error means.

Table 6 *In vitro* digestibility (48 h) of pelleted diets with binders inclusion (Experiment II).

Digestibility (%)	Experimental diets*					SEM	*p*-value
	NCTL	PCTL	10AALB+M	24AALB+M	38AALB+M		
DM	32.7^{b}	32.9^{b}	33.9^{b}	35.8^{a}	36.1^{a}	0.190	<0.01
OM	29.8^{d}	32.5^{c}	35.2^{b}	36.1^{b}	38.9^{a}	0.270	<0.01

* NCTL: negative control; PCTL: positive control with 2% of molasses; 10AALB+M: 1% of AALB from paper processing combined with 2% of molasses; 24AALB+M: 2% of AALB combined with 2% of molasses; 38AALB+M: 3.8% of AALB combined with 2% of molasses.
[a,b,c] Different superscripts in the same row are statistically different ($p < 0.05$).

38AALB+M ($p = 0.03$). On the other hand, the PCTL and 10AALB+M did not differ from all the other diets. There was no effect of the diets on the number of approaches, no eating, or time spent on non-nutritive behavior or inactivity ($p = 0.22$; $p = 0.60$, respectively).

There is no previous report in the literature about changes in rumen digestibility or *in vitro* rumen degradation when binders are used in the pelleting process. However, we observed that 48 h *in vitro* DM digestibility was greater for 24AALB+M and 38AALB+M compared with that for all other treatments ($p < 0.01$, Table 6). In addition, 38AALB+M showed the greatest 48 h *in vitro* OM digestibility, followed by 24AALB+M and 10AALB+M, PCTL, and NCTL ($p < 0.01$). The difference in the OM digestibility might be attributed to two factors. The first factor is the lignin binder inclusion because the diets with AALB showed a greater OM digestibility than the positive (molasses only) and negative control diets (no AALB and no molasses).

The second factor is attributed to molasses addition because the diets with molasses showed a greater digestibility than the NCLT. Regarding the AALB, we speculate that the modifications in pellet properties possibly led to an improvement in digestibility. On the other hand, molasses containing approximately 62% of sugar [31], might impact degradation because it provides carbon to improve the efficiency of microbial protein synthesis in the rumen.

Time spent ruminating over 60 min was the longest for animals fed 10AALB+M and NCTL, followed by PCTL and 24AALB+M, whereas 38AALB+M showed the shortest time spent ruminating ($p < 0.01$). Time spent ruminating was complementary to the time spent eating. The diets for which the animals spent more time eating (PCTL, 24AALB+M, and 38AALB+M) were consequently the diets for which the animals spent less time ruminating over 60 min of feeding behavior observation.

4. Conclusion

The inclusion of 1.6% of new alkaline aqueous lignin by-product (AALB) from paper processing showed a greater feed intake (preference) over 60 min compared with other diets (Exp. I). However, the inclusion of 2.4% and 3.8% of AALB combined with molasses in pelleted TMR led to a greater 48 h *in vitro* digestibility, preference, and intake over 60 min for dairy heifers (Exp. II). Overall, it appears that AALB could be used as an alternative in the pelleting process. Further investigations are needed to better understand the effect of AALB on physical and chemical characteristics of pelleted diets, animal productive measures, and the positive associative effect of AALB and molasses when used to improve pellet quality of total mixed ration.

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Conflict of Interest

The authors declare no conflict of interest.

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