



By-products replacing ground corn for dairy cows rotationally grazing elephant grass: milk yield and composition

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Abstract. This study investigated the effects of different by-products used as feedstuffs to replace ground corn for supplementing Holstein dairy cows rotationally grazing intensively managed elephant grass. Five experiments were conducted to evaluate various levels of pelleted citrus pulp (PCP), corn gluten feed (CGF), whole cottonseed (WCS), wheat middlings (WHM), and soybean hulls (SBH) as alternatives to ground corn in iso-proteic and iso-energetic supplements. The experiments followed a Latin square design and assessed blood and performance parameters such as milk yield (MY) and milk composition. Blood parameters were not affected ($p > 0.05$) by any by-products. The inclusion of PCP, CGF and SBH up to 75 %, and WHM up to 50 % in place of corn did not affect ($p > 0.05$) either MY or milk composition. However, replacing the inclusion of ground corn at the level of 75 % of WCS resulted in a significant reduction ($p < 0.05$) in yields of milk, 3.5 % fat-corrected milk, protein, and milk solids, along with an increase ($p < 0.05$) in milk urea nitrogen. Results obtained suggest that the replacement of ground corn by PCP, CGF and SBH up to 75 % and WHM and WCS up to 50 % in the supplement have no adverse short-term effects on MY and composition in Holstein cows grazing intensively managed elephant grass.

Keywords: blood parameters, coproducts, forage quality, Holstein, *Cenchrus purpureus*, tropical pastures.

Sustitución del maíz molido por subproductos en vacas lecheras en pastoreo rotacional de pasto elefante: producción y composición de la leche

Resumen. Este estudio investigó los efectos de reemplazar el maíz molido por diferentes subproductos utilizados como suplementos para vacas lecheras Holstein en pasturas de pasto elefante manejadas intensivamente bajo pastoreo rotacional. Se realizaron cinco experimentos para evaluar diferentes niveles de pulpa de cítricos peletizada (PCP), gluten de maíz (CGF), semilla entera de algodón (WCS), afrecho de trigo (WHM) y cascarilla de soya (SBH) como alternativas al maíz molido. Los experimentos siguieron un diseño de Cuadrado Latino y evaluaron parámetros sanguíneos y productivos, tales como la producción y composición de la leche. Los parámetros sanguíneos no se vieron afectados ($p > 0,05$) por ninguno de los subproductos. La inclusión de PCP, CGF y SBH hasta un 75 % y de WHM hasta un 50 % en sustitución del maíz no afectó ($p > 0,05$) la producción ni la composición de la leche. Sin embargo, la sustitución del maíz molido por 75 % de WCS resultó en una reducción significativa ($p < 0,05$) en la producción de leche, leche corregida al 3,5 % de grasa, proteína y sólidos lácteos, y un incremento ($p < 0,05$) en el nitrógeno ureico de la leche. Los resultados obtenidos sugieren que la sustitución del maíz como suplemento por PCP, CGF y SBH hasta un 75 %, y por WHM y WCS hasta un 50%, no tiene efectos adversos a corto plazo sobre la producción y la composición de la leche de las vacas Holstein pastoreando pasto elefante manejado intensivamente.

Palabras clave: parámetros sanguíneos, coproductos, calidad del forraje, Holstein, *Cenchrus purpureus*, pasturas tropicales.

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Substituição de milho moído por co-produtos para vacas leiteiras em pastejo rotacionado de capim-elefante: produção e composição do leite

Resumo. Este estudo investigou os efeitos de diferentes subprodutos usados como ração para substituir o milho moído no suplemento de vacas leiteiras holandesas em pastagens de capim-elefante manejado intensivamente sob pastejo rotacionado. Cinco experimentos foram conduzidos para avaliar polpa cítrica peletizada (PCP), farelo de glúten de milho (CGF), caroço de algodão inteiro (WCS), farelo de trigo (WHM) e casca de soja (SBH) como alternativas ao milho moído em suplementos isoproteicos e isoenergéticos. Os experimentos seguiram um delineamento de quadrado latino e avaliaram parâmetros sanguíneos e de desempenho, como produção e composição do leite. Os parâmetros sanguíneos não foram afetados ($p > 0,05$) por nenhum subproduto. A inclusão de PCP, CGF e SBH até 75 % e de WHM até 50 % em substituição ao milho, não afetou ($p > 0,05$) a produção nem a composição do leite. Entretanto, a substituição de milho moído por 75 % de WCS resultou em redução significativa ($p < 0,05$) na produção de leite, leite corrigido para 3,5% de gordura, proteína e sólidos totais, além de aumento ($p < 0,05$) na ureia do leite. Os resultados sugerem que a substituição do milho por PCP, CGF e SBH até 75 % e por WHM e WCS até 50 % não causa efeitos adversos de curto prazo sobre a produção e composição do leite de vacas Holandesas em pastejo rotacionado de capim-elefante manejado intensivamente.

Palavras chave: parâmetros sanguíneos, coprodutos, qualidade da forragem, Holstein, *Cenchrus purpureus*, pastagens tropicais.

Introduction

The bulk of dry matter (DM) consumed by grazing dairy cows comes from the grazed forage; however, a significant portion of the energy utilized for maintenance and lactation may derive from non-structural carbohydrates and lipids supplied by supplements (Vazquez and Smith, 2000; NRC, 2001). Corn is the main feed used as supplement for grazing dairy cows in Brazil (Carmo *et al.*, 2015) and approximately 72 % of the corn DM is starch (NRC, 2001). However, depending on corn type and processing methods, the starch content can be as high or above 90 % DM (Ferraretto *et al.*, 2013). Therefore, the use of high levels of corn as supplement can cause nutritional disorders, such as acidosis (Bramley *et al.*, 2008), if nutrients coming from grazed forages and supplements are not well balanced.

More recently, the inclusion of human-edible grains in ruminant diets has been intensely debated in the light of food security concerns (Wilkinson and Lee, 2018). Contextually, feeding by-products can supply valuable nutrients to livestock and allow the use of feedstuffs that would otherwise be treated as waste and sent to landfills (Eastridge, 2006). Several by-products have then been tested as alternatives to replace protein or energy sources in ruminant diets, especially for dairy cows (Harvatine *et al.*, 2002; Kononoff *et al.*, 2006; Bales *et al.*, 2024). However, those studies have been mostly developed for confined cows fed total mixed rations, and there are considerably

fewer studies conducted under grazing conditions, particularly in tropical climates, highlighting the need for research that addresses existing gaps for grazing dairy systems.

The food industry is wide with several options of by-products to feed ruminants becoming available, especially in countries devoted to agricultural production such as Brazil (Silva *et al.*, 2019). Citrus pulp is a by-product of the citrus industry, and its energy content varies from 85 to 90 % of that of corn (NRC, 2001). It has been shown that citrus pulp inclusions up to 10 % of diet in DM-basis can successfully replace ground corn in dairy cows' diets improving milk yield (Hartinger *et al.*, 2024). In the context of tropical agriculture, the effects of increasing levels of inclusion of citrus pulp replacing flint corn are likely to be different). In beef cattle, citrus pulp has been successfully used as a supplement to improve growth performance of beef cattle (Costa *et al.*, 2019). Citrus pulp has high contents of soluble carbohydrates and pectin and low of lignin, which makes its ruminal fermentation distinct from that of corn. Its acetic fermentation profile decreases risks of acidosis (García-Rodríguez *et al.*, 2020). Soybean hulls (SBH), a byproduct from the soybean processing industry, has little value either as human food and for industrial uses (Ipharraguerre and Clark, 2003). The nutrient composition of SBH is variable, but the product is known for having high pectin content and high digestible fiber, but low starch content.



Whole cottonseed (WCS) is a common by-product from cotton production (Arieli, 1998). Despite having a high energy content because of lipids in its composition, WCS has been classified effective at stimulating chewing during eating and rumination because of the linter fraction (Harvatine *et al.*, 2002). Furthermore, the low density of the oil in WCS likely assists with this ingredient floating in and above the rumen mat, therefore touching the rumen walls and stimulating motility. Similarly to WCS, corn gluten feed (CGF) and wheat middlings (WHM) are by-products noted by their high energy and protein

contents (Armentano and Dentine, 1988; Duncan *et al.*, 2024). Yet, CGF contains low lignin content being a source of highly digestible fiber with relatively low concentration of rapidly fermentable carbohydrates, decreasing the risk of ruminal acidosis (Kononoff *et al.*, 2006). Therefore, our objective was to assess milk yield and composition of dairy cows rotationally grazing elephant grass pastures and fed increasing levels of pelletized citrus pulp (PCP), corn gluten feed (CGF), whole cottonseed (WCS), wheat middlings (WHM), and soybean hulls (SBH), replacing ground corn as concentrate ingredients.

Materials and Methods

This study comprises a set of five supplementary feeding experiments carried out from March 2002 to June 2006 in the same area at the University of Sao Paulo, Luiz de Queiroz College of Agriculture, Piracicaba, SP, Brazil (22°42'S, 47°38'W, 546 m.a.s.l.). The pastures were divided into 40 paddocks (2000 m² each). The perennial elephant grass (*Cenchrus purpureus* (Schumach.) Morrone syn. *Pennisetum purpureum* Schumach.) cv. Cameroon and cv. Napier pastures were established in early 1970, in a high fertility Eutroferric Red Nitossol soil (NVef) (Batalha *et al.*, 2022b). According to the Köppen classification, the climate of the experimental area is humid sub-tropical with hot summers and dry winters (Alvares *et al.*, 2013). The rainy season runs from October to March and the dry season from April to September. The historical (1917-2023) average mean air temperature and accumulated rainfall during both rainy and dry seasons are 24.0 °C and 992 mm and 19.6 °C and 282 mm, respectively (Série de Dados Climatológicos do Campus Luiz de Queiroz de Piracicaba, 2024).

Paddocks were rotationally grazed with fixed stocking and rest periods. In Experiment 1, the stocking and rest periods were 3 and 37 days, respectively, whereas for the remaining experiments herds were kept only one to two days grazing each paddock and 23 days resting. Sward surface height was monitored from ground level to the top leaf horizon by 20 readings per paddock using an adapted sward stick (Carnevalli *et al.*, 2021). Ten hand plucked samples simulating what the animals grazed were taken per paddock at pre-grazing during the grazing cycles and mixed as a composite sample used to determine forage nutrient composition (Batalha *et al.*, 2022a) (Table 1). Pastures were fertilized with 80 kg N ha⁻¹ (as urea, 45 % of N) after each grazing event. In all experiments replacing ground corn with PCP, CGF, WCS, WHM, or SBH, supplements were iso-proteic and iso-energetic.

Table 1. Pre- and post-grazing sward heights and nutrient composition of pre-grazing hand pluck forage samples for all five experiments.

Item	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Mean
Pre-grazing sward height, cm	154.0	130.0	100.0	142.7	149.0	135.1
Post-grazing sward height, cm	129.5	66.0	60.0	72.0	67.3	79.0
Nutrient composition, %DM						
Dry matter	20.3	18.3	18.6	18.5	18.2	18.8
Ash	10.5	10.6	10.9	12.7	11.3	11.2
Crude protein	13.7	18.5	14.7	17.6	17.1	16.3
Neutral detergent fiber	62.9	61.4	63.9	61.5	60.8	62.1
Acid detergent fiber	33.3	32.0	33.5	33.4	33.3	33.1
Ether extract	2.5	2.7	2.5	3.0	2.7	2.7
Lignin	3.3	2.8	3.2	3.2	3.3	3.2
<i>In vitro</i> DM digestibility	68.8	72.9	67.4	70.2	70.3	69.9

Supplements formulation

The supplements of all experiments were formulated using the Nutrient Requirements of Dairy Cattle model

(NRC, 2001), considering a forage containing 18 % of crude protein (DM basis) for dairy cows yielding 20 kg d⁻¹ of milk, similar to other experiments carried out in the same set of paddocks (Danes *et al.*, 2013; Chagas *et*



al., 2021). The formulation of the diets was determined based on the total diet, which accounted for both the estimated forage intake and the known daily supplement consumption. The treatments' nomenclature refers to the percentage that ground corn was substituted by each byproduct in each experiment.

Experiment 1 - Citrus pulp

Between March and June 2002, 8 multiparous Holstein dairy cows averaging (\pm SD) 523 ± 82 kg body weight (BW), 82 ± 27 days in milk (DIM), and 20.0 ± 1.5 kg d⁻¹ milk yield (MY) at the beginning of the experiment, were used in a duplicated ($n = 2$) 4×4 Latin square design. Cows were grouped into 2 squares based on their DIM, MY and BW characteristics. The experiment consisted of four 20-day periods, with 15 days for adaptation and 5 for data collection. Cows within each square were randomly assigned to the treatments based on the inclusion levels of finely ground and pelletized citrus pulp (PCP): 0, 25, 50 and 75 % of PCP (PCP0, PCP25, PCP50 and PCP75, respectively). The ingredients and nutrient composition of experimental concentrates are presented in Table A1. Cows were offered the supplements individually (6.3 kg DM d⁻¹), in two equal portions after the morning and before the afternoon milking.

Milk yield was recorded daily during the evaluation period with samples collected in vials containing a bronopol preservative pill. Blood samples were taken from the coccygeal vessels of each cow four hours after the morning concentrate feeding on the last day of the sampling period and stored in vacutainers containing potassium oxalate as an anticoagulant. The body condition score (BCS) was assessed on the first and last day of each period based on a 5-point scale, as proposed by Wildman *et al.* (1982).

Experiment 2 - Corn gluten feed

Sixteen multiparous Holstein dairy cows averaging 468 ± 52 kg BW, 141 ± 52 DIM, and 13.9 ± 2.2 kg d⁻¹ MY at the beginning of the experiment, were used in a quadruplicated ($n = 4$) 4×4 Latin square design with four periods of 20 days each (15 + 5 days for adaptation and evaluation, respectively) from November 2004 to February 2005. Cows were grouped into 4 squares based on their DIM, MY, and BW attributes. The treatments consisted of increasing levels of dried corn gluten feed (CGF) replacing ground corn in concentrate supplements as follows: 0, 25, 50 and 75 % of CGF (CGF0, CGF25, CGF50 and CGF75, respectively). The ingredients and nutrient composition of experimental concentrates are presented in Table A2. Each cow

received 4.9 kg DM d⁻¹ of the supplementary feed, and the provision of those, as well as the measurement of animal performance and metabolic parameters, was similar to that in Experiment 1.

Experiment 3 - Whole cottonseed

Twelve multiparous Holstein dairy cows averaging 544 ± 73 kg BW, 143 ± 14 DIM, and 20.1 ± 2.5 kg d⁻¹ MY at the beginning of the experiment, were used in a triplicated ($n = 3$) 4×4 Latin square design with four periods of 20 days each (15 + 5 days for adaptation and evaluation, respectively) from March to June 2005. Cows were grouped into 3 squares based on DIM, MY, and BW. The treatments consisted of increasing levels of whole cottonseed (WCS) replacing ground corn in the concentrate supplement as follows: 0, 25, 50 and 75 % of WCS (WCS0, WCS25, WCS50 and WCS75, respectively) to correspond to inclusion levels of approximately 0, 7, 14 and 21 % of WCS in the total diet. The ingredients and nutrient composition of the experimental concentrates are shown in Table A3. Each cow received 5.9 kg DM d⁻¹ of the supplementary feeds, and the provision of those, as well as the measurement of animal performance and metabolic parameters was similar to that in Experiment 1.

Experiment 4 - Wheat middlings

Twelve multiparous Holstein dairy cows averaging 532 ± 34 kg BW, 84 ± 13 DIM, and 20.1 ± 1.8 kg d⁻¹ MY at the beginning of the experiment, were used in a triplicated ($n = 3$) 4×4 Latin square design with four periods of 20 days each (15 + 5 days for adaptation and evaluation, respectively) from November 2005 to February 2006. Cows were grouped into 3 squares based on DIM, MY, and BW. The treatments consisted of increasing levels of wheat middlings (WHM) replacing ground corn in the concentrate supplement as follows: 0, 25, 50 and 75 % of WM (WHM0, WHM25, WHM50 and WHM75, "respectively". Each cow received 5.6 kg DM d⁻¹ of supplementary feeds, and the provision of those, as well as the measurement of animal performance and metabolic parameters was similar to that in Experiment 1. The ingredients and nutrient composition of experimental concentrates are presented in Table A4.

Experiment 5 - Soybean hulls

Twelve multiparous Holstein dairy cows averaging 509 ± 57 kg BW, 91 ± 11 DIM, and 20.6 ± 1.4 kg d⁻¹ MY at the beginning of the experiment, were used in a triplicated ($n = 3$) 4×4 Latin square design with four periods of 20 days each (15 + 5 days for adaptation and



periods of 20 days each (15 + 5 days for adaptation and evaluation, respectively) from March to June 2006. Cows were grouped into 3 squares based on DIM, MY, and BW. The treatments consisted of increasing levels of soybean hulls (SBH) replacing ground corn in concentrate supplements as follows: 0, 25, 50 and 75 % of SBH (SBH0, SBH25, SBH50 and SBH75, respectively). Each cow received 5.8 kg DM d⁻¹ of the supplementary feed, and the provision of those, as well as the measurement of animal performance and metabolic parameters was similar that in Experiment 1. Ingredients and nutrient composition of experimental concentrates are shown in Table A5.

Laboratory analysis

Forage and concentrate samples were ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA) before analysis of chemical composition. Dry matter, ash, and ether extract (EE) were determined according to Helrich (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by an automatic fiber analyzer (ANKOM Technology Corp., Macedon, NY, USA) adding sodium sulfite and heat-stable α -amylase as proposed by Van Soest *et al.* (1991). Lignin was also determined according to Van Soest *et al.* (1991). Total nitrogen (N) content was determined by the Dumas combustion method using a N analyzer (Leco FP-2000; Leco Instruments Inc., St. Joseph, MI, USA), and crude protein (CP) was calculated as $N \times 6.25$. Forage *in vitro*

DM digestibility (IVDMD) was determined by the two-stage procedure of Tilley and Terry (1963) modified by Goering and Van Soest (1970).

Milk samples were analyzed for fat, protein, lactose, total solids, and urea nitrogen (MUN) using infrared procedures (MilkoScan FT+; Foss North America Inc., Eden Prairie, MN). Blood samples were centrifuged (3,000 × g, 4°C, 20 min) and plasma analyzed for glucose (YSI 2700 Select, Biochemistry analyzer, Yellow Spring, OH, USA), urea-N (PUN; Kit N 535, Sigma Chemical Co., St. Louis, MO, USA), and non-esterified fatty acids (NEFA; NEFA-C kit, Wako Chemicals GmbH, Richmond, VA, USA) modified by Johnson and Peters (1993).

Statistical analysis

All data were analyzed with SAS (release 8.0, SAS Institute Inc.). Data were examined for outliers using the REG procedure and outliers were removed based on an absolute studentized residue value > 3. Then all datasets were tested for model additivity, independence of errors, normality, and homoscedasticity before analysis of variance. Analysis of variance for all five experiments was performed using the GLM procedure. Means were obtained using the least squares method and compared using the Tukey's test. Differences were declared significant at $p \leq 0.05$, and trends were declared at $0.05 \leq p \leq 0.10$.

Results and Discussion

Experiment 1 - Citrus pulp (PCP)

Increasing levels of PCP up to 75 % replacing ground corn in the supplement did not affect ($p > 0.05$) performance, milk composition and blood parameters in mid-lactation Holstein cows grazing intensively managed elephant grass (Table 2). These results agree with those from Assis *et al.* (2004) who reported no differences in nutrient intake, BW change as well as MY and composition of Holstein cows fed total mixed rations with increasing levels of PCP replacing up to 100 % of corn. In cows maintained in pens, some authors have reported an increase in milk fat content due to the higher inclusion levels of dried citrus pulp in the supplementary feed, at the expense of corn (Drude *et al.*, 1971; Van Horn *et al.*, 1975). This increase in milk fat content, usually observed when the lipids

content in the basal diet was less than 3.5 %, is likely due to a more efficient rumen fermentation because of a greater proportion of highly degradable NDF and readily fermentable soluble sugars found in PCP (García-Rodríguez *et al.*, 2020), which is the case of the diets in this study (Table 1). Conversely, other studies have shown no increase in milk fat content when the lipid content in the basal diet was higher than 3.5 % (Harvatine *et al.*, 2002).

Experiment 2 - Corn gluten feed (CGF)

Increasing levels of CGF in supplements did not influence ($p > 0.05$) the production performance, milk composition and blood parameters of the dairy cows rotationally grazing elephant grass pastures (Table 3).



Table 2. Performance, milk, and blood parameters of dairy cows rotationally grazing elephant grass and supplemented with increasing levels of pelletized citrus pulp (PCP) replacing ground corn.

Item ⁽¹⁾	PCP0 ⁽²⁾	PCP25	PCP50	PCP75	SEM ⁽³⁾	p-value
BCS	2.50	2.53	2.53	2.47	0.04	0.61
Milk yield, kg d ⁻¹	18.3	18.9	18.7	18.8	0.44	0.78
3.5% FCM, kg d ⁻¹	18.2	18.8	18.7	19.0	0.43	0.62
Fat, kg d ⁻¹	0.630	0.660	0.650	0.670	0.01	0.57
Protein, kg d ⁻¹	0.550	0.580	0.560	0.560	0.01	0.57
Milk solids, kg d ⁻¹	2.30	2.46	2.51	2.42	0.12	0.83
Fat, %	3.53	3.50	3.54	3.55	0.06	0.95
Protein, %	3.06	3.12	3.06	3.02	0.03	0.27
Lactose, %	4.38	4.43	4.37	4.35	0.02	0.12
Total solids, %	12.9	12.9	13.3	12.9	0.43	0.83
MUN, mg dL ⁻¹	15.1	15.1	14.8	14.9	0.35	0.90
Plasma glucose, mg dL ⁻¹	76.5	76.1	73.4	77.6	1.38	0.20
Plasma urea, mg dL ⁻¹	36.3	36.7	36.0	36.4	0.81	0.94
PUN, mg dL ⁻¹	16.9	17.1	16.8	16.9	0.72	0.99
NEFA, μ Eq L ⁻¹	492	429	443	461	38.3	0.68

⁽¹⁾BCS, body condition score; 3.5 % FCM (fat-corrected milk) = [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN, milk urea nitrogen; PUN, plasma urea nitrogen; NEFA, non-esterified fatty acids. ⁽²⁾PCP0, 0 % of PCP; PCP25, 25 % of PCP; PCP50, 50 % of PCP; PCP75, 75 % of PCP. ⁽³⁾SEM, standard error of the mean.

Table 3. Performance, milk, and blood parameters of dairy cows rotationally grazing elephant grass and supplemented with increasing levels of corn gluten feed (CGF) replacing ground corn.

Item ⁽¹⁾	CGF0 ⁽²⁾	CGF25	CGF50	CGF75	SEM ⁽³⁾	p-value
BCS	2.45	2.44	2.42	2.36	0.03	0.18
Milk yield, kg d ⁻¹	12.4	12.6	12.4	12.2	0.23	0.58
3.5% FCM, kg d ⁻¹	12.3	12.5	12.2	12.3	0.23	0.73
Fat, kg d ⁻¹	0.425	0.434	0.420	0.430	0.09	0.72
Protein, kg d ⁻¹	0.396	0.407	0.395	0.398	0.08	0.70
Milk solids, kg d ⁻¹	1.46	1.49	1.45	1.45	0.27	0.68
Fat, %	3.50	3.50	3.47	3.60	0.07	0.67
Protein, %	3.30	3.31	3.31	3.35	0.08	0.55
Lactose, %	4.26	4.22	4.25	4.20	0.02	0.13
Total solids, %	11.9	11.9	11.9	11.9	0.07	0.78
MUN, mg dL ⁻¹	14.3	13.9	14.2	14.0	0.22	0.52
Plasma glucose, mg dL ⁻¹	66.7	66.7	66.1	65.0	1.69	0.84
Plasma urea, mg dL ⁻¹	41.5	42.3	41.0	39.0	1.88	0.61
PUN, mg dL ⁻¹	19.4	19.8	19.1	18.2	0.90	0.61
NEFA, μ Eq L ⁻¹	385	373	353	357	16.6	0.51

⁽¹⁾BCS, body condition score; 3.5 % FCM (fat-corrected milk) = [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN, milk urea nitrogen; PUN, plasma urea nitrogen; NEFA, non-esterified fatty acids. ⁽²⁾CGF0, 0 % of CGF; CGF25, 25 % of CGF; CGF50, 50 % of CGF; CGF75, 75 % of CGF. ⁽³⁾SEM, standard error of the mean.

The results obtained showed that CGF can replace corn up to 75 % in the supplement with no side effects in the case of the late lactating dairy cows grazing intensively managed elephant grass. These results are similar to others obtained in with high-producing Holstein cows fully maintained in corrals (Armentano and Dentine, 1988; Pedroso *et al.*, 2009). Other authors have reported increased MY when CGF was included in the diet (Kononoff *et al.*, 2006; Firkins *et al.*, 1991); however, in those studies, CGF replaced not only the

ground corn but also other roughage and concentrate ingredients, resulting in higher DM intake, which explained greater MY. The results obtained in other studies for milk composition parameters, especially the protein, fat, and urea-N contents, were variable, with some studies showing changes (Pedroso *et al.*, 2009) whilst others did not (Firkins *et al.*, 1991). Such variability is likely due to which ingredients were replaced by CGF, was only corn or corn plus other forage and concentrate ingredients.



Experiment 3 – Whole cottonseeds (WCS)

Dairy cows fed supplements including 75 % of WCS had lower ($p < 0.05$) yields of milk, 3.5 % FCM, protein,

and milk solids compared to other levels of inclusion (Table 4). The inclusion of WCS in supplements increased MUN ($p < 0.05$) of dairy cows regardless of the level.

Table 4. Performance, milk, and blood parameters of dairy cows rotationally grazing elephant grass and supplemented with increasing levels of whole cottonseed (WCS) replacing ground corn.

Item ⁽¹⁾	WCS0 ⁽²⁾	WCS25	WCS50	WCS75	SEM ⁽³⁾	p-value ⁽⁴⁾
BCS	2.29	2.25	2.33	2.28	0.03	0.37
Milk yield, kg d ⁻¹	17.7 ^a	17.4 ^a	16.9 ^a	15.3 ^b	0.31	<0.01
3.5% FCM, kg d ⁻¹	17.8 ^a	17.8 ^a	17.8 ^a	16.2 ^b	0.38	0.01
Fat, kg d ⁻¹	0.627	0.635	0.648	0.593	0.01	0.18
Protein, kg d ⁻¹	0.512 ^a	0.494 ^a	0.470 ^a	0.426 ^b	0.01	< 0.01
Milk solids, kg d ⁻¹	2.06 ^a	2.03 ^a	2.00 ^a	1.80 ^b	0.04	< 0.01
Fat, %	3.55	3.65	3.80	3.80	0.11	0.27
Protein, %	2.90	2.84	2.80	2.83	0.03	0.06
Lactose, %	4.23	4.20	4.17	4.00	0.07	0.06
Total solids, %	11.6	11.6	11.7	11.5	0.16	0.81
MUN, mg dL ⁻¹	13.4 ^b	15.0 ^a	15.9 ^a	15.8 ^a	0.46	< 0.01
Plasma glucose, mg dL ⁻¹	49.4	51.2	52.0	53.8	1.71	0.10
Plasma urea, mg dL ⁻¹	49.8	48.3	49.0	51.8	1.48	0.16
PUN, mg dL ⁻¹	23.2	22.6	22.9	24.2	0.69	0.16
NEFA, μ Eq L ⁻¹	368.3	371.5	365.3	362.3	18.2	0.60

⁽¹⁾BCS, body condition score; 3.5 % FCM (fat-corrected milk) = [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN, milk urea nitrogen; PUN, plasma urea nitrogen; NEFA, non-esterified fatty acids. ⁽²⁾WCS0, 0 % of WCS; WCS25, 25 % of WCS; WCS50, 50 % of WCS; WCS75, 75 % of WCS. ⁽³⁾SEM, standard error of the mean.

⁽⁴⁾Means followed with the same lowercase letter in the rows do not differ by Tukey's test, at 5 % probability.

The substitution of ground corn at the level of 75 % of WCS in the supplement decreased yields of milk, 3.5 % FCM, protein, and milk solids of dairy cows in the current study. This is likely due to a decrease in the total DM intake and its consequences on microbial synthesis and the supply of metabolizable protein to the duodenum (Clark *et al.*, 1992). Milk protein content tended to be lower with increasing levels of WCS, which suggests either a lower supply of microbial protein to the duodenum or a poorer amino acid profile of this protein source. Previous studies with housed dairy cows have shown that inclusion of WCS between 15-18 % in the diet did not have negative effects on MY and composition (Fernandes *et al.*, 2002). For dairy cows yielding 40-50 kg d⁻¹ of milk, WCS levels up to 16 % have increased milk and fat yields compared with 0 and 24 % WCS (Bales *et al.*, 2024). We also observed an increase in MUN when WCS was fed. It corroborates previous studies, and it is likely due to the high degradability of the WCS protein (Arieli, 1998). Even though diets had the same crude protein and net energy for lactation contents, the increase in MUN when WCS was added to the diets might be explained due to poorer synchronism of protein and energy as the energy provided by WCS comes from lipids and not carbohydrates.

Experiment 4 – Wheat Middlings (WHM)

Increasing levels of WHM in the supplement of dairy cows rotationally grazing elephant grass had no influence ($p > 0.05$) on animal performance and blood parameters (Table 5). The substitution of ground corn for 75 % of WHM resulted in a slight decline in MY and 3.5 % FCM ($p < 0.06$), and the MUN tended to increase along with the level of substitution of maize with WHM ($p < 0.05$) in dairy cows.

Overall, dairy cows were not affected by increasing levels of WHM in the supplement replacing corn, which is consistent with previous research with housed animals (Soares *et al.*, 2004; Tufarelli and Laudadio, 2010). Conversely, Acedo *et al.* (1987) reported a decrease in MY of dairy cows when WHM was fed at 60 %. The latter aligns with this study with a high level of WCS inclusion reached with the 75 % substitution. Further, cows fed WHM at 75 % of corn substitution had higher MUN compared to other levels. This is likely due to the greater ruminal degradability of WHM protein and the lower availability of fermentable carbohydrates in the rumen (Soares *et al.*, 2004). Other studies have shown that this greater protein degradability linked to lower energy availability in the rumen provided by higher levels of WHM inclusion also resulted in an increased PUN (Acedo *et al.*, 1987); however, the results obtained in this study did not corroborate such findings.

Table 5. Performance, milk, and blood parameters of dairy cows rotationally grazing elephant grass and supplemented with increasing levels of wheat middlings (WHM) replacing ground corn.

Item ⁽¹⁾	WHM0 ⁽²⁾	WHM25	WHM50	WHM75	SEM ⁽³⁾	p-value ⁽⁴⁾
BCS	2.25	2.31	2.23	2.27	0.02	0.11
Milk yield, kg d ⁻¹	19.6	19.7	19.6	18.6	0.30	0.06
3.5% FCM, kg d ⁻¹	19.1	19.5	19.4	18.5	0.38	0.29
Fat, kg d ⁻¹	0.653	0.676	0.673	0.646	0.02	0.55
Protein, kg d ⁻¹	0.576	0.594	0.586	0.558	0.01	0.10
Milk solids, kg d ⁻¹	2.05	2.09	2.10	1.98	0.05	0.28
Fat, %	3.35	3.46	3.45	3.52	0.06	0.34
Protein, %	2.96	3.04	3.00	3.02	0.04	0.51
Lactose, %	4.19	4.24	4.20	4.23	0.03	0.53
Total solids, %	10.5	10.6	10.7	10.7	0.17	0.73
MUN, mg dL ⁻¹	13.8 ^b	14.3 ^b	14.6 ^b	15.9 ^a	0.30	< 0.01
Plasma glucose, mg dL ⁻¹	69.8	68.8	73.5	68.3	3.52	0.72
Plasma urea, mg dL ⁻¹	39.3	41.3	46.0	44.3	1.75	0.11
PUN, mg dL ⁻¹	18.3	19.3	21.5	20.7	0.82	0.11
NEFA, μ Eq L ⁻¹	262	268	280	238	12.4	0.12

⁽¹⁾BCS, body condition score; 3.5 % FCM (fat-corrected milk) = [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN, milk urea nitrogen; PUN, plasma urea nitrogen; NEFA, non-esterified fatty acids. ⁽²⁾WHM0, 0% of WHM; WHM25, 25 % of WHM; WHM50, 50 % of WHM; WHM75, 75 % of WHM. ⁽³⁾SEM, standard error of the mean. ⁽⁴⁾Means followed with the same lowercase letter in the rows do not differ by Tukey's test, at 5 % probability.

Experiment 5 - Soybean hulls (SBH)

The animal performance and blood parameters in dairy cows rotationally grazing elephant grass were

not affected ($p > 0.05$) by increasing levels of SBH replacing ground corn in the supplement up to 75 % (Table 6).

Table 6. Performance, milk, and blood parameters of dairy cows rotationally grazing elephant grass and supplemented with increasing levels of soybean hulls (SBH) replacing ground corn.

Item ⁽¹⁾	SBH0 ⁽²⁾	SBH25	SBH50	SBH75	SEM ⁽³⁾	p-value
BCS	2.17	2.25	2.23	2.19	0.05	0.63
Milk yield, kg d ⁻¹	17.8	17.8	17.4	17.3	0.31	0.50
3.5% FCM, kg d ⁻¹	16.5	16.8	16.6	16.4	0.48	0.92
Fat, kg d ⁻¹	0.540	0.560	0.557	0.547	0.03	0.93
Protein, kg d ⁻¹	0.500	0.517	0.485	0.479	0.02	0.54
Milk solids, kg d ⁻¹	1.73	1.78	1.73	1.68	0.05	0.59
Fat, %	3.06	3.21	3.22	3.20	0.13	0.80
Protein, %	2.81	2.92	2.78	2.78	0.09	0.70
Lactose, %	3.95	4.02	3.97	3.82	0.13	0.74
Total solids, %	9.71	10.0	9.90	9.70	0.23	0.65
MUN, mg dL ⁻¹	15.7	15.9	15.0	14.5	0.54	0.24
Plasma glucose, mg dL ⁻¹	75.3	81.0	75.5	76.8	1.29	0.06
Plasma urea, mg dL ⁻¹	29.8	30.8	34.3	32.8	1.28	0.16
PUN, mg dL ⁻¹	13.9	14.4	16.0	15.3	0.60	0.16
NEFA, μ Eq L ⁻¹	358	371	361	342	18.8	0.75

⁽¹⁾BCS, body condition score; 3.5 % FCM (fat-corrected milk) = [(0.4324 × milk yield) + (16.216 × fat yield)]; MUN, milk urea nitrogen; PUN, plasma urea nitrogen; NEFA, non-esterified fatty acids. ⁽²⁾SBH0, 0 % of SBH; SBH25, 25 % of SBH; SBH50, 50 % of SBH; SBH75, 75 % of SBH. ⁽³⁾SEM, standard error of the mean.

Previous research has tested SBH replacing corn in the concentrate to supply 0, 10, 20, 30 or 40 % of the dietary DM of housed Holstein cows (Ipharraguerre *et al.*, 2002), and found that the yields of 3.5 % FCM, protein, and total solids as well as protein content and MUN were not affected by such levels of replacement. On the other hand, milk fat and total solids contents as well as fat yield increased linearly with increasing levels of SBH. Ipharraguerre and Clark (2003) did not report any significant effects of replacing corn with

SBH in dairy cow diets using a meta-analysis approach. The authors have attributed the results to the average dietary inclusion level of SBH in the dataset. However, based on a smaller group of studies, the latter authors speculated that levels greater than 30 % of dietary DM as SBH in high grain diets (≥ 50 %), would limit the physically effective fiber. It is important to emphasize that the latter may increase the concentration of acids in the rumen resulting in reduced DM intake (Ipharraguerre and Clark, 2003).



Conclusions

The use of PCP, CGF and SBH replacing corn with up to 75 % of the supplement and WCS and WHM up to 50 %, has no adverse short-term effects on milk yield

and composition of Holstein cows grazing intensively managed elephant grass.

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APPENDIX

Table A1. Ingredients and nutrient composition of experimental supplements including increasing levels of pelletized citrus pulp (PCP) - Experiment 1.

Item	PCP0 ⁽¹⁾	PCP25	PCP50	PCP75
Ingredient, % DM				
Ground corn	70.0	51.4	33.5	16.4
Pelletized citrus pulp	-	17.1	33.5	49.1
Soybean meal	24	25.5	27.0	28.5
Urea	1.0	1.0	1.0	1.0
Minerals and vitamins	5.0	5.0	5.0	5.0
Nutrient composition ⁽²⁾				
Crude protein, %DM	21.8	21.8	21.8	21.8
NEL, Mcal kg ⁻¹ DM	2.0	2.0	2.0	2.0

⁽¹⁾PCP0, 0 % of PCP; PCP25, 25 % of PCP; PCP50, 50 % of PCP; PCP75, 75 % of PCP indicating the level of substitution of ground corn by PCP. ⁽²⁾According to NRC (2001); NEL, net energy for lactation.

Table A2. Ingredients and nutrient composition of experimental supplements including increasing levels of corn gluten feed (CGF) - Experiment 2.

Item	CGF0 ⁽¹⁾	CGF25	CGF50	CGF75
Ingredient, % DM				
Ground corn	80.1	61.3	43.4	23.6
Corn gluten feed	-	20.4	43.4	70.8
Soybean meal	12.4	11.7	7.2	-
Urea	1.9	1.0	0.4	-
Minerals and vitamins	5.6	5.6	5.6	5.6
Nutrient composition ⁽²⁾				
Crude protein, %DM	19.0	19.0	19.0	19.0
NEL, Mcal kg ⁻¹ DM	2.0	2.0	2.0	2.0

⁽¹⁾CGF0, 0 % of CGF; CGF25, 25 % of CGF; CGF50, 50 % of CGF; CGF75, 75 % of CGF indicating the level of substitution of ground corn by CGF. ⁽²⁾According to NRC (2001); NEL, net energy for lactation.

Table A3. Ingredients and nutrient composition of experimental supplements including increasing levels of whole cottonseed (WCS) - Experiment 3.

Item	WCS0 ⁽¹⁾	WCS25	WCS50	WCS75
Ingredient, % DM				
Ground corn	80.1	63.2	43.4	23.6
Whole cottonseed	-	21.1	43.4	70.7
Soybean meal	12.4	8.7	7.1	-
Urea	1.9	1.4	0.5	0.1
Minerals and vitamins	5.6	5.6	5.6	5.6
Nutrient composition ⁽²⁾				
Crude protein, %DM	19.0	19.0	19.0	19.0
NEL, Mcal kg ⁻¹ DM	2.0	2.0	2.0	2.0

⁽¹⁾WCS0, 0 % of WCS; WCS25, 25 % of WCS; WCS50, 50 % of WCS; WCS75, 75 % of WCS indicating the level of substitution of ground corn by WCS. ⁽²⁾According to NRC (2001); NEL, net energy for lactation

Table A4. Ingredients and nutrient composition of experimental supplements including increasing levels of wheat middlings (WHM) – Experiment 4.

Item	WHM0 ⁽¹⁾	WHM25	WHM50	WHM75
Ingredient, % DM				
Ground corn	80.1	60.6	40.7	20.4
Wheat middlings	-	20.1	40.7	61.1
Soybean meal	12.4	12.4	12.3	12.9
Urea	1.9	1.3	0.7	-
Minerals and vitamins	5.6	5.6	5.6	5.6
Nutrient composition ⁽²⁾				
Crude protein, %DM	19.0	19.0	19.0	19.0
NEL, Mcal kg ⁻¹ DM	2.0	2.0	2.0	2.0

⁽¹⁾WCS0, 0 % of WCS; WCS25, 25 % of WCS; WCS50, 50 % of WCS; WCS75, 75 % of WCS indicating the level of substitution of ground corn by WCS. ⁽²⁾According to NRC (2001); NEL, net energy for lactation

Table A5. Ingredients and nutrient composition of experimental supplements including increasing levels of soybean hulls (SBH) – Experiment 5.

Item	SBH0 ⁽¹⁾	SBH25	SBH50	SBH75
Ingredient, % DM				
Ground corn	80.1	61.1	40.7	19.8
Wheat middlings	-	20.4	40.7	59.5
Soybean meal	12.4	11.2	11.6	14.5
Urea	1.9	1.7	1.4	0.6
Minerals and vitamins	5.6	5.6	5.6	5.6
Nutrient composition ⁽²⁾				
Crude protein, %DM	19.0	19.2	19.3	19.2
NEL, Mcal kg ⁻¹ DM	2.0	1.9	1.9	1.8

⁽¹⁾SBH0, 0 % of SBH; SBH25, 25 % of SBH; SBH50, 50 % of SBH; SBH75, 75 % of SBH indicating the level of substitution of ground corn by SBH. ⁽²⁾According to NRC (2001); NEL, net energy for lactation.

Table A6. Nutrient composition of the feed ingredients.

Nutrient composition, %DM	GC ⁽¹⁾	SBM	PCP	CGF	WCS	WHM	SBH
Dry matter	89.2	89.8	93.9	89.9	93.7	90.0	90.0
Ash	1.1	6.1	8.5	3.9	3.4	4.3	4.2
Crude protein	10.4	49.0	7.6	23.9	23.7	19.0	10.8
Neutral detergent fiber	9.7	10.4	25.2	35.8	60.5	37.0	60.5
Acid detergent fiber	3.2	6.7	24.4	14.8	38.7	12.8	44.6
Ether extract	4.1	1.2	-	3.3	19.2	4.4	2.5

⁽¹⁾GC, ground corn; SBM, soybean meal; PCP, pelletized citrus pulp; CGF, corn gluten feed; WCS, whole cottonseed; WHM, wheat middlings; SBH, soybean hulls.