Ileal digestibility of energy and amino acids in three faba bean cultivars (*Vicia faba* L.) planted and harvested early or late in broiler chickens

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ABSTRACT A concern of both pulse growers and poultry producers is how the timing of planting and harvesting affect the nutritional quality of faba bean for broiler chickens. To investigate, half of the seed of 2 zero-tannin cultivars (Snowbird and Snowdrop) and 1 low vicine and convicine cultivar (Fabelle) were planted at a single site either in early May and harvested in late September (EARLY) or planted in late May and harvested in late October (LATE). Diets of the 3 EARLY or LATE cultivars (95% inclusion) were fed to 756 broiler chickens (Ross 708) from d 15. Chickens were housed in 56 floor pens (13−14 birds/pen) in a 3 × 2 factorial arrangement (7 pens/diet). A nitrogen-free diet to correct for endogenous amino acid (**AA**) losses was fed to broilers in 14 pens from d 20. Ileal digesta was collected after euthanizing birds on day 23 or 24. Planting and harvesting LATE vs. EARLY increased the proportion of immature beans from 5 to 64% for Snowbird, 7 to 79% for Snowdrop, and 22 to 80% for Fabelle. Planting and harvesting LATE vs. EARLY increased the proportion of frost-damaged beans from 20 to 83% for Snowbird, 36 to 88% for Snowdrop, and 5 to 29% for Fabelle. Planting and harvesting LATE vs. EARLY increased (P < 0.001) apparent ileal digestibility (**AID**) of gross energy (**GE**) by 45% and standardized ileal digestibility (**SID**) of CP by 13%. Planting and harvesting LATE vs. EARLY increased ($P < 0.001$) SID of AA by 11% except Cys. Snowbird and Snowdrop had greater ($P < 0.05$) SID of AA by 4.5% except Thr and Trp compared with Fabelle. Fabelle had 13% greater ($P < 0.001$) SID of Trp compared with Snowbird or Snowdrop. Results indicate that planting and harvesting LATE vs. EARLY increased GE, CP, and AA digestibility possibly by frost interrupting bean ripening on the field. Hull tannin content may have reduced the AA digestibility of Fabelle compared with Snowbird or Snowdrop.

Key words: broiler chicken, faba bean cultivar, frost damage, ileal digestibility, immature beans

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INTRODUCTION

The single largest cost of broiler chicken meat production for human consumption is feed (Alltech, 2018). The 2 components that account for most of the cost in broiler feed are starch as source of energy and protein as source of amino acids (**AA**). Faba bean (*Vicia faba L.*) grain is rich in both starch (38%) and crude protein (**CP**) (28%; Crépon et al., 2010), making it a potential substitute for conventional feedstuffs in broiler feed. Faba bean is a high-yield pulse that grows well in temperate climate zones (north of the 50° parallel) where neither corn nor soybean cultivation is optimal (Henriquez et al., 2018). Surplus food- and feed-grade quality faba bean tonnage is available regionally (Clancey, 2020). Locally grown faba bean represent an opportunity to poultry producers in temperate regions to reduce feed cost by replacing higher priced ingredients like imported corn grain and soybean meal.

Canada's faba bean production consists of beans intended for human consumption, mostly for export, and those produced for animal feed markets (Clancey, 2018). Preference for human consumption is largely based on visual acceptance and regional traditions (e.g., large or midsize bean, shiny brown, or light tan color), whereas cost and low content of antinutritional factors (**ANF**) in faba bean are more important for animal feeding. Condensed tannins (proanthocyanidins) tie up both bean protein and starch, as well as endogenous gastric secretions such as mucus or digestive enzymes. To mitigate the negative effects of bean tannins on animal feed intake and nutrient digestibility, white-flowered, low-tannin (so-called zero-tannin) cultivars of faba bean

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have been developed (Duc, 1997; Crépon et al., 2010). Zero-tannin faba bean cultivars showed greater apparent ileal digestibility of AA in broilers than color-flowered, tannin-containing cultivars and may be a better source of both starch and protein for broiler chickens (Woyengo and Nyachoti, 2012). Other ANF of interest in faba bean are vicine and convicine, which can cause an acute hemolytic anemia named favism in humans with an erythrocyte-located genetic deficiency of glucose-6-phosphate dehydrogenase (Arese et al., 2012), and may also cause blood spots in table eggs when fed to laying hens (Muduuli et al., 1981). New faba bean cultivars termed 'double zero' are now being developed that are both low in tannin and low in vicine and convicine content (Khazaei et al., 2019) but these cultivars are not commercially available yet.

A concern of both pulse growers and poultry producers is how the timing of planting and harvesting affect the feeding quality of faba bean grain. Faba bean has a growth cycle more than 2 wk longer than field pea, the predominant pulse crop in Western Canada, making it susceptible to fall frost damage if planted late due to a wet spring. Moreover, zero-tannin faba bean cultivars are less tolerant to early frost at fall harvest time (Henriquez et al, 2018), increasing the chance of frost-damaged beans being available for the animal feed market instead of receiving a premium price for human food export. Suboptimal growing conditions can also result in a high percentage of immature beans at harvest time. Immature, blackened hull (testa) beans would not make human food grade, but instead would be destined for animal feeding.

Previous reports showed that broilers can be fed diets containing different faba bean cultivars at relatively high dietary inclusions (up to 36% Cho et al., 2019; up to 40% Kopmels et al., 2020). However, these research trials fed high quality faba bean. Little is known about the effect of feeding frost damaged and immature beans on digestibility of gross energy (**GE**), CP, and AA for broiler chickens, yet pulse growers phase this harvest challenge almost every year. Therefore, the objective of this study was to compare the effect of feeding diets including different faba bean cultivars planted and harvested either EARLY (mostly ripen grain, no frost damage) or LATE (high proportion of immature beans, blackened hulls) on digestibility of GE, CP, and AA in broiler chickens. Our null hypothesis was that feeding different faba bean cultivars planted and harvested either EARLY or LATE would result in no difference in nutrient digestibility.

MATERIALS AND METHODS

Animal use was approved and experiment procedures were reviewed by the University of Alberta Animal Care and Use Committee for Livestock. Study conduct followed principles established by the Canadian Council on Animal Care (**CCAC**, 2009). The experiment was carried out at the Poultry Research and Technology Centre (**PRTC**) located at the University of Alberta, South Campus (Edmonton, Alberta, Canada).

Housing

The experiment was conducted in a growout room with 2 rows of 28 floor pens separated by a center alley. Pens (1.44 m length × 1.04 m width) had concrete floor, 1 concrete sidewall and 3 sides that consisted of a fence made from plastic mesh strung over a polyvinyl chloride pipe frame. Pens were bedded with newspapers throughout the experiment and were equipped with heat lamps for the first 7 d. Birds had ad libitum access to feed from height-adjustable, round hopper feeders and continuous access to water from adjustable height bars providing 3 to 4 nipple drinkers per pen. For the first 6 d, chicks were also provided feed in rectangular, plastic feeding troughs and water in shallow dishes to encourage feed and water consumption. Automated controllers and timers specific to the test room adjusted temperature, ventilation, and lighting as programmed. Temperature of the room was reduced as birds aged as per the Ross 708 production manual (Aviagen, 2018) adjusted for low relative humidity. Lighting schedule was according to the PRTC's Standard Operating Procedures and followed guidelines set forth by the National Farm Animal Care Council Code of Practice (2016) providing a minimum of 10 to 15 lux with 20L:4D throughout the trial.

Ingredients and Diets

High quality (#1 certified) faba bean seed of 2 zero-tannin cultivars (Snowbird and Snowdrop) and 1 tannin-cultivar low in vicine and convicine (Fabelle) was sourced. Snowbird originated from Galloway Seeds (Fort Saskatchewan, Alberta, Canada), Snowdrop from Shewchuk Seeds (Blaine Lake, Saskatchewan, Canada), and Fabelle from Stamp Seeds Ltd. (Enchant, Alberta, Canada). Seed of the 3 cultivars was planted at a single site (53°38′52.2″ N 113°21′09.2″ W) at the Crop Diversification Centre North (Edmonton, Alberta, Canada). Half of the seed of each cultivar was sown in early May, chemically desiccated (to dry down the crop fast to reduce time to harvest and avoid uneven ripening) in the middle of September, and harvested in late September (EARLY), whereas the other half was sown in late May, desiccated in early October, and harvested in late October (LATE) to purposely increase the proportion of frost-damaged beans. After harvesting, beans were cleaned using a combination of mesh sieving and blowing air in a custom-made, pilot-scale seed cleaner. Beans were then ground through a 4.76-mm screen using a hammer mill (Model DLK1, Carter Day International; Minneapolis, MN) at the University of Alberta Environment and Metabolism Unit (Edmonton, Alberta, Canada). Analyzed nutrient content of the faba bean grain cultivars either planted and harvested EARLY or LATE is shown in Table 1. Bulk density and proportion of frost-damaged (blackened hull) and immature (green

Table 1. Analyzed nutrients, antinutritional factors content, gross energy value, and particle size of 3 faba bean cultivars planted and harvested either EARLY or LATE[1] (as is).

	Snowbird		Snowdrop		Fabelle	
	Early	Late	Early	Late	Early	Late
Analyzed nutrients, %						
Moisture	14.48	12.39	13.05	14.46	13.39	17.29
Starch	36.05	41.92	38.86	36.53	36.40	36.33
Crude protein	23.71	25.35	25.82	25.05	26.18	25.43
Neutral detergent fiber	11.71	10.50	11.83	12.47	11.16	10.20
Acid detergent fiber	10.31	8.84	9.77	10.27	10.23	9.40
Crude fiber	9.16	7.29	7.65	9.10	8.38	8.63
Ash	3.10	3.26	3.25	3.22	2.94	2.86
Crude fat	1.33	1.60	1.25	1.57	1.20	0.99
Indispensable amino acids, %						
Arginine	1.86	1.93	2.08	2.04	2.24	2.42
Histidine	0.58	0.59	0.63	0.57	0.64	0.67
Isoleucine	1.03	1.10	1.07	1.05	1.14	1.15
Leucine	1.74	1.84	1.83	1.75	1.96	1.98
Lysine	1.56	1.59	1.63	1.51	1.68	1.71
Methionine	0.16	0.18	0.21	0.20	0.20	0.20
Phenylalanine	1.03	1.11	1.09	1.05	1.17	1.15
Threonine	0.80	0.81	0.85	0.78	0.86	0.88
Tryptophan	0.20	0.20	0.21	0.20	0.20	0.17
Valine	1.09	1.17	1.16	1.15	1.24	1.25
Dispensable amino acids, %						
Alanine	0.95	1.19	1.04	1.29	1.12	1.12
Aspartic acid	2.50	2.53	2.60	2.47	2.67	2.81
Cysteine	0.27	0.26	0.30	0.28	0.25	0.31
Glutamic acid	3.81	4.00	4.12	3.76	4.26	4.48
Glycine	0.98	0.97	1.05	0.98	1.06	1.11
Proline	0.99	1.03	1.04	1.01	1.06	1.13
Serine	0.97	1.03	1.03	0.98	1.06	1.10
Tyrosine	0.77	0.78	0.77	0.81	0.86	0.82
Gross energy, MJ/kg	15.82	16.29	16.15	15.99	16.37	15.64
Antinutritional factors, g/kg						
Proanthocyanidins[2]	0.24	ND[3]	0.22	0.16	6.84	4.61
Degree of polymerization	6.42		6.82	6.75	6.06	6.58
Vicine	5.3	4.4	5.4	3.8	0.6	0.7
Standard deviation	0.10	0.06	0.15	0.12	0.02	0.01
Convicine	1.6	1.2	1.7	1.1	0.1	0.1
Standard deviation	0.00	0.06	0.06	0.06	0.01	0.01
Particle size, μm	1088	809	864	802	942	872
Standard deviation	2.32	2.54	2.32	2.43	2.41	2.30

[1]Half of the seed of each cultivar was sown in early May, chemically desiccated in mid-September, and harvested in late September (EARLY), whereas the other half was sown in late May, desiccated early October (LATE), and harvested in late October to purposely increase the proportion of frost-damaged beans. Snowbird and Snowdrop were white-flowered, zero-tannin cultivars whereas Fabelle was a color-flowered cultivar, low in vicine and convicine content.

[2]Condensed tannins plus monomeric flavan-3-ols.

[3]Not detected (≤0.05 g/kg).

and soft) beans per planting and harvesting occasion are listed in Table 2. To quantify frost damage, a sample was spread on a tabletop, 100 beans were separated by riffle cuts as conducted in grain grading and the number of beans that had high (blackened hull), intermediate, low or no damage were counted. The same 100 beans were broken apart (cut through) and marked as immature (if they were green and soft when rolled), or normal (if they were yellowish or white and hard).

Diets (Table 3) consisted of 1 of the 6 faba bean cultivar × planting and harvesting combinations (95% inclusion) and included vitamins and minerals to meet the

Table 2. Bulk density and proportion of frost damaged (blackened hull) and immature (green and soft) beans of 3 faba bean cultivars planted and harvested either EARLY or LATE[1] (as is).

	Snowbird		Snowdrop		Fabelle	
	Early	Late	Early	Late	Early	Late
Bulk density, g/L	794.0	680.0	808.4	652.2	722.4	578.1
Frost damage[2], %						
High	6	25	13	14	0	6
Intermediate	14	58	23	74	5	23
No/low damage	80	17	64	12	95	71
Maturity[3], %						
Immature	5	64	7	79	22	80
Normal	95	36	93	21	78	20

[1]Half of the seed of each cultivar was sown in early May, chemically desiccated in mid-September, and harvested in late September (EARLY), whereas the other half was sown in late May, desiccated early October (LATE), and harvested in late October to purposely increase the proportion of frost-damaged beans. Snowbird and Snowdrop were white-flowered, zero-tannin cultivars whereas Fabelle was a color-flowered cultivar, low in vicine and convicine content.

[2]To quantify frost damage, a sample was spread on a tabletop, 100 beans were separated by riffle cuts as conducted in grain grading and the number of beans that had high (blackened hull), intermediate, low or no damage were counted.

[3]The same 100 beans were broken apart (cut) and marked as immature (if they were green and soft when rolled), or normal (if they were yellowish or white and hard).

nutrient requirements according to age as per the Ross 708 broiler nutrition specifications (Aviagen, 2019). The seventh was a nitrogen (**N**) free diet with ingredient composition based on that reported by Adedokun et al. (2015) but with cornstarch:dextrose ratio increased to 1:1 to facilitate cold pelleting. Indigestible marker (0.3% TiO_2), minerals and micro ingredients were weighed separately and then combined before adding to diets to ensure proper mixing. Diets were mixed in a 60-kg capacity, stainless steel mixer (model PB35, A&M Process Equipment Ltd., Ajax, Ontario, Canada) at the University of Alberta Environment and Metabolism Unit. The N-free diet was pelleted through a flat die pellet press (Model PM30, Buskirk Engineering; Ossian, IN) and then crumbled in a tandem twin roller mill (model CHD 8.5 × 12, Iowa Farm Automation Ltd, Stanley, IA). Faba bean diets were fed as dry mash whereas the N-free diet was fed as crumbles.

From placement, chicks had ad libitum access to 2 commercial starter diets sequentially (21% CP Poultry Starter, HI-PRO Feeds, Sherwood Park, Alberta, Canada; 19% CP CO-OP Chick Starter, Federated Co-operatives Ltd., Saskatoon, Saskatchewan, Canada).

Animals and Experimental Design

In total, 756 male Ross 708 broiler chickens (Avigen, Huntsville, AL; Lilydale hatchery, Spruce Grove, Alberta, Canada) originating from the same flock and hatched on the same day were used in the experiment. On d 0, chicks were randomly distributed among 56 pens, 13 to 14 chicks per pen. The 56 pens were divided into 7 area blocks of 8 pens each by location along the rectangular room. Each block included feeding each faba

Table 3. Ingredient composition (as fed), analyzed nutrient content (standardized to 88% dry matter), and particle size of test diets including 1 of 3 faba bean cultivars planted and harvested either EARLY or LATE[1,2], and nitrogen (N)-free diet[3].

	Snowbird		Snowdrop		Fabelle		
	Early	Late	Early	Late	Early	Late	N-free
Ingredients, %							
Snowbird, early harvest	95.23	-	-	-	-	-	-
Snowbird, late harvest	-	95.23	-	-	-	-	-
Snowdrop, early harvest	-	-	95.23	-	-	-	
Snowdrop, late harvest	-	-	-	95.23	-	-	-
Fabelle, early harvest	-	-	-	-	95.23	-	-
Fabelle, late harvest	-	-	-	-	-	95.23	-
Dextrose	-	-	-	-	-	-	41.80
Cornstarch	-	-	-	-	-	-	41.80
Solkafloc (cellulose)	-	-	-	-	-	-	5.00
Canola oil	-	-	-	-	-	-	5.00
Mono-/di-calcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	2.50
Limestone	1.70	1.70	1.70	1.70	1.70	1.70	1.40
Salt	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Broiler premix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Potassium chloride	-	-	-	-	-	-	0.50
Magnesium oxide	0.20	0.20	0.20	0.20	0.20	0.20	0.35
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride 60%	0.075	0.075	0.075	0.075	0.075	0.075	0.15
Analyzed nutrient content, %							
Crude protein	24.02	23.83	24.34	24.26	25.69	24.91	0.48
Crude fiber	6.99	9.52	8.50	8.49	7.09	11.74	1.06
Crude fat	0.79	0.66	0.55	0.91	0.42	0.51	3.89
Ash	7.48	7.23	7.24	7.50	6.69	7.09	4.76
Titanium	0.183	0.166	0.174	0.184	0.157	0.189	0.171
Indispensable amino acids, %							
Arginine	1.92	1.78	2.01	1.94	2.34	2.16	0.03
Histidine	0.59	0.55	0.61	0.55	0.64	0.57	0.01
Isoleucine	1.06	0.99	1.05	1.00	1.10	1.06	0.03
Leucine	1.78	1.68	1.77	1.67	1.91	1.80	0.04
Lysine	1.58	1.46	1.56	1.45	1.64	1.51	0.03
Methionine	0.16	0.14	0.17	0.16	0.16	0.14	0.01
Phenylalanine	1.06	1.02	1.05	1.01	1.11	1.07	0.02
Threonine	0.80	0.74	0.81	0.74	0.85	0.76	0.02
Tryptophan	0.20	0.18	0.20	0.18	0.18	0.17	0.02
Valine	1.12	1.08	1.13	1.09	1.21	1.14	0.03
Dispensable amino acids, %							
Alanine	0.96	1.12	1.01	1.23	1.07	1.29	0.03
Aspartic acid	2.53	2.36	2.50	2.37	2.69	2.59	0.04
Cysteine	0.26	0.26	0.29	0.26	0.29	0.24	0.01
Glutamic acid	3.93	3.71	4.01	3.64	4.35	4.04	0.07
Glycine	0.99	0.92	1.02	0.94	1.06	0.97	0.02
Proline	0.98	0.90	0.96	0.91	1.02	0.96	0.04
Serine	0.98	0.95	0.98	0.96	1.06	1.01	0.02
Tyrosine	0.77	0.75	0.76	0.77	0.81	0.82	0.01
Gross energy, MJ/kg	13.296	13.683	13.564	13.239	13.749	12.925	13.702
Particle size, μm	811	658	812	762	640	828	1,827
Standard deviation, μm	2.54	2.78	2.63	2.67	2.84	2.60	2.38

[1]Half of the seed of each cultivar was sown in early May, chemically desiccated in mid-September, and harvested in late September (EARLY), whereas the other half was sown in late May, desiccated early October (LATE), and harvested in late October to purposely increase the proportion of frost-damaged beans. Snowbird and Snowdrop were white-flowered, zero-tannin cultivars whereas Fabelle was a color-flowered cultivar, low in vicine and convicine content.

[2]Faba bean diets were fed from d 15 to 23 or 24.

[3]Nitrogen-free diet was fed from d 20 to 24 to calculate endogenous amino acid losses.

[4]Trouw Nutrition (Ponoka, Alberta, Canada). Provided the following per kg of feed: retinyl acetate 3440 ug, cholecalciferol 100 μg, dl-alpha tocopheryl acetate 50 mg, thiamine 4 mg, riboflavin 10 mg, pantothenic acid 15 mg, biotin 0.2 mg, folic acid 2 mg, vitamin B_{12} 0.02 mg, niacin 65 mg, pyridoxine 5 mg, menadione 4 mg, manganese 120 mg, iron 80 mg, copper 20 mg, zinc 100 mg, selenium 0.3 mg, iodine 1.65 mg.

bean diet to 1 pen and the N-free diet to 2 pens, distributed per block as a randomized complete block design for a total of 7 replicate pens per treatment. Digesta of the 2 pens fed N-free diet per block were combined, resulting in 7 replicates for the N-free diet as well. Birds were weighed as groups by pen. On d 15, 42 of the 56 pens were switched to feed 1 of the 6 faba bean diets. To minimize feeding a protein-depleted diet for too long, 14 pens stayed on the commercial starter diet until they were switched to the N-free diet on d 20. In the morning of d 23 (blocks 1 to 4) or d 24 (blocks 5 to 7), individual birds were euthanized by cervical dislocation. The ileum was excised by making cuts approximately 25-mm distal to the Meckel's diverticulum and approximately 25-mm proximal to the ileocecal junction. Digesta was collected by gently squeezing out the ileal content into plastic bags and stored in a −20°C freezer. Digesta from all birds in a pen was pooled to obtain 1 sample per pen.

Samples of the 2 pens within block fed the N-free diet were also combined to obtain enough digesta for analyses. Digesta samples were lyophilized using a freeze-dryer at the University of Alberta main campus (Edmonton, Alberta, Canada) and were then sent for laboratory analyses.

Measurements and Calculations

Throughout the trial, broilers found dead, ill, or injured were promptly removed, euthanized, and individually weighed, and the suspect reason for death or removal was written down.

The apparent ileal digestibility (**AID**) of GE, CP, and AA in diets was calculated using Equation 1 (Stein et al., 2007):

$$AID,\ \% = \left[1 - \left(\frac{\%\ Nutrient_{digesta}}{\%\ Nutrient_{assay\ diet}}\right) \times \left(\frac{\%\ Marker_{assay\ diet}}{\%\ Marker_{digesta}}\right)\right] \times\ 100 \quad (1)$$

Where AID is the digestibility of a nutrient in the diet as %, % $Nutrient_{digesta}$ is the proportion of a nutrient in digesta, % $Nutrient_{assay\ diet}$ is the proportion of a nutrient in the assay diet, % $Marker_{assay\ diet}$ is the proportion of marker in the assay diet, and % $Marker_{digesta}$ is the proportion of marker in digesta. Mean Ti of test diets (except N-free) was used for calculations instead of Ti of individual diets as premix added to test diets was the same.

The endogenous flow, also known as the endogenous losses of CP and each AA at the terminal ileum was calculated from feeding the N-free diet using Equation 2 (Stein et al., 2007):

$$IEAA,\ g/kg\ DMI = AA_{digesta},\ g/kg \times [(Marker_{diet},\ g/kg)/(Marker_{digesta},\ g/kg)] \quad (2)$$

Where IEAA is the ileal endogenous CP or AA content in g per kg of DM intake, $AA_{digesta}$ is the content of CP or AA in digesta in g per kg, $Marker_{diet}$ is the content of marker in the diet in g per kg, and $Marker_{digesta}$ is the content of marker in digesta in g per kg DM.

Standardized ileal digestibility (SID) was calculated for CP and each AA using Equation 3 (Lemme et al., 2004):

$$SID, \% = AID,\ \% + \left[100 \times \left(\frac{IEAA,\ \ g/kg\ of\ DMI}{CP\ or\ AA\ diet,\ \ g/kg\ of\ DM}\right)\right] \quad (3)$$

Where SID is the SID of CP or AA as %, AID is the AID of CP or AA as %, IEAA is the ileal endogenous of CP or AA in g per kg of DM intake, and CP or AA_{diet} is the content of CP or AA in the diet in g per kg DM.

Lastly, the SID of the different faba bean cultivars was adjusted by accounting for the minor difference in DM content between the diet and the test ingredient as per Equation 4:

$$SID, \% = SID_{diet}, \% \times \left(\frac{DM_{diet}, \%}{DM_{ing},\ \ \%}\right) \quad (4)$$

Where SID is the SID of CP or AA as % in the test ingredient, SID_{diet} is the SID of CP or AA as % in the diet, DM_{diet} is the proportion of DM in the diet as %, and DM_{ingr} is the proportion of DM in the test ingredient as %.

Chemical Analyses

Faba bean, diets, and lyophilized digesta samples were ground through a 0.5-mm screen in a centrifugal mill (ZM 200, Retsch GmbH, Haan, Germany). Samples were analyzed at the Agricultural Experimental Station Chemical Laboratories (University of Missouri, Columbia, MO) using the Association of Official Analytical Chemists (AOAC, 2006) methods unless stated otherwise. Faba bean, diet, and digesta samples were analyzed for DM (method 934.01), CP (method 990.03), and AA (method 982.30 E [a, b, c]) content. Titanium content was measured in diets and digesta samples according to the method described by Myers et al. (2004). Moreover, faba bean and diet samples were analyzed for crude fat (method 920.39 (A)), ash (method 942.05), and crude fiber (**CF**; method 978.10) content. Faba bean samples were also analyzed for starch (assay kit STA-20; Sigma, St. Louis, MO), acid detergent fiber (**ADF**; method 973.18 [A-D]), and neutral detergent fiber (**NDF**; Holst, 1973).

GE for faba bean, diets, and digesta samples was measured in duplicate using bomb calorimetry (Model 6050, Parr Instrument Company, Moline, IL) utilizing benzoic acid as a standard. Faba bean and diet particle size was determined using a Ro-Tap (model RX-29, W.S. Tyler, Ontario, Canada) equipped with 13 sieves and a pan following the method of American Society of Agricultural and Biological Engineers (2008). Faba bean samples were analyzed for vicine and convicine content using a slight modification of the extraction procedure described by Purves et al. (2018) (Organic Residue Laboratory, Alberta Agriculture and Forestry, Edmonton, AB, Canada) as described by Cho et al. (2019).

Faba bean samples were also analyzed at the Natural Resources Institute Finland (LUKE; Jokionen, Finland) for proanthocyanidins (**CT**; mostly condensed tannin plus some monomeric flavan-3-ols) using HPLC after thiolytic degradation as described by Ivarsson and Neil (2018).

Statistical Analyses

Data residuals were tested for normality using the Univariate procedure and for homogeneity of variance using the Bartlett and Levine test in SAS Ver. 9.4 (SAS Institute, Cary, NC). The AID and SID of nutrients were analyzed as a 3 × 2 factorial arrangement with a generalized linear mixed model (GLIMMIX procedure) using a normal distribution and the identity link function. Individual birds were the sampling unit but pen was the experimental unit for all variables. The model included cultivar (Snowbird, Snowdrop, and Fabelle), planting and harvesting time (EARLY and LATE), and interaction as fixed effects; block was the random term in the model. Mean separation was conducted using the PDIFF option in the LSMEANS statement. Treatment differences were considered significant if $P < 0.05$, and a trend if $P < 0.10$.

RESULTS

Nutrient Content of Faba Bean Cultivars

Faba bean from the 3 different cultivars planted and harvested either EARLY or LATE were close to each other in analyzed nutrient content with some exceptions (Table 1): LATE harvested Fabelle had greater moisture content than other combinations. LATE harvested Snowbird had greater starch content than other combinations. Both EARLY and LATE harvested Fabelle had slightly greater AA content than Snowbird and Snowdrop. Fabelle contained more proanthocyanidins and less vicine and convicine than Snowbird or Snowdrop regardless of planting and harvesting time.

Bulk density was lower for LATE vs. EARLY faba bean for all cultivars (Table 2). LATE planting and harvesting also increased the proportion of intermediate and high frost-damaged (blackened hull) beans, as well as the proportion of immature (green and soft) beans compared with EARLY harvested faba bean regardless of cultivar. Fabelle had lower bulk density, lower proportion of frost-damaged beans, and a greater proportion of immature beans compared with Snowbird and Snowdrop.

Nutrient Digestibility

There were no interactions between faba bean cultivar and timing of planting and harvesting. Therefore, both fixed effects are presented separately (Table 4). Faba bean cultivar did not affect the AID of GE and CP of diets. Diets including LATE had greater AID of GE and CP than diets including EARLY planted and harvested faba bean.

The SID of CP, Thr, and Ala was not affected by faba bean cultivar. For all other AA, Fabelle had lower SID than Snowbird and Snowdrop except for Trp, for which

Table 4. Apparent ileal digestibility (AID) of gross energy (GE) and crude protein (CP) in diets including 3 faba bean cultivars planted and harvested either EARLY or LATE[1], and standardized ileal digestibility (SID)[2] of CP and amino acids (AA) of 3 faba bean cultivars planted and harvested either EARLY or LATE.

	Cultivar				Planted and harvested			*P* value		
	Snowbird	Snowdrop	Fabelle	SEM[3]	Early	Late	SEM	Cultivar	Harvest	Interaction
AID of diets, %										
GE	31.78	33.74	36.22	2.09	27.71	40.12	1.71	0.3331	<0.0001	0.5393
CP	66.99	67.25	67.07	1.33	63.01	71.20	1.08	0.9894	<0.0001	0.9015
SID of faba bean, %										
CP	70.69	70.96	70.81	1.34	66.47	75.16	1.09	0.9902	<0.0001	0.8682
Indispensable AA										
Arg	88.75[a]	89.07[a]	85.98[b]	0.68	84.96	90.91	0.59	0.0007	<0.0001	0.8833
His	82.01[a]	80.69[a]	75.19[b]	1.03	76.72	81.88	0.91	<0.0001	<0.0001	0.6514
Ile	78.36[a]	77.44[a]	73.79[b]	1.14	71.73	81.33	0.99	0.0064	<0.0001	0.9494
Leu	81.23[a]	80.69[a]	76.67[b]	1.07	74.64	84.42	0.94	0.0020	<0.0001	0.9840
Lys	84.93[a]	84.16[a]	80.04[b]	1.03	79.98	86.11	0.91	0.0005	<0.0001	0.6623
Met	64.77[a]	67.36[a]	56.12[b]	2.35	59.17	66.33	2.06	0.0008	0.0034	0.1105
Phe	81.97[a]	81.23[a]	76.32[b]	1.05	75.26	84.41	0.93	0.0001	<0.0001	0.9941
Thr	73.77	72.77	70.26	1.41	68.22	76.31	1.23	0.1123	<0.0001	0.7060
Trp	76.43[b]	76.56[b]	86.74[a]	1.40	75.94	83.88	1.23	<0.0001	<0.0001	0.4324
Val	76.69[a]	76.09[a]	72.73[b]	1.20	70.09	80.25	1.04	0.0266	<0.0001	0.9443
Dispensable AA										
Ala	80.10	81.12	78.47	1.00	73.83	85.96	0.87	0.1200	<0.0001	0.8809
Asp	81.72[a]	81.14[a]	77.79[b]	0.97	76.28	84.16	0.85	0.0040	<0.0001	0.9204
Cys	50.45[a]	49.88[a]	39.33[b]	1.87	45.49	47.61	1.53	0.0002	0.3355	0.7094
Glu	87.63[a]	87.59[a]	84.30[b]	0.72	83.38	89.63	0.63	0.0006	<0.0001	0.7681
Gly	66.17[a]	66.92[a]	60.83[b]	1.77	60.66	68.62	1.44	0.0416	0.0005	0.7714
Pro	77.55[a]	76.75[a]	72.17[b]	1.08	71.23	79.74	0.93	0.0007	<0.0001	0.7243
Ser	79.73[a]	78.62[ab]	76.11[b]	1.07	74.06	82.25	0.92	0.0333	<0.0001	0.9662
Tyr	80.15[a]	77.49[b]	74.47[c]	1.10	74.92	79.81	0.96	0.0006	<0.0001	0.9143
Total AA	79.85[a]	79.43[a]	76.29[b]	1.01	74.48	82.56	0.87	0.0164	<0.0001	0.9910

[a,b,c]Least squares means within a row without a common superscript differ ($P < 0.050$).

[1]Half of the seed of each cultivar was sown in early May, chemically desiccated in mid-September, and harvested in late September (EARLY), whereas the other half was sown in late May, desiccated early October (LATE), and harvested in late October to purposely increase the proportion of frost-damaged beans. Snowbird and Snowdrop were white-flowered, zero-tannin cultivars whereas Fabelle was a color-flowered cultivar, low in vicine and convicine content.

[2]Ileal endogenous losses (g/kg): CP 8.11, Arg 0.28, His 0.14, Ile 0.32, Leu 0.48, Lys 0.38, Met 0.09, Phe 0.29, Thr 0.44, Trp 0.06, Val 0.48, Ala 0.32, Asp 0.62, Cys 0.25, Glu 0.72, Gly 0.40, Pro 0.44, Ser 0.41, Tyr 0.20, total AA 7.34.

[3]Standard error of the mean.

Fabelle had greater SID than Snowbird and Snowdrop. Snowbird and Snowdrop had no different SID for all AA except for Tyr, for which Snowdrop had lower SID than Snowbird. LATE planted and harvested faba bean had greater SID of CP and all AA than EARLY planted and harvested faba bean, except for Cys, for which timing of planting and harvesting had no effect (Table 4).

DISCUSSION

Bean Maturity and Frost Damage

The objective of planting and harvesting faba beans either EARLY, which was in fact the recommended times for this location, or 2 to 3 wk later (LATE) was to produce 1 batch of each cultivar that would be of high quality and 1 batch that would be of lower quality due to frost exposure. Because of an unseasonably cold and wet summer, even the EARLY planted faba bean was affected by frost. Depending on pod height on the plant, frost exposure affects beans differently. Beans in pods at the top of the plant are more exposed to frost, even more in clear nights. Beans in pods at the bottom of the plant protected by biomass above, keep warm by radiated heat from the soil at night. Biomass density, spacing between plant rows, daytime heating, wind velocity, and humidity affect radiated heat from the soil, thus more heat is trapped among these tall plants at night (Henriquez et al., 2018). That is the reason why frost-damaged beans are not visually uniform having beans ranging widely in color from light gray to black. These beans can be color sorted by optical scanners to maximize economic return. Generally, early and intermediate maturing cultivars have less damaged beans after frost exposure than late maturing cultivars (Henriquez et al., 2018). Indeed, in our study planting and harvesting faba bean LATE considerably increased the proportion of frost damage and immature beans for all cultivars. It seems that frost interrupted bean ripening on the field, and this happened to a greater extent for LATE than EARLY planted faba bean, resulting in more blackened hulls, as well as green and soft immature cotyledons at harvest time. Despite frost exposure even to EARLY planted and harvested beans, we did manage to create 2 distinct batches of faba bean that differed in quality, with the LATE planted and harvested batch having a greater proportion of frost damage and immature beans.

The proportion of visually frost-damaged beans was remarkably lower for Fabelle than Snowbird and Snowdrop, which was likely because of the frost-protective effect of tannins. Their activity as a supercooling promoting agent or anti-ice nucleating agent likely prevented intracellular ice formation and therefore a blackened hull (Koyama et al., 2014). Henriquez et al. (2018) also found that tannin-containing cultivars showed lower proportions of frost-damaged beans when compared with zero-tannin cultivars, even after accounting for differences in bean crop maturity. Fabelle had greater proportion of immature (green and soft) cotyledons than Snowbird and Snowdrop beans, likely because Snowbird and Snowdrop are early maturing cultivars vs. Fabelle, which is a medium maturing cultivar (Alberta Agriculture and Forestry, 2019).

Nutrient Quality of Faba Bean

Although Snowbird and Snowdrop are considered zero-tannin faba bean cultivars, some proanthocyanidins were found in Snowdrop and the early harvested Snowbird. As expected, Fabelle, which is known as a tannin-containing cultivar, had much greater proanthocyanidin content than Snowbird and Snowdrop beans. The proanthocyanidin content of Fabelle beans was within the expected range for tannin-containing cultivars (Duc et al., 1999; Mayer Labba et al., 2021). Fabelle was developed as a low vicine and convicine cultivar. Indeed, vicine and convicine content were low for both the early and late planted and harvested Fabelle. In contrast, both Snowbird and Snowdrop beans had greater vicine and convicine content within the range reported by Duc et al. (1999) and Mayer Labba et al. (2021).

Planting and harvesting faba beans 2 to 3 wk later than recommended practice not only increased the proportion of frost damage and immature cotyledons, but also resulted in lower proanthocyanidin content in all faba bean cultivars, and lower vicine and convicine content in Snowbird and Snowdrop. The degree of polymerization of condensed tannins in faba bean changes during seed development presumably related to bird and pest repellence. Condensed tannins in the testa reached maximum content around 30 d after flowering and then declined (Martín et al., 1991). Proanthocyanidins oxidation is related to the darkening of the seed coat of some faba bean and dry beans like pinto and cranberry beans that grade lower and are less marketable than non-darkened counterparts. Testa proanthocyanidin levels increased with plant maturation in a darkening-susceptible cranberry bean recombinant inbred line (**RIL**), whereas these metabolites were absent in seeds of the non-darkening RIL plants (Freixas Coutin et al., 2017). Anthocyanins content was enriched substantially in the last red-to-black stage and peaked in full ripe blackberries whereas proanthocyanidin content gradually decreased (Chen et al., 2012). Reports on how fall frost affects tannin, vicine and convicine content of faba bean at harvest time are lacking in the literature.

Nutrient Digestibility

There was no effect of cultivar on AID of GE despite differences in bean tannin and vicine and convicine content. Both ANF have been shown to affect energy digestibility (Vilariño et al., 2009; Crepón et al., 2010). Vilariño et al. (2009) showed that AMEn was reduced by 0.5 MJ/kg DM for faba bean averaging 9.9 vs. 1.3 g/kg tannin content and by 0.35 MJ/kg DM for faba bean averaging 10.1 vs. 0.7 g/kg vicine and convicine content; the negative effects of both ANF on energy were additive. Snowbird and Snowdrop beans had high vicine and convicine content and low tannin content, whereas for Fabelle it was the opposite. The AID of GE may have been affected in Snowbird and Snowdrop beans by their vicine and convicine content and in Fabelle by its tannin content, resulting in no difference among cultivars.

The AID of GE was greater for LATE vs. EARLY planted and harvested beans because of disparity in bulk density and potentially the less ripe LATE planted and harvested cotyledons may have had a greater proportion of highly digestible starch (e.g., lower amylose: amylopectin ratio), mono- or disaccharides (glucose, fructose, and sucrose) and a lower proportion of the non-digestible oligosaccharides (raffinose, stachyose, and verbascose) than EARLY planted and harvested beans (Landry et al., 2016). Indeed, Hejdysz et al. (2016) showed negative correlations between the AME_n of faba bean and content of oligosaccharides ($r = -0.80$) or raffinose ($r = -0.79$). They also reported similar negative correlations with AID of DM, starch, CP, and AA. α-Galactosides accumulate during the final stage of ripening to stabilize cell membranes at desiccation and again when the seed rehydrates during germination (McPhee et al., 2002) but vanish soon after (Guillon and Champ, 2002). Stachyose and verbascose content in faba bean can decrease under optimal irrigation (Szukala et al., 2001). The lower proportions of proanthocyanidin content in all cultivars and lower vicine and convicine content in Snowbird and Snowdrop in LATE vs. EARLY planted and harvested beans likely played a role in improving energy digestibility. No other effects of frost and stage of grain maturity on energy and nutrient digestibility of pulses for poultry could be found.

The SID of Met and Cys were lower than those reported by AMINOD at 5.0 (Evonik Degussa GmbH; Hanau-Wolfgang, Germany) and by Witten et al. (2018) but were similar to those reported by Masey O'Neill et al. (2012) and Olukosi et al. (2019). Witten et al. (2018) reported much greater SID of AA in faba bean compared with SID they cited from the literature, partially because they used the regression method to estimate endogenous losses rather than feeding a N-free diet as per Olukosi et al. (2019) and the current trial. Using the regression method generally results in greater estimates of endogenous losses than feeding a N-free diet (Lemme et al., 2004).

Lower SID for most AA in Fabelle vs. Snowbird or Snowdrop grain was likely due to Fabelle's condensed tannin content given its low vicine and convicine content. Condensed tannins reduce both protein and AA digestibility in broiler chickens by interfering with digestive enzyme activity and by forming tannin-protein complexes (Vilariño et al., 2009). These authors showed that AID of CP was reduced from 86 to 75% for faba bean with 1.3 vs. 9.9 g/kg tannin content and found no interaction with vicine and convicine content. Lower SID coefficients in Fabelle vs. Snowbird and Snowdrop grain were offset by a greater AA content (not shown), resulting in similar SID content in Fabelle as Snowbird and Snowdrop for most AA. Fabelle had greater SID content (not shown) of arginine and leucine than Snowbird and Snowdrop grain.

Two major classes of protein are typically found in pulse grains: albumins and globulins. Albumins encompass functional proteins like amylase inhibitors, enzymatic proteins, lectins, and protease inhibitors, whereas legumin and vicilin are the main storage globulins (69.5 −78.1% in faba bean; Boye et al., 2010). The proportion of albumins:globulins and legumin:vicilin vary with cultivar. Pulse protein is high in Lys, Leu, Asp, Glu, and Arg, but low in Met, Cys, and Trp (Crepón et al., 2010; Mayer Labba et al., 2021). Burbano et al. (1995) showed that vicine and convicine levels were highest in young green cotyledons of faba bean but then content declined to a constant level when seed achieve approximately 40% moisture. The faba bean we fed to broilers in this experiment ranged from 12 to 17% moisture. Agronomic conditions, location, and ripening stage affect AA content (Boye et al., 2010). No information on how frost or stage of grain maturity affect CP and AA digestibility in pulses for poultry could be found.

The SID for most AA in EARLY planted and harvested faba bean were similar to those observed by Masey O'Neill et al. (2012) except that the SID of Thr was lower and that of Trp was greater in the current trial than theirs. Compared with the SID given in AMINODat 5.0, EARLY planted and harvested faba bean was lower for Ile, Lys, Met, Cys, Thr, and Val, similar for Arg, His, Leu, and Phe and greater for Trp. However, SID of LATE planted and harvested faba bean were greater than those given by AMINODat 5.0 except for Met and Cys. This information is of practical relevance to animal nutritionists, who should adjust their SID feed formulation matrix when a batch of faba bean has blackened hulls and immature cotyledons.

Although LATE planted and harvested faba bean had greater digestibility, this does not imply that the seed embryo was spared from frost damage. Frost damaged beans would likely have poor germination if planted as seed, which is a good reason why it would make sense to feed frost damaged faba bean to poultry instead. Care should be taken regarding the extent of frost damage. These results should not encourage the feeding of extremely damaged, heated or rotten beans to poultry, as predictable growth performance would not be guaranteed. It would be recommended instead to limit inclusion level or dilute a batch of heavily frost-damaged, immature beans with beans that show no frost damage.

CONCLUSIONS

Compared with planting and harvesting faba beans at the recommended time for the region (early May), delaying planting and harvesting by 2 to 3 wk resulted in beans with greater proportions of frost-damaged (blackened) hull and immature grain (green and soft) cotyledons. Late planted and harvested faba bean had both lower condensed tannin and vicine and convicine content than early planted and harvested faba bean.

Contrary to that expected, the results of this digestibility trial indicate that LATE vs. EARLY planting and harvesting faba bean increased GE, CP, and AA digestibility possibly by frost interrupting bean ripening on the field. Tannin content may have reduced the AA digestibility of Fabelle compared with Snowbird or Snowdrop faba bean cultivars.

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DISCLOSURES

The authors declare that no financial or other contractual agreements exist that might cause conflicts of interest or be perceived as causing conflicts of interest.

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