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Effects of different grain types on nutrient apparent digestibility, glycemic responses, and fecal VFA content in weaned foals

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Abstract

Background China's equine industry has shifted from traditional rough grazing to modern intensive farming, expanding the roles of horses into eventing, leisure, tourism, and meat and dairy production. Concurrently, equine nutrition has evolved from a forage-based diet to a more diverse regimen incorporating grain supplements to meet the heightened energy demands of intensive farming. However, nutrient digestibility and glycemic response vary considerably based on grain type, starch content, composition, and structural properties. Optimal grain selection is therefore essential for energy supplementation across developmental stages to sustain growth and performance. This study examines the impact of diets incorporating steam-flaked grains (corn, oats, and barley) on apparent nutrient digestibility, glycemic response, and fecal volatile fatty acid (VFA) composition in the weaned Kazakh foals.

Methods Eighteen male Kazakh foals, weaned at 5 months, were randomly assigned to three groups ($n = 6$ per group) based on grain type: corn group (CG), oats group (OG), and barley group (BG). The daily starch intake for the foals was set at 2 g starch (DM)/kg body weight per day to determine the amount of concentrate supplements to be fed, based on the principle of equal grain starch intake over a 60-day feeding trial.

Results Results indicated that the apparent nutrient digestibility was lower in OG than in CG and BG ($P > 0.05$). However, amylose intake and digestibility were significantly higher in OG compared to CG ($P < 0.01$). Plasma glucose and glucagon levels were elevated in CG relative to OG and BG ($P < 0.01$), while the insulin/glucose ratio was highest in the BG. Additionally, BG increased fecal lactic acid and total VFA (TVFA) concentrations while reducing fecal pH.

Conclusions For weaned Kazakh foals, steam-flaked corn could be recommended in advance of steam-flaked oats and barley in cereal-based energy supplementation alongside basal forage diets. It may reduce amylose intake, improve glycemic responses, increase plasma glucose levels and reduce fecal lactic acid content.

Keywords Grains, Weaned foals, Nutrient digestibility, Glycemic response, VFA

Background

Artificial weaning, commonly practiced in stall-confined horses, involves separating foals from their mothers earlier and more abruptly than natural weaning, typically between 4 and 7 months of age [1, 2]. This process represents one of the most stressful periods in a horse's life, influencing feeding behavior, physiological and emotional responses, immune function, and overall growth and development [3, 4]. Following weaning, the foal's diet shifts from mare's milk and fodder to complete

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forage and concentrate supplements [5]. Given their rapid growth and high energy demands, foals require a diet with a higher caloric density [6]. which is typically achieved through grain supplementation [7]. As herbivorous non-ruminants with cecal fermentation [8], horses exhibit limited starch digestion due to the relatively low activity and concentration of starch-digesting enzymes in the small intestine of *Equus* animals [9]. Consequently, unprocessed grain starch, irrespective of grain type is not efficiently hydrolyzedfby digestive enzymes and instead reaches the large intestine for fermentation [10]. This process promotes the proliferation of amylolytic bacteria, such as *Lactobacillus* and *Streptococcus*, increasing lactic acid concentrations, lowering pH, and reducing cellulolytic bacteria populations. The resulting hindgut acidosis predisposes horses to colic, laminitis, and impaired fiber utilization, ultimately compromising overall health [11]. Maximizing starch digestibility in the small intestine is therefore essential. Studies have shown that thermal processing (including cooking, baking, puffing, steam-flaking and steam-explosion) can gelatinize grain starch and increase its enzymatic capacity, thereby improving starch digestibility in the small intestine [12]. Starch types and structure influence postprandial glycemic and insulin responses [13]. With blood glucose and insulin concentrations serving as key indicators of starch digestibility [13]. Hoekstra et al. [14] reported that compared to crushed corn, steam-pressed corn induces a higher glycemic response in horses due to starch gelatinization during heatin.

Current research on weaned foals primarily explores the weaning methods and the effects of weaning on behavioral and physiological characteristics, with limited studies on the apparent nutrient digestibility and the glycemic responses to processed grains. The Kazakh horse, an indigenous breed from Xinjiang, China, is valued for its meat and milk production [15]. Common dietary energy supplements include grains such as corn, oats, barley, and wheat. This study aimed to assess the effects of steam-flaked cereal-based diets (corn, oats, and barley) with equivalent starch content in the concentrate supplements on the apparent nutrient digestibility, glycemic response, and fecal volatile fatty acid (VFA) composition in weaned Kazakh foals. And to reveal the effects of these cereal grains on the digestive physiological parameters of weaned foals.

Materials and methods

Animals and experimental design

In this study, 18 healthy Kazakh equine male foals born in March 2019 (± 5 d, selected on the basis of the foal's birth record), an average weight of 112.4 ± 7.75 kg, were selected. They were weaned at 5 months of age (August 2019) and had no history of systemic disease. All foals originated from the same local pasture and were clinically healthy at the time of the inspection. The experiment was conducted from August to October 2019 for 60 days. The foals were divided into three dietary groups based on the source of the grains in their concentrate supplement: corn group(CG), oats group(OG), and barley group(BG), each with six foals. Before the start of the experiment (day 0) and on day 30, the foals were weighed on an empty stomach before the morning feed and the daily concentrate supplement was calculated based on their body weight. On day 45 of the experiment, jugular vein blood samples were collected from the foals at different time points before and after feeding to assess the glucose, insulin, glucagon and lactic acid levels. On days 50–55 of the experimental, a 6-d collected total faecal digestion experiment was conducted to evaluate the apparent digestibility of dietary nutrients in foals (Fig. 1).

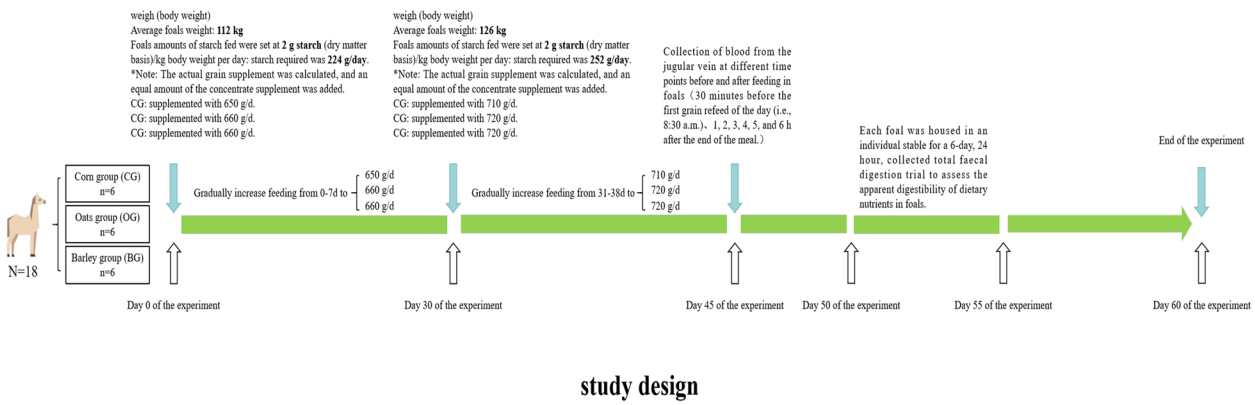

Fig. 1 Study design

Experimental diets

The foals were provided a diet comprising concentrate supplements and forage grass, formulated according to the NRC (2007) nutritional guidelines for horses [16]. To ensure equivalent starch intake, the daily starch allowance was set at 2 g starch (DM)/kg body weight [17]. Before experiment commencement (day 0), foals had an average body weight of 112 ± 7.75 kg, establishing a target daily starch intake of 224 g. The required grain supplement was calculated accordingly, with additional nutrients balanced to maintain dietary consistency. During days 0–30 of the experiment, foals in the CG, OG, and BG were fed concentrate supplements containing 650 g of steam-flaked corn, 660 g of steam-flaked unhulled oats, and 660 g steam-flaked of barley, respectively. From days 0 to 7, the concentrate supplement was incrementally increased to reach the target intake. By day 30, foal body weight averaged 126 ± 7.52 kg, necessitating an adjusted daily starch intake of 252 g. The grain supplement was recalculated, with additional nutrients adjusted accordingly. From days 31 to 60, foals in the CG, OG, and BG were fed concentrate supplements containing 710 g of steam-flaked corn, 720 g of steam-flaked unhulled oats, and 720 g steam-flaked of barley, respectively. With a gradual increase in concentrate intake from days 31 to 38 to meet the final target. The composition and nutritional profile of the concentrate supplements are detailed in Table 1.

Steam flaking of grains was conducted by Junli Agriculture and Animal Husbandry Technology Co., Ltd. (Zhaosu County, Xinjiang, China). Corn, unhulled oats, and barley underwent tempering in a steam box at 95 ℃ for 60 min, maintaining a moisture content of ≤ 14% and a final flake thickness of 2.5 mm. After pressing, the grains were subjected to hot-air drying, yielding a final density of 360 g/L.

The roughage consisted of alfalfa hay and grass hay of local origin, fed as a mixture at a 2:1 ratio, chopped to 3–5 cm. Foals were allowed free access to roughage, and the daily feeding amount is set at 1.5 times their actual intake to ensure an adequate supply. Details on forage intake and nutritional composition are presented in Tables 2 and 3.

Table 1 Composition and nutrition level of concentrate supplement

The raw material (%)	CG	OG	BG
Steam-flaked corn	60	–	–
Steam-flaked oats	–	66	–
Steam-flaked barley	–	–	66
Soybean meal	36	30	30
Calcium hydrogen phosphate	2	2	2
Limestone	1	1	1
Premix[a]	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Total	100.00	100.00	100.00
Nutrition levels[b] (Dry Matter, DM basis)			
Dry Matter, DM (%)	92.44	93.28	91.93
Organic Matter, OM (%)	94.46	93.32	93.90
Crude Protein, CP (%)	26.26	26.09	26.86
Gross Energy,GE (MJ/kg)	18.25	18.29	18.26
Starch (%)	37.68	37.00	38.27
Neutral Detergent Fiber, NDF (%)	24.46	27.04	24.79
Acid Detergent Fiber, ADF (%)	5.10	9.09	5.71
Calcium, Ca (%)	1.06	1.45	1.14
Phosphorus, P (%)	0.94	1.02	1.02

CG Corn group ($n = 6$), *OG* Oats group ($n = 6$), *BG* Barley group ($n = 6$)

[a] The premix provided the following per kg of concentrate supplement: Vitamin A 3000 IU, Vitamin B_1 20 mg, Vitamin B_2 20 mg, Vitamin B_6 6 mg, Vitamin C 20 mg, Vitamin D 1000 IU, Vitamin E 500 IU, Pantothenic acid 10 mg, Nicotinamide 100 mg, Cu 25 mg, Fe 107 mg, Mn 81 mg, Zn 74 mg, I 6 mg, Se 14 mg, Co 3 mg, Choline chloride 120 mg

[b] Nutrition levels were measured values

Table 2 Nutrient levels of forage grass (Dry Matter, DM basis)

Nutrient[a]	Hay	Alfalfa
DM (%)	89.75	87.56
OM (%)	91.80	92.21
CP (%)	10.75	20.56
GE (MJ/kg)	18.93	19.53
Starch (%)	3.63	4.46
NDF (%)	60.00	52.60
ADF (%)	41.82	45.21
Ca (%)	0.33	1.50
P (%)	0.27	0.25

CG Corn group ($n = 6$), *OG* Oats group ($n = 6$), *BG* Barley group ($n = 6$)

[a] Nutrition levels were measured values

Feeding management

All foals underwent deworming with commercial-grade ivermectin before the experiment. Throughout the 60-day study, each foal received its daily concentrate supplement in three equal portions at 09:00, 15:00, and 21:00, administered via individually assigned horse muzzle feed bag. From 09:00 to 21:00, foals roamed freely in an 80 m × 40 m outdoor dry-land activity field, where they had unrestricted access to clean water and forage. From 21:00 to 09:00, each foal was kept in a separate stable (4 m × 3 m, stables with wood shavings as bedding), where forage and water were provided in a trough. During the fecal collection period (days 50–55), each foal was stabled all day.

Table 3 DM, Digestible Energy, and Starch Intake

Item		CG	OG	BG	SEM
Concentrate	Intake (kg/d)	0.71	0.72	0.72	0.00
	DM intake (kg/d)	0.66	0.67	0.66	0.00
Forage grass	Intake (kg/d)	5.62	5.52	5.67	0.22
	DM intake (kg/d)	4.96	4.87	5.00	0.19
Total DM intake (kg/d)		5.62	5.54	5.67	0.19
GE intake (MJ, DM basis)		107.90	106.44	108.79	3.66
Fecal energy (MJ, DM basis)		44.59	48.59	48.49	1.60
DE[a] (MJ/kg, DM basis)		11.16	10.41	10.60	0.46
Total starch intake (g/d, DM basis)		454.73	451.86	462.37	7.93
Amylopectin intake (g/d, DM basis)		318.78	326.72	329.16	5.12
Amylose intake (g/d, DM basis)		28.55[C]	40.19[A]	37.77[B]	0.11

CG Corn group ($n=6$), *OG* Oats group ($n=6$), *BG* Barley group ($n=6$)

SEM standard error of means

[a] DE = (GE intake-fecal energy)/total DM intake

[A, B, C] Mean values with no same superscript in a row were highly significantly different ($P<0.01$)

Sample collection and analysis

Blood sampling and analysis

On day 45, blood samples were collected to assess glucose and insulin responses pre- and post-feeding. An intravenous catheter (Baihe Medical Technology Co., Ltd., Guangdong Province, China) was inserted into the jugular vein following local neck disinfection, prior to morning concentrate feeding (before 09:00). Blood samples were drawn at baseline (30 min before feeding, 08:30) and at 1, 2, 3, 4, 5, and 6 h post-feeding. At each preset time point, 6 mL of blood was collected and divided into two vials. For plasma glucose analysis, 3 mL of was transferred into 5 mL sodium fluoride tubes (Kangjian Medical, Jiangsu Province, China), centrifuged at 1500 ×g for 10 min, and the plasma was aliquoted into two Eppendorf tubes (Eppendorf [Shanghai] International Trade Co., Ltd., Shanghai, China). For insulin, glucagon, and lactic acid measurements, 3 mL was collected in an anticoagulant-free container, centrifuged under identical conditions, and the serum was separated into two aliquots. All samples were stored at − 80 ℃ for subsequent analysis.

Plasma glucose levels were determined using a glucose kit (glucose oxidase method; A154 - 1–1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based assay. Serum lactic acid concentration was quantified via a colorimetric lactate assay kit (A019 - 2–1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Thawed serum samples were mixed with the reaction solution and incubated at 37 ℃ for 10 min, and absorbance was measured at 530 nm with a 1 cm optical path length.

Serum insulin and glucagon concentrations were measured using commercial ELISA kits (insulin: H203 - 1–2; glucagon: H183;Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Samples were thawed, mixed with the respective reaction solutions, and incubated at 37 ℃ following the manufacturer's protocols. Absorbance was measured at 450 nm using an ELISA reader (DG5033 A ELISA instrument; Nanjing Huadong Electronics Group Medical Equipment Co, Nanjing, China).

Concentrate supplements, forage, and fecal sample collection

During days 50–55 of the experiment, each foal was housed individually in a stable (4 m× 3 m, with stall mats used as bedding), Throughout this six-day period, all fecal samples were collected for the digestion experiment. Additionally, 100 g of feed samples were obtained from each grain group's concentrate supplement and stored in sealed bags for subsequent chemical analysis. Forage samples, consisting of a 1:1 mixture of alfalfa and pasture hay, were also collected using a five-point sampling method. Before morning feeding (09:00) each day, the total amount of feed and concentrate supplement provided to each foal was weighed. At the same time the following morning, any leftover feed from each foal's trough was collected, weighed, and used to calculate daily feed intake. Since each foal's concentrate supplement was divided into three equal feedings and all were consumed, no leftovers were observed. After collection, forage samples were air-dried under ambient conditions (20–25 ℃, 40–60% relative humidity) until they reached equilibrium moisture content. The dried weight was then recorded, and the samples were sealed for later chemical analysis. Fecal collection was carried out daily using custom-made nylon collection bags that did not

hinder the foals'movement or ability to lie down. The total fecal output was weighed each day over the six-day period. Samples were homogenized, and 5 g of feces was immediately diluted in 15 mL of distilled water (1:3 ratio of feces to water). This mixture was stirred for 3–5 min at room temperature [18]. Fecal pH was then measured using a portable pH meter (FiveEasy22-Meter, Mettler-Toledo International Trading [Shanghai] Co.), calibrated to an accuracy of 0.01. The average pH over the six-day collection period for each foal was recorded as the final fecal pH. Additionally, 200 g of fecal subsamples were collected daily in sterile plastic containers and stored at − 20 ℃. At the end of the fecal sampling period, the 6-d fecal subsamples from each foal were homogenized. Two copies of 100 g subsamples were taken; one was stored at − 20 ℃ for nutrient analysis, and the other at − 80 ℃ for VFA analysis.

Chemical analysis of forage, concentrate, and fecal samples

Chemical analyses of all samples in this study were carried out at the Laboratory of Herbivore Nutrition for Meat & Milk Production (Autonomous Region Key Laboratory, Xinjiang Urumqi, China).

Fecal subsamples, stored at − 20 ℃, were thawed, transferred to an aluminum box, and dried in an electric thermostatic blast drying oven at 65 ℃ for 48 h. The collected concentrate supplements, forage, forage leftovers, and dried feces were then pulverized using a multi-functional high-speed grinder(400 g upright type; Tohe Electromechanical Technology [Shanghai] Co., Ltd., Shanghai, China) and sieved through a 1 mm mesh. Subsequently, the dry matter (DM), crude ash (ash), and crude protein (CP) contents were determined using the international AOAC method [19] in conjunction with the Chinese national standard methods for the measurement of conventional nutrient contents [20]. Gross energy (GE) were measured using a highly accurate calorimeter (OR2014, Shanghai Ou Rui Instrument Equipment Co., Ltd., Shanghai, China). Organic matter (OM) content was calculated using the equation: OM% = (DM%—Ash%). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified using the method outlined by Van Soest et al. [21] with an automated fiber analyzer (ANKOM-2000, USA; Shanghai Longjie Instrument Equipment Co., Ltd., Shanghai, China).

The total starch content of the samples was determined using acid hydrolysis combined with the anthrone colorimetric method [22]. Crushed concentrate, forage, forage residue, and fecal samples were extracted with 80% ethanol in a water bath at 80 ℃ for 30 min to separate soluble sugars and starch. The extracts were then cooled and centrifuged at 3000 × g for 10 min, after which the supernatant was discarded. The precipitate was gelatinized in a water bath at 95 ℃ for 15 min, cooled, and subsequently hydrolyzed with concentrated sulfuric acid at 95 ℃. Anthrone coloring solution was added to the reaction mixture and incubated for 10 min before cooling. The solution was centrifuged at 3000 × g for 10 min, and the supernatant was collected for analysis. Absorbance content assessed was measured at 620 nm using a spectrophotometer to determine the total starch content. Amylose content was assessed using iodine colorimetry combined with a single-wavelength method [23]. Samples were extracted with 80% ethano at 80 ℃ for 30 min to isolate soluble sugars and starch, then centrifuged at 3000 × g for 5 min at 25 ℃, and the supernatant was discarded. Ether was added to remove lipids, followed by shaking for 5 min and another centrifugation under the same conditions. The supernatant was discarded, and the precipitate was treated with 0.5 mol/L KOH solution at 95 ℃ for 10 min. After cooling, the solution was acidified with 0.1 mol/L hydrochloric acid (HCl) to pH 3.5, treated with iodine reagent, and analyzed spectrophotometrically at 620 nm to determine amylose content. Amylopectin concentration was determined using iodine colorimetry combined with a dual-wavelength method [23]. Sample processing followed the amylose determination procedure, with final absorbance readings taken at 550 and 743 nm to quantify amylopectin content.

Fecal VFA concentrations, including acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate, were quantified using gas chromatography. Fecal subsamples stored at − 80 ℃, were thawed and homogenized. prior to analysis. A 10 g fecal subsample was mixed with 10 mL of ultrapure water, vortexed at 4 ℃ for 30 min, and filtered through four layers of gauze. The filtrate was centrifuged at 5000 × g for 10 min, and the supernatant was incubated at 4 ℃ for 12 h. Following incubation, 0.6 mL of the supernatant was combined with 0.6 mL of 19% trichloroacetic acid (Sigma Aldrich [Shanghai] Trading Co.) and 0.1 mL of a 60 mmol internal standard solution (crotonic acid, Sigma Aldrich [Shanghai] Trading Co.). The mixture was vortexed, left to stand at 4 ℃ for 20 min, and then centrifuged at 20,000 × g for 15 min. A 1 μL aliquot of the resulting solution was used for chromatographic analysis. VFA quantification was performed using a gas chromatograph (Agilent 7890 A, Agilent Technologies [China] Ltd., Beijing, China) equipped with an HP-FFAP capillary column (50 m × 0.20 mm i.d. × 0.33 μm film thickness, Agilent Technologies [China] Ltd., Beijing, China). Chromatographic conditions included a temperature gradient of 10 ℃/min, increasing from 60 ℃ to 220 ℃ over 12 min. The injector and detector temperatures were maintained at 240 ℃ and 280 ℃, respectively. Nitrogen was used as the carrier gas at a flow rate of 5.0 mL/min.

Statistical analysis

The data obtained from assessing nutrient digestibility, starch digestibility, fecal VFA and pH of foals were analyzed as a complete randomized design using the General Linear Models (GLM) procedure in SAS software (version 8.1, SAS Inst.Inc., Cary, NC, USA), based on the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is observation (apparent nutrient digestibility, starch digestibility, fecal VFA, and pH), μ is the general mean, T_i is the effect of sources of grains in the experimental diet, and e_{ij} is the standard error term.

Glycaemic response data were analyzed as repeated measurements using the MIXED procedures of SAS, based on the following statistical model:

$$Y_{ijk} = \mu + T_i + H_j + (TH)_{ij} + e_{ijk}$$

Where Y_{ijk} is observation (glycemic response), μ is the general mean, T_i is the effect of sources of grains in the experimental diet, H_j is the effect of sampling hours, $(TH)_{ij}$ is the interaction between the effect of experimental diets and sampling hours and e_{ijk} is the standard error of term. Means were compared by the Duncan multiple comparison tests at $P < 0.05$.

Changes in plasma glucose, insulin, glucagon, and lactate concentrations were calculated for each postprandial period. The areas under the curve (AUC) of the postprandial glucose, insulin, glucagon, and lactic acid responses (and respective incremental responses) were calculated by the trapezoidal numerical integration method using the software GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as P value < 0.05 and the difference was highly significant at P value < 0.01.

Results

Apparent nutrient digestibility

The results of feed intake and apparent nutrient digestibility are shown in Fig. 2. There was no significant difference in the intake and digestibility of different nutrients between the grain groups ($P > 0.05$) (Table 3, Fig. 2).

Total starch and amylopectin intake and digestibility did not differ significantly among dietary treatments ($P > 0.05$) (Table 3, Fig. 2G, H). However, amylose intake was significantly higher in OG compared to CG and BG ($P < 0.01$), and in BG compared to CG ($P < 0.01$) (Table 3). Amylose digestibility was also higher in OG and BG than in CG ($P = 0.0001$) (Fig. 2I).

Glycemic responses

As shown in Table 4, postprandial glycemic response varied significantly among grain groups, as indicated by differences in glucose, insulin, glucagon, and lactic acid levels ($P < 0.05$ or $P < 0.01$). Plasma glucose concentration was higher in CG than in BG ($P < 0.01$), while serum insulin levels were elevated in the BG compared to CG and OG ($P = 0.0039$). Serum glucagon levels were significantly higher in CG than in OG and BG ($P < 0.01$), whereas serum lactic acid concentration was higher in BG than in CG ($P = 0.0301$).

The response on different grain sources in post-meal levels of mean glucose, insulin, glucagon, and lactic acid in weaned foals are shown in Fig. 3. Glucose levels in the plasma of foals peaked at 1 h post-meal in OG and BG, whereas in CG, the peak occurred at 2 h. At 2, 3 and 4 h post-meal, the mean glucose concentrations were significantly higher in CG than in BG ($P < 0.01$; $P < 0.05$, respectively) (Fig. 3A).

Serum insulin levels peaked at 2 h post-meal in the OG of foal and at 3 h in CG and BG of foals. However, no significant differences in insulin response were detected between dietary treatments at any time point (Fig. 3B).

Serum glucagon concentrations across the dietary treatment groups reached their lowest point at 1 h post-meal, peaking at 5 h post-meal. At 6 h post-meal, the mean glucagon concentration was significantly higher in the CG of foal compared to the BG of foal ($P < 0.05$). Serum glucagon concentrations in CG foal were consistently higher than those in OG and BG foals ($P > 0.05$) (Fig. 3C).

In all dietary treatment groups, foal serum lactic acid levels were lowest at 2 h post-meal, peaking at 5 h post-meal. Throughout the 3–6 h postprandial period, serum lactic acid concentrations were consistently higher in BG foals than in CG and OG foals ($P > 0.05$) (Fig. 3D).

Table 5 presents the area under the curve (AUC) values (mean ± standard error) for each glycemic indicator based on different dietary treatments and time points. The glucose AUC was significantly higher in CG compared to OG and BG, with OG showing a higher AUC than BG ($P < 0.01$). The insulin AUC was higher in BG than in CG and OG ($P < 0.01$). The glucagon AUC was significantly higher in CG than inOG and BG ($P < 0.01$), and the lactic acid AUC was higher in BG than in CG and OG ($P < 0.01$).

Insulin-to-glucose ratio (Insulin/Glucose, I/G)

The insulin/glucose ratio is depicted in Fig. 4. After a meal, this ratio declined sharply before rising again, reaching its lowest point at 1 h postprandially in all diet-treated group. From 1 to 6 h post-meal, the I/G

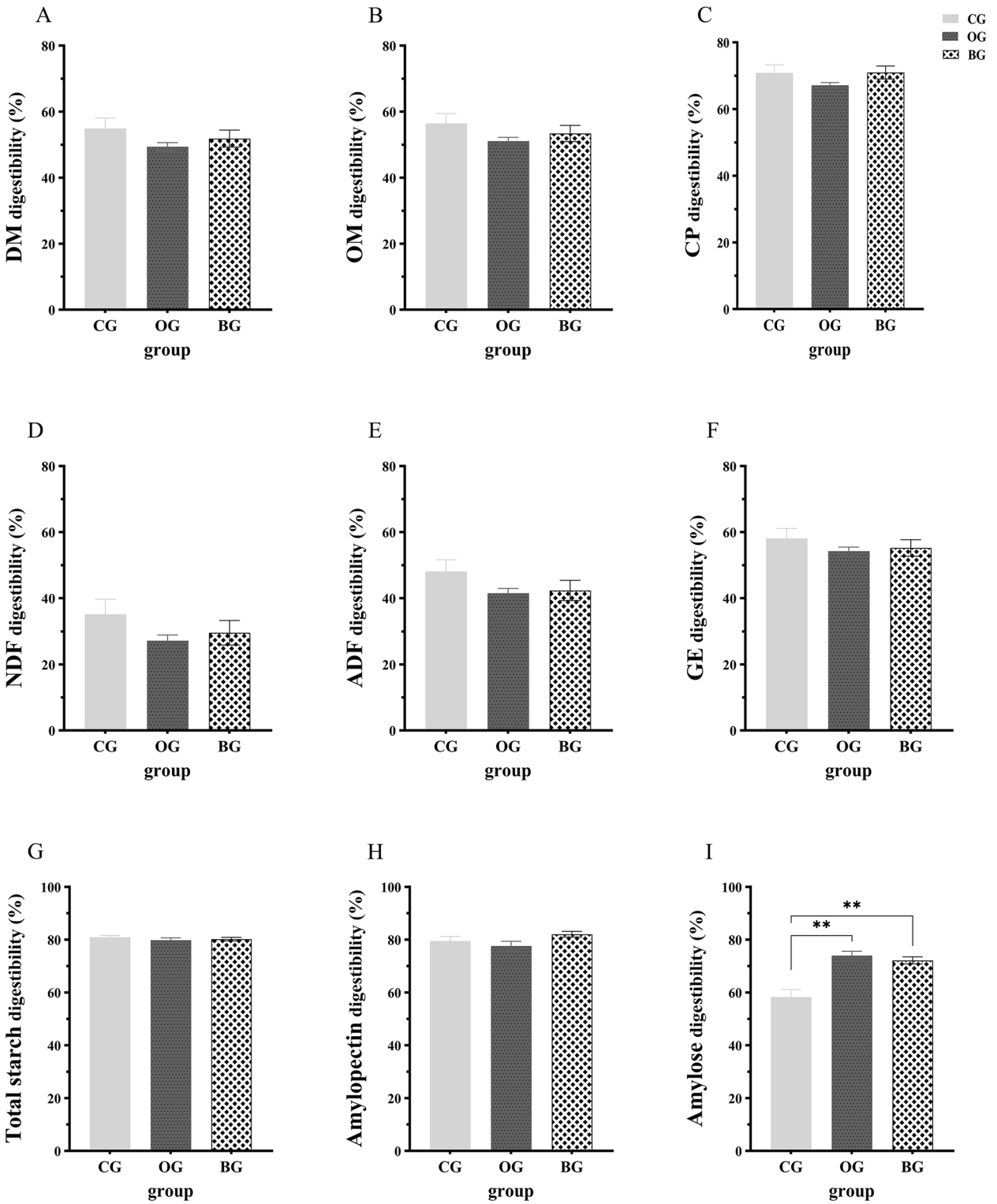

Fig. 2 Effect of different steam-flaked grains on the apparent nutrient digestibility of weaned foals (Means ± standard error) (DM basis). Dry matter (DM) digestibility (**A**); organic matter (OM) digestibility (**B**); crude protein (CP) digestibility (**C**); neutral detergent fiber (NDF) digestibility (**D**); acid detergent fiber (ADF) digestibility (**E**); gross energy (GE) digestibility (**F**); total starch digestibility (**G**); amylopectin digestibility (**H**); amylose digestibility (**I**). Each bar represents mean ± standard error of the mean (SEM). ** $P < 0.01$. CG: Corn group ($n = 6$); OG: Oats group ($n = 6$); BG: Barley group ($n = 6$). Note: Total starch, amylopectin and amylose are actual values measured using to different methods

Table 4 Effect of different steam-flaked grains on plasma glucose, serum insulin, glucagon and lactic acid in weaned foals

Item	Diet			SEM	*P* value		
	CG	OG	BG		Treatment diets	Date	Treatment diets*Date
Glucose (mmol/L)	7.31^{A}	7.10^{AB}	6.51^{B}	0.13	< 0.0001	< 0.0001	0.2475
Insulin (mIU/L)	12.43^{B}	12.62^{B}	13.51^{A}	0.24	0.0039	< 0.0001	0.9531
Glucagon (ng/L)	325.91^{A}	268.18^{B}	259.39^{B}	11.30	< 0.0001	< 0.0001	0.9954
Lactic acid (mmol/L)	2.78^{b}	2.84^{ab}	3.10^{a}	0.09	0.0301	0.0007	0.5487

CG Corn group ($n = 6$), *OG* Oats group ($n = 6$), *BG* Barley group ($n = 6$)

SEM standard error of means

$^{a, b}$ Mean values with no same superscript in a row were significantly different ($P < 0.05$)

$^{A, B}$ Mean values with no same superscript in a row were highly significantly different ($P < 0.01$)

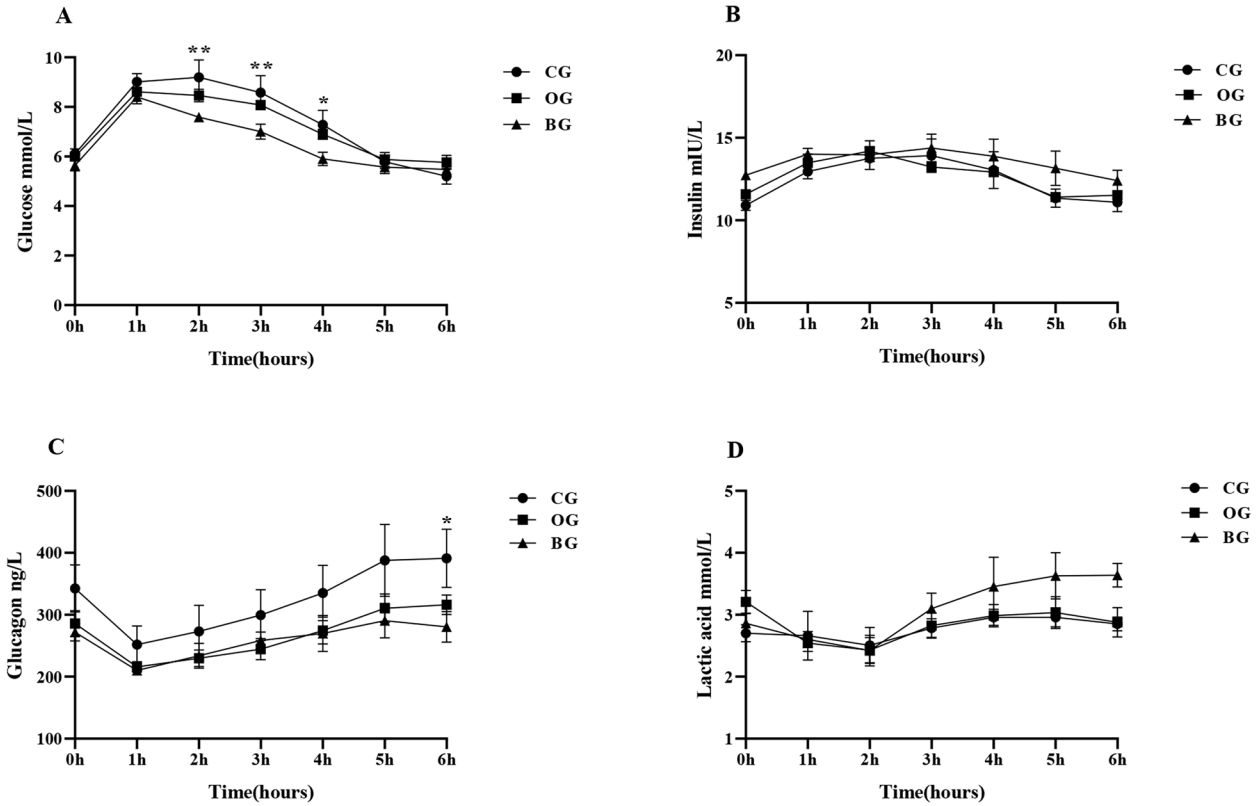

Fig. 3 Curves of the effect of different steam-flaked grains on postprandial plasma glucose, serum insulin, glucagon and lactic acid in weaned foals (Means ± standard error). Changes in mean plasma glucose level (**A**), mean serum insulin level (**B**), mean serum glucagon level (**C**) and mean serum lactic acid level (**D**) in the blood of weaned foals at different time points after feeding different grains. CG: Corn group ($n = 6$); OG: Oats group ($n = 6$); BG: Barley group ($n = 6$). * indicates that the CG was significantly higher than the BG ($P < 0.05$); ** indicates that the CG was highly significantly higher than the BG ($P < 0.01$)

ratio in BG was higher than in CG and OG. Notably, at 3 and 5 h post-meal, the I/G ratio in BG surpassed that in both CG and OG ($P < 0.05$). At 4 h post-meal, I/G in BG was significantly higher than in CG and OG ($P < 0.01$) (Fig. 4).

Fecal pH and VFA levels

Table 6 shows the impact of different grain sources on the fecal VFA composition and pH of foals. The inclusion of different grain sources did not significantly alter fecal pH, acetate, isobutyrate, valerate, isovalerate, or

Table 5 Effect of different steam-flaked grains on the area under the curve of plasma glucose, serum insulin, glucagon and lactic acid in weaned foals

Item		Diet			*P* value
		CG	OG	BG	
Glucose AUC_{0-6h}, mmol/L × hours	Mean[a]	45.51 ± 2.16[A]	43.82 ± 0.93[B]	40.03 ± 1.08[C]	< 0.0001
	range	41.27 to 49.75	42.00 to 45.63	37.92 to 42.13	
Insulin AUC_{0-6h}, mIU/L × hours	mean	76.02 ± 3.21[B]	76.78 ± 1.68[B]	81.97 ± 3.19[A]	< 0.0001
	range	69.73 to 82.30	73.48 to 80.08	75.72 to 88.21	
Glucagon AUC_{0-6h}, ng/L × hours	mean	1914 ± 186.40[A]	1576 ± 75.40[B]	1539 ± 89.97[B]	< 0.0001
	range	1549 to 2280	1428 to 1724	1363 to 1716	
Lactic acid AUC_{0-6h}, mmol/L × hours	mean	16.65 ± 0.98[B]	16.86 ± 0.86[B]	18.46 ± 1.27[A]	< 0.0001
	range	14.73 to 18.57	15.17 to 18.55	15.97 to 20.94	

CG Corn group ($n = 6$), *OG* Oats group ($n = 6$), *BG* Barley group ($n = 6$)

AUC Area under the curve

[a] The results of the data are expressed as the mean ± standard error deviation

[A, B, C] Mean values with no same superscript in a row were highly significantly different ($P < 0.01$)

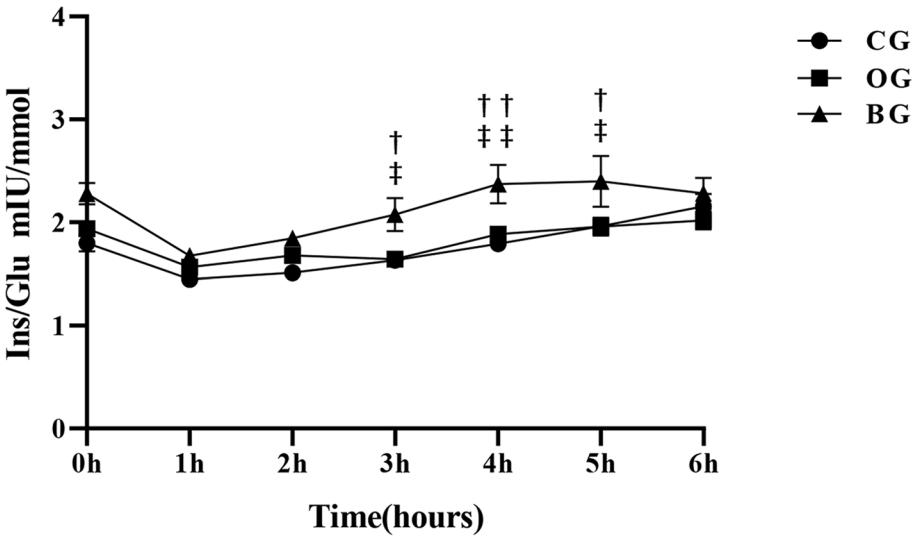

Fig. 4 Effect of different different steam-flaked grains on insulin/glucose ratio in weaned foals. CG: Corn group ($n = 6$); OG: Oats group ($n = 6$); BG: Barley group ($n = 6$). † indicates that the BG was significantly higher than the CG ($P < 0.05$); †† indicates that the BG was highly significantly higher than the CG ($P < 0.01$). ‡ indicates that the BG was significantly higher than the OG ($P < 0.05$); ‡‡ indicates that the BG was highly significantly higher than the OG ($P < 0.01$)

total fecal VFA concentrations in foals ($P > 0.05$). However, fecal pH was lower in the BG compared to the CG and OG ($P > 0.05$). Lactic acid concentrations in feces were significantly elevated in the BG relative to the CG and OG ($P = 0.0053$). Propionate levels were higher in OG than in both CG and BG, while BG exhibited more propionate than CG ($P = 0.0003$). Butyrate concentration was greater in the CG than in BG ($P = 0.0064$). Additionally, the acetate/propionate ratio was significantly higher in the CG than in the OG ($P = 0.0278$).

Discussion

Apparent nutrient digestibility

Horses, as non-ruminant herbivores, are primarily sustained on roughage-based diets, consisting of either fresh or dried forage [24]. However, in intensive management systems where feeding practices shift from continuous grazing to restricted daily rations, or during critical physiological stages (e.g., weaning, growth, gestation, and lactation), insufficient roughage availability or poor forage quality may not meet the equine nutritional requirements of equines. In such cases, additional supplemental

Table 6 Effect of different steam-flaked grains on fecal pH and fecal VFA concentration in weaned foals

Item	Diet			SEM	*P* Value
	CG	OG	BG		
pH	6.51	6.40	6.35	0.13	0.6559
Lactic acid (mmol/g)	0.36^{B}	0.42^{B}	0.63^{A}	0.05	0.0053
Acetate (mol/L)	23.31	25.90	26.47	1.69	0.3942
Propionate (mol/L)	1.86^{C}	2.70^{A}	2.32^{B}	0.11	0.0003
Butyrate (mol/L)	0.42^{A}	0.27^{AB}	0.13^{B}	0.05	0.0064
Isobutyrate (mol/L)	0.57	0.74	0.76	0.07	0.1477
Valerate (mol/L)	0.19	0.14	0.13	0.02	0.2696
Isovalerate (mol/L)	0.65	0.67	0.77	0.08	0.5339
Acetate/propionate	12.67^{a}	9.61^{b}	11.52^{ab}	0.72	0.0278
Total VFA (mol/L)	27.00	30.42	30.57	1.80	0.3128

CG Corn group ($n = 6$), *OG* Oats group ($n = 6$), *BG* Barley group ($n = 6$)

SEM standard error of means

VFA volatile fatty acid

$^{a, b}$ Mean values with no same superscript in a row were significantly different ($P < 0.05$)

$^{A, B, C}$ Mean values with no same superscript in a row were highly significantly different ($P < 0.01$)

feed concentrates are required to meet their nutritional needs [24, 25]. Starch, the primary component of grains, plays a pivotal role in determining the nutritional value of grains in horses, as their digestibility directly impacts their overall nutritional benefit [26]. Several studies have shown that variations in starch content and structure across different grains can influence animal growth performance and nutrient digestibility [27, 28]. Hussein et al [29]. found that when horses were supplemented with different grains (barley, corn, naked oats, and oats) in an alfalfa-cube diet, the apparent nutrient digestibility of DM, OM, CP, ether extract, NDF, and ADF was lower in the oats group compared to the other grain groups. Similarly, Direkvandi et al. [30] reported that replacing more than 60% of the forage in horse diets with oats resulted in reduced cellulose and ADF digestibility. In our study, the apparent nutrient digestibility in the diet of foals in the OG was lower than that of the CG and BG, although the difference was no statistically significant. Additionally, Särkijärvi and Saastamoinen [31] assessed the nutritional value of four oat diets for horses, including untreated oats, hulled oats, autoclave-processed oats, autoclave-processed hulled oats, Dantoaster-processed oats, and Dantoaster-processed hulled oats. Their results indicated that hulled oat had superior feeding value and improved nutrient digestibility compared to untreated oat group. However, thermal treatments (autoclave and Dantoaster processing) did not enhance total tract nutrient digestibility. Our findings align with these previous studies. In our experiment, the oats used were unhulled and underwent steam flaking. Oat hulls typically constitute 21.0% to 24.2% of the whole grain (dry matter) and are almost entirely composed of fiber (84%, dry matter) [32]. The physicochemical properties of fibers, including particle size, hydration, and rheological characteristics (such as viscosity), directly influence the nutrient digestion and the physical properties of chyme. These factors, in turn affect chyme transit, nutrient hydrolysis, and absorption [33]. Studies have shown that increases in NDF and ADF in the diet lead to decreaseed nutrient digestibility in horses [34]. In our study, the OG diet had higher levels of ADF (9.09%) and NDF (27.04%) compared to the other two groups, which may explain the observed results. However, microorganisms in the hindgut (cecum and colon) of foals can ferment fibers to produce VFA, which are absorbed and serve as direct energy sources for the horse [35]. Research indicates that cellulolytic bacteria appear in foals as early as 4–7 days after birth, with foals capable of digesting plant fibers by 2 months of age. The gut microbiota stabilizes and becomes more similar to that of adult horses, particularly in terms of community structure [36–39]. Kalantariet et al. [40] found that increasing concentrate and grain levels (wheat, corn, oats, and barley) in the diet of Turkmen horses improved nutrient digestibility. They also observed that horses fed processed grains (micronized, steam-flake, or extruded) exhibited significantly higher digestibility of OM, CP, NDF, and DE compared to those fed unprocessed grains. Specifically, steam flaking has been shown to enhance digestibility by altering the physical form of the grain, disrupting starch granule structure, and increasing surface area, thus allowing greater interaction with digestive and microbial enzymes in the gastrointestinal tract, which improves pre-caecal utilization [41, 42]. In our study, all grain underwent identical steam-pressing processing, resulting in steam-flaked grains. Consequently, there were no significant differences in the apparent digestibility of nutrients among the foals in each grain group.

Digestibility of starch

The digestive utilization of starch in grains is influenced by factors such as starch structure, the amylopectin-to-amylose ratio, digestive enzyme activity in the animal's intestine, and the processing method [43]. Ma et al. [44] demonstrated that steam-flaked corn contains higher levels of soluble non-starch polysaccharides and exhibits greater starch gelatinization than normal corn, enhancing starch digestion and utilization in growing pigs. White's [45] reported that high-temperature, high-pressure extrusion of wheat resulted in higher starch digestibility in young piglets small intestines compared to raw wheat. This is because steam flaking can release a certain quantity of starch and protein from the starch-protein

matrix and granule structure, resulting in improved starch digestibility [46]. In our study, no significant differences were found in the digestibility of total starch and amylopectin across the three grain types in foals, suggesting that starch digestibility was not affected by grain type when processed similarly. However, our values were lower than those reported by Julliand et al. [41], likely due to differences in breed, age, and physiological stage of the horses in the studies. Foals at the age < 5–6 month of age likely have a digestive system with limited capacity for starch breakdown and uptake of glucose [47]. Notably, in our study, amylose digestibility was significantly higher in the OG compared to the CG and BG. This variability in amylose digestion can primarily be attributed to differences in starch processing, structure, and properties. Oat starch particles are small and typically range from 7.0 to 7.8 μm in size, whereas corn starch particles range from 3 to 14 μm, and barley starch particles range from 6.96 to 30.0 μm [48–50]. Previous studies have shown that under thermal processing conditions, amylose in oat starch particles reorganizes with amylopectin, allowing the starch chains to unfold completely and improve their digestibility [51]. Potter et al. [52] observed higher starch digestibility in oats than in corn and barley, while Shamekh et al. [53] found that, at 95 ℃, the amount of straight-chain starch dissolved in oats exceeded that of other cereals. Therefore, in our study, the higher amylose digestibility observed in the OG likely stems from the smaller size of oat starch granules and the steam-press processing technique (steam temperature > 100 ℃).

Glycemic responses

For equine animals, starch digestion in grains within the stomach and small intestine depends on both the grain source and feed processing methods [41]. The hydrolysis of starch in the gastrointestinal tract results in distinct metabolic products, which vary depending on the anatomical site of digestion and the predominant hydrolytic mechanism—endogenous enzymatic action or microbial fermentation. Enzymatic digestion produces glucose in the stomach and small intestine, whereas microbial fermentation in the large intestine (cecum and colon) primarily generates VFA and lactic acid. For a given cereal, feed processing significantly influences the extent of pre-cecal starch digestion, with the degree of digestion being determined by enzymatic activity and the retention time of the digesta in the foregut [39]. Research has shown that alpha-amylase activity in the equine mouth is minimal, rendering starch digestion in the mouth negligible. However, starch digestibility in the gastrointestinal tract is measurable [54]. Meyer et al. [55] reported that the pre-ileal starch digestibility of oats (whole, rolled) ranged between 76.6% and 90.4%, that of corn (whole, crushed, ground, expanded) between 28.9% and 90.1%, and that of barley (crushed) between 21.5% and 30.3% in small horses, respectively. Insulin, the only hormone with a hypoglycemic effect, suppresses hepatic glucose production postprandially and promotes the uptake and utilization of glucose by tissues.Thus, insulin levels serve as an indicator of blood glucose regulation. As a primary carbohydrate in the diet, the digestion and absorption of grain starch directly impact blood glucose levels and insulin metabolism. In our study, CG foals exhibited higher plasma glucose concentrations (7.31 mmol/L), plasma glucose levels at 0–6 h postprandially, and a larger area under the plasma glucose curve (45.51 mmol/L ×h) compared to OG (7.10 mmol/L, 43.82 mmol/L ×h) and BG (6.51 mmol/L, 40.03 mmol/L ×h) foals. Vervuert et al. [26, 42, 56] confirmed that processing methods (such as untreated, finely ground, micronized, steamed, steam-flaked, or popped) for corn, oats, and barley result in distinct glucose and insulin responses in adult horses. After steam-flaking, the AUC for glucose in plasma followed the order: oats > corn > barley, and the AUC for insulin mirrored this pattern. Furthermore, compared to the untreated group, steam-flaked corn and oats led to a reduction in plasma glucose AUC, while barley showed the opposite trend. Jose-Cunilleras et al. [57] also reported that corn intake caused greater fluctuations in blood glucose compared to oat groats and barley, with blood glucose levels peaking higher in the corn group at 2–3 h post-intake. These findings align with the results of the present study. In equines, the elevated glucose responses observed with corn are attributed to its higher digestibility in the equine digestive tract [58]. Additionally, amylose content has been shown to influence starch digestibility and the efficiency of blood glucose production [59]. Thus, the lower amylose intake in the CG of this study may contribute to the higher plasma glucose production efficiency observed. In our study, serum insulin levels (13.51 mIU/L) and the area under the insulin curve (81.97 mIU/L ×h) were higher in the BG compared to the CG (12.43 mIU/L, 76.02 mIU/L ×h) and OG (12.62 mIU/L, 76.78 mIU/L ×h). Furthermore, the insulin/glucose ratio was consistently higher in the BG at all time points post-meal, with significant differences observed at 3, 4, and 5 h post-meal compared to the CG and OG. Barley-fed foals exhibited heightened insulin sensitivity compared to those fed corn or oats. This enhanced insulin response is likely associated with the high β-glucan content in barley. Barley kernels contain significantly more β-glucan than other cereals, and this component is crucial for influencing its nutritional efficiency [60]. β-glucan in barley has been shown to significantly reduce fasting blood glucose levels and glycated serum proteins in type II diabetic mice, while also

promoting insulin and enteroglucagon secretion. It regulates glucose and lipid metabolism, exerting a hypoglycemic effect [61]. Research shows that upon fermentation by the gut microbiota, β-glucan produces short-chain (volatile) fatty acids (SCFAs), which activate FFAR2 and FFAR3 receptors on small intestinal epithelial cells and pancreatic β-cells. This activation inhibits insulin secretion through coupling with Gi-type G proteins. Subsequently, by regulating the levels of endocrine hormones such as insulin-dependent glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide- 1 (GLP- 1), it can suppress appetite, enhance insulin sensitivity, increasing insulin levels, thereby regulating blood glucose [62–64]. This may explain the potential reason for the high insulin sensitivity observed in the barley-fed foals in our study, which warrants further in-depth investigation. These findings align with those of the present study. In addition, when blood glucose levels drop post-feeding, the pancreas secretes glucagon to stimulate liver glycogen breakdown and provide energy. However, when glycogen stores are insufficient, glucagon promotes the conversion of protein to glucose and fat to glycerol and fatty acids to sustain energy level [65]. In our study, serum glucagon levels and the AUC were significantly higher in the CG than in the OG and BG, consistent with the glycemic response results. This suggests leads to a rapid increase in blood glucose, and the low glucose concentrations at 5–6 h post-feeding likely induce a hypoglycemic state, prompting glucagon secretion to mobilize energy reserves. In contrast, oats and barley facilitate a slower release of blood glucose in the digestive tract, thereby helping to maintain stable blood glucose levels in the foals [30].

Lactic acid is an intermediate product of carbohydrate fermentation in the gastrointestinal tract, typically produced through glycolysis or microbial fermentation [54]. The ability of cereal starches to be hydrolyzed, digested, and absorbed by digestive enzymes in the horse's stomach and small intestine are limited and depends on the starch source [41, 54]. In this study, the amylose content in the OG and BG was higher than in the CG, and higher amylose content generally leads to the formation of more resistant starch. Resistant starch resists enzymatic hydrolysis by digestive enzymes and is not easily digested and absorbed in the small intestine [66]. Furthermore, amylose that has undergone processing-induced denaturation and retrogradation is particularly difficult to hydrolyze due to changes in its spatial structure, which reduces its accessibility to digestive enzymes [67]. This may explain why barley in this study is less hydrolyzed by digestive enzymes in the foal's stomach and small intestine, resulting in a lower glucose production. Conversely, as mentioned above, oats, with their smaller starch granules, enhance the enzymatic digestion during thermal processing, thereby promoting glucose production. This aligns with the results that the plasma glucose levels in foals from the CG and OG were higher than those in the BG. On the other hand, previous studies have shown that starch-degrading bacteria (*Lactobacillus* and *Streptococcus*) exist in the horse's stomach and small intestine [54, 68]. These bacteria can ferment starch that has not been hydrolyzed by digestive enzymes to produce lactic acid. Any undigested starch in the stomach and small intestine is transported to the hindgut (cecum and colon), where it is further fermented by microorganisms generating lactic acid and VFA. In our study, the lactic acid content in the plasma of foals in the BG was higher than in the CG and OG. This suggests that barley, compared with corn and oats, is less efficiently hydrolyzed by digestive enzymes to produce glucose, as reflected by the lower plasma glucose levels in BG foals (Table 4, Table 5). Consequently, a larger proportion of the starch in barley undergoes fermentation in the gastrointestinal tract, producing lactic acid. However, whether this lactic acid contributes to the body's energy metabolism via the oxidative phosphorylation or is converted into glucose and glycogen through the gluconeogenesis remains to be investigated further.

Fecal pH and VFA levels

The equine large intestine (cecum and colon) serves as a key site for microbial fermentation, hosting a diverse microbial community [54]. Undigested dietary starch from the small intestine is transported to the hindgut along with the intestinal chyme, where it undergoes microbial fermentation, leading to the production of VFAs [54]. Research indicates that VFA production in the cecum can meet up to 30% of the horse's energy requirements during maintenance, with additional VFAs generated in the colon [69]. Fecal samples offer an indirect but reliable reflection of the equine hindgut contents (cecum and colon) [70]. Hussein et al. [29] observed that adding barley grain to a diet based on alfalfa cubes (dry matter provision at 1% of BM) resulted in reduced fecal pH and increased total VFA(TVFA) levels in horses compared to corn, naked oats, and oats. In our study, foals in the BG had higher concentrations of lactic acid and TVFA in their feces and lower pH compared to the CG and OG, aligning with the findings of Hussein et al. [29]. However, our results are lower than those reported by Hussein et al. [29]. This may be attributed to the level of starch fed (i.e., 2.0 vs 2.9 g·kg of $BW^{-1}\cdot d^{-1}$), the age-physiological stage (weaned foals vs mature geldings) of the horses, and the type of barley (steam-pressed vs rolled) administered. In addition, fecal lactic acid and TVFA concentrations in the BG of foals were consistent with the above

glycemic response results in this study. This may indicate that compared to corn and oats, there are differences in the utilization pathways of starch in barley in the gastrointestinal tract of foals (more of the amylase digestion shifts towards microbial fermentation). The starch in barley may be limited in its enzymatic digestion in the gastrointestinal tract of foals, thus reducing glucose levels. It is likely that more of the starch that has not been digested by enzymes enters the hindgut and is fermented by microorganisms, thereby increasing the lactic acid and TVFA levels. Notably, butyrate concentrations were significantly higher in the feces of CG foals than in BG foals. Research suggests that butyrate produced in the hindgut (cecum, colon) of mammals can stimulate the growth of hindgut epithelial cells, thereby promoting intestinal health [71]. While fecal VFA concentrations may not precisely reflect actual hindgut VFA concentrations, they can provide valuable information regarding increases or decreases in VFA production [72]. Unfortunately, this aspect was not further investigated in the present study.

Conclusions

In weaned Kazakh horse foals, when designing cereal-based energy supplements to complement basal forage diets, steam-flaked corn is preferentially recommended over steam-flaked oats and barley. This is because steam-flaked corn may reduce the intake of amylose, improve glycemic responses, increase plasma glucose levels, and decrease the lactic acid content in feces.

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Authors' contributions
XinXin Huang., contributed significantly to article conception and design, data acquisition, data analysis and interpretation; XiaoBin Li involved in the critical revision of important knowledge and content in the manuscript; Xuanyue Li, Chao Li, Jiahao Li, Hongxin Jing, Fan Yang, performed animals feed and sample collection. Qian Li and Linjiao He, carried out sample analysis and other related work. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All protocols were approved by the Animal Care and Use Committee of Xinjiang Agricultural University (permission number 2018012). Informed consent—Owners gave informed consent for their animals' inclusion in the study. All methods were carried out in accordance with relevant guidelines and regulations for the use of animal subjects. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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