Received: 14 June 2020 | Revised: 15 August 2020 | Accepted: 3 September 2020

DOI: 10.1111/jpn.13457

ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition | WILEY

Effects of dietary energy on antioxidant capacity, glucose–lipid metabolism and meat fatty acid profile of Holstein bulls at different ages

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Funding information
National Key R&D Program of China, Grant/Award Number: 2018YFD05018000; Key Technology R&D Program of Ningxia, Grant/Award Number: 2017BY078 and 2018BFF02001; National Natural Science Foundation of China, Grant/Award Number: 31802084; China Agriculture Research System, Grant/Award Number: CARS-37.

Abstract

This experiment was conducted to investigate the effects of dietary energy on antioxidant capacity, liver glucose–lipid-related gene expressions and meat fatty acid of Holstein bulls. Thirty-six Holstein bulls (age, 17.0 ± 0.49 months; body weight, 493.3 ± 39.7 kg) were randomly allocated to three dietary treatments. The metabolizable energy of diets was 10.12, 10.90 and 11.68 MJ/kg. Bulls in each dietary treatment were sampled at the age of 20, 23 or 26 months. Results showed that serum glutathione peroxidase and superoxide dismutase decreased with the increasing age. Dietary energy and age had interaction effects on the expressions of fatty acid synthase, peroxisome proliferator-activated receptor alpha, acyl coenzyme A oxidase 1 and carnitine palmitoyl-transferase 1 alpha. Besides, the increase of age and dietary energy increased the expression of liver phosphoenolpyruvate carboxykinase 1. The expressions of liver glucose-6-phosphatase, tumour necrosis factor alpha and sterol regulatory element binding protein 1 increased with the increasing age. The increase of age and dietary energy increased the proportions of C18:1*cis*-9, C18:2n-6*trans* and monounsaturated fatty acid. In summary, the increase of age and dietary energy enhanced the intensity of metabolic changes and inflammatory responses. Dietary energy and age affected the expressions of liver lipid metabolism-related genes, further affected meat fatty acid composition of Holstein bulls.

KEYWORDS
antioxidant capacity, dietary energy, Holstein bull, liver metabolism, meat fatty acid

1 | INTRODUCTION

Increasing numbers of consumers are aware of the links between well-being and diet, leading to the growth of the food market. Saturated fatty acid (SFA) is associated with increased risk of obesity and cardiovascular disease, leading nutrition advisers to recommend a higher intake of polyunsaturated fatty acids (PUFA) (Smit et al., 2009). Ruminant-derived products are characterized by the presence of fatty acids that are mainly derived from the ruminant metabolism of fat, which are not present in the feed (Toral et al., 2018). Different lipid and fatty acid compounds present in ruminant products are indeed potentially negative or positive factors for consumers' health (Salter, 2013). Meat, particularly beef, usually has a negative health image because of its relatively low PUFA and

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high SFA contents (Demeyer & Doreau, 1999). Therefore, replacing SFA with monounsaturated fatty acids (MUFA) and PUFA in ruminant products has been a prime target for animal nutritionists. The concentration of health-promoting fatty acids in beef could be effectively affected by nutrition, age and breed (Smith et al., 2009).

High-concentrate diets are often used to feed finishing bulls to meet the energy requirement for rapid growth and fat deposition. However, feeding diets with high concentrate would lead to rapid ruminal pH reduction, and the prolonged low ruminal pH would promote the release of endotoxin, and further stimulate the release of proinflammatory cytokines (Tian et al., 2019). During an inflammatory response, macrophages and neutrophils will participate in phagocytosis, produce reactive oxygen species (ROS), biosynthesis of oxylipid from the PUFA (Sordillo, 2016). An imbalance between body antioxidants and the ROS may lead to damage in lipids, proteins and DNA and lead to increased level of various metabolites which are final products of lipid peroxidation (Sordillo & Aitken, 2009). Besides, ROS are also important signalling molecules that activate the nuclear factor kappa B (NFκB) pathway and further promote inflammatory response (Abuelo et al., 2016).

In ruminant animals, dietary unsaturated fatty acids are primarily affected by the biohydrogenation within the rumen microbial ecosystem (Jenkins et al., 2008). In addition, unsaturated fatty acids could further be metabolized in tissues (liver and adipose tissues) involved in fatty acids metabolism (Gruffat et al., 2011). The long-chain fatty acids from non-esterified fatty acid of blood and the hydrolysis of triglycerides are subjected to oxidation or esterification in liver (Brzozowska & Oprządek, 2016). Bovine liver plays vital roles in lipid metabolism, especially during periods of lipid metabolism that occur in several nutritional and physiological situations (Bauchart et al., 1996). Consequently, the regulation of liver metabolic pathways may affect fatty acids composition of ruminant products. We previously reported that Holstein bulls fed diet with the metabolizable energy at 10.90 MJ/kg and slaughtered at the age of 26 months or Holstein bulls fed diet with the metabolizable energy at 11.68 MJ/kg and slaughtered at the age of 23 or 26 months have higher growth and slaughter performance, and higher IMF content (Wang, Li et al., 2019), but the metabolism and beef fatty acid are still unknown. Therefore, this experiment was to evaluate the antioxidant capacity and glucose–lipid metabolism and beef fatty acid profiles of Holstein bulls.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sampling

Thirty-six Holstein bulls (initial age, 17.0 ± 0.49 months; body weight, 493.3 ± 39.7 kg) were bought from the Beijing Capital Agribusiness Group (Beijing, China). The bulls were randomly allocated into three diet treatments, and diets were designed according to the nutrient requirements of beef cattle (NRC, 2016) to achieve metabolizable energy contents of 10.12 (low energy, LE), 10.90 (medium energy, ME) and 11.68 (high energy, HE) MJ/kg. Bulls were individually fed the total mixed rations at 07:00 and 16:30, and the dry matter intake was reported in our previous research (Wang, Li et al., 2019). The dietary ingredients and nutrient composition are shown in Table 1. Four bulls from each group were randomly selected for sampling at the age of 20, 23 or 26 months. At each sampling period, before morning feeding, the tail vein blood was collected and centrifuged at 3,000 × *g* for 15 min to separate serum. Liver sample was collected immediately after slaughter and placed in a screw-capped micro-centrifuge tubes containing RNA store reagent. The *longissimus thoracis* samples were collected from the 6th and 7th ribs after being aged for 48 hr.

2.2 | Serum biochemical parameters analysis

The serum biochemical parameters including glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analysed according to the instructions of the commercial test kits (Beijing Strong Biotechnologies).

TABLE 1 Ingredients and nutrient composition of experimental diets

Item	Diets		
	LE	ME	HE
Ingredient (g/kg dry matte basis)			
Corn grain	248.0	407.0	576.0
Distillers dried grain with solute	40.0	45.0	50.0
Extruded soybean	30.4	32.5	35.0
Soybean meal	30.8	20.0	8.1
Corn silage	499.0	365.0	230.0
Wheat straw	81.0	68.0	54.0
Peanut hay	60.0	47.0	26.0
Mineral–vitamin premix[a]	3.6	5.2	6.9
$NaHCO_3$	3.6	5.2	6.9
NaCl	3.6	5.2	6.9
Chemical composition			
Metabolizable energy (MJ/kg)	10.12	10.90	11.68
Organic matter (g/kg DM)	926.4	932.6	939.9
Crude protein (g/kg DM)	106.1	106.2	105.9
Ether extract (g/kg DM)	38.9	40.1	41.4
Neutral detergent fibre (g/kg DM)	383.9	317.1	245.9

Abbreviations: HE, high energy; LE, low energy; ME, medium energy.
[a]The mineral–vitamin premix provides per kg: Fe, 6.75 g; Cu, 2.5 g; Zn, 4.2 g; Mn, 10.34 g; Co, 30 mg; I, 90 mg; Se, 54 mg; vitamin A, 625,000 IU and Vitamin D, 100,000 IU.

2.3 | Antioxidant activity and oxidative stress analysis

Serum ROS and glutathione peroxidase (GSH-Px) were measured according to the kit instruction (Shanghai Enzyme-linked Biotechnology). Serum total antioxidant capacity (TAC) was assayed using the ABTS radical cation. Serum superoxide dismutase (SOD) was measured by a microtiter plate assay using a water-soluble tetrazolium salt (WST-1). Serum catalase activity was assayed according to the method described by Goth (Goth, 1991). The malonaldehyde (MDA) concentration was measured by the thiobarbituric acid method. The oxidative stress index (OSI) was calculated by the formula:

$$\text{OSI(arbitrary unit)} = \text{ROS(IU/ml)}/\text{TAC(mmol Trolox equivalent/L)}$$

2.4 | Gene expression analysis

Total hepatic RNA extraction was performed following the procedure recommended by the EASYspin plus kit (Aidlab Biotechnologies). The NanoDrop 2000 spectrophotometer (Technologies) was used to determine the quantity and purity of RNA. The cDNA was synthesized using a THERMO script RT Kit (Aidlab Biotechnologies). Quantitative analysis of PCR was carried out using a Stratagene MX3000p (Agilent Technologies), and the reaction system was 10 μl of 2 × SYBR SuperReal PreMix Plus (Tiangen Biotech), 0.6 μl of each primer (10.0 μM), 7.8 μl of RNase-free ddH_2O and 1.0 μl of DNA template. The conditions were 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The primer sequence can be seen in Table A1. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of mRNA of the target (Livak & Schmittgen, 2001).

2.5 | Meat fatty acid analysis

Meat fatty acids were measured according to our previously referenced method (Wang, He et al., 2019). Briefly, a glass tube was filled with about 100 mg beef, 4 ml ethyl chloride and methanol (1:10; v/v), and 2 ml margaric acid (1 mg/ml), then heated at 80°C for 2 hr. The glass was cooled then 4 ml potassium carbonate solution (100 g/L) was added, shaken and centrifuged at 3,000 × *g* for 10 min. Finally, the fatty acid methyl ester could be attained from the supernatant.

The fatty acid methyl ester was used in the gas chromatograph analysis. Nitrogen was used as a carrier gas at a pressure of 358.2 K pa. The injection volume was 1 μl. The inlet temperature was 250°C, and detector temperature was 280°C. The initial oven temperature was held at 140°C for 5 min, after that the temperature was increased at a rate of 3.5°C/min to 230°C and held for 15 min. The relative retention times of the detected fatty acids and the standard (18919-1AMP, Sigma Chemical Co.) were used to identify the target fatty acid. The chromatographic peak area and internal standard-based calculations were used to quantify fatty acid methyl ester (Vahmani et al., 2015).

2.6 | Statistical analysis

All data were analysed by the proc GLM of SAS 9.2 (SAS Inst.) using the following model: $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$, where Y_{ij} is dependent variable; μ is population mean; α_i is fixed effect of the diet energy (i = 1, 2 or 3), β_j is fixed effect of the slaughter age (j = 1, 2 or 3), $(\alpha\beta)_{ij}$ is the interaction between diet energy and slaughter age, ε_{ij} is the random error. Least squares means in the event of a dietary energy × age interaction were assessed *post hoc* using the Tukey test. $p < .05$ was considered as significant, and $.05 < p < .10$ was discussed as a tendency.

3 | RESULTS

3.1 | Serum biochemical parameters

As shown in Table 2, dietary energy had no effects on serum biochemical parameters of Holstein bulls. The increase of age increased the concentrations of serum glucose, triglycerides, cholesterol,

TABLE 2 Effects of dietary energy and age on serum biochemical parameters of Holstein bulls

	20			23			26				*p*-value		
Item	LE	ME	HE	LE	ME	HE	LE	ME	HE	*SEM*	Diet	Age	Diet × Age
Glucose (mM)	3.80	3.79	3.85	3.87	4.16	4.46	5.13	5.03	5.28	0.185	.206	<.001	.616
Triglyceride (mM)	0.18	0.17	0.18	0.22	0.20	0.25	0.22	0.23	0.27	0.025	.255	.009	.865
Cholesterol (mM)	2.96	3.39	3.48	4.08	4.27	4.26	3.94	4.02	4.18	0.305	.445	.002	.971
HDL-C (mM)	0.98	1.17	1.13	1.25	1.26	1.35	1.28	1.31	1.39	0.080	.206	.003	.799
LDL-C (mM)	0.60	0.68	0.68	0.85	0.88	0.89	0.80	0.83	0.87	0.062	.470	<.001	.991
ALT (U/L)	17.0	17.5	15.3	16.5	19.8	18.0	16.3	16.5	16.8	1.130	.286	.174	.450
AST (U/L)	67.3	54.8	58.8	66.0	81.0	86.0	75.5	79.0	80.8	5.358	.444	<.001	.084
ALP (U/L)	102.3	107.0	112.3	153.5	135.3	146.3	164.8	149.5	150.0	10.78	.557	<.001	.740

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; HE, high energy; 20, 23 and 26 represent the ages were 20, 23 and 26 months, respectively; LDL-C, low-density lipoprotein cholesterol; LE, low energy; ME, medium energy.

HDL-C and LDL-C, and the activities of serum AST and ALP ($p < .05$). There were no interaction effects between age and dietary energy on serum biochemical parameters ($p > .05$).

3.2 | Antioxidant activity and oxidative stress

The serum antioxidant activity and oxidative stress of Holstein bulls are presented in Table 3. The concentration of serum GSH-Px in bulls at the ages of 23 months was lower than 20 and 26 months ($p < .05$). Age and dietary energy had an interaction effect on the SOD activity ($p < .05$), at the age of 20 and 23 months, the activities of SOD were similar; at the age of 26 months, the activities of SOD of the ME group were lower than those of the LE and HE group. Besides, the increase of age tended to increase the concentrations of TAC ($p = .061$), decrease the concentration of ROS ($p = .075$).

3.3 | Liver glucose–lipid-related gene expression

The mRNA expressions of gluconeogenesis and inflammation-related genes in liver are shown in Figure 1. Age and dietary energy had interaction effects on the expressions of pyruvate carboxylase (*PC*) and insulin-like growth factor 1 (*IGF1*) ($p < .05$). At the age of 20 months, bulls in the LE and ME groups had higher expressions of *PC* than those in the HE group; at the age of 23 months, bulls in the LE group had higher expressions of *PC* than those in the ME and HE groups; at the age of 26 months, bulls in the LE and HE groups had higher expressions of *PC* than those in the ME group. At the age of 20 months, bulls in the LE group had higher expressions of *IGF1* than those in the ME and HE groups; at the age of 23 months, the expressions of *IGF1* were similar with the increasing dietary energy; at the age of 26 months, bulls in the LE group had lower expressions of *IGF1* than those in the ME group. The expressions of cytosolic phosphoenolpyruvate carboxykinase (*PCK1*) increased with the increasing dietary energy and age ($p < .05$). The increase of age increased the expressions of glucose-6-phosphatase (*G6PC*) and tumour necrosis factor alpha (*TNFα*) ($p < .05$).

The mRNA expressions of liver lipid synthesis and metabolism-related genes are shown in Figure 2. Age and dietary energy had interaction effects on the expressions of fatty acid synthase (*FASN*), peroxisome proliferator-activated receptor alpha (*PPARα*), acyl coenzyme A oxidase 1 (*ACOX1*) and carnitine palmitoyl-transferase 1 alpha (*CPT1α*) ($p < .05$). At the age of 20 and 23 months, bulls in the LE and ME groups had lower expressions of *FASN* than those in the HE group; at the age of 26 months, bulls in the LE group had lower expressions of *FASN* than those in the ME and HE groups. At the age of 20 months, bull in the LE group had higher expressions of *PPARα* than those in the ME and HE groups; at the age of 23 and 26 months, the expressions of *PPARα* were similar with the increasing dietary energy. At the age of 20 and 23 months, the expressions of *ACOX1* were similar with the increasing dietary energy; at the age of 26 months, bulls in the HE group had higher expressions of *ACOX1* than those in the LE and ME groups.

TABLE 3 Effects of dietary energy and age on serum antioxidant activity and oxidative stress of Holstein bulls

Item	20			23			26			*SEM*	*p*-value[a]		
	LE	ME	HE	LE	ME	HE	LE	ME	HE		Diet	Age	Diet × Age
TAC (Trolox Eq/L)	0.68	0.66	0.65	0.64	0.65	0.66	0.68	0.69	0.69	0.020	.316	.061	.169
ROS (IU/ml)	66.43	67.52	69.04	58.63	63.61	63.67	65.63	58.85	69.99	2.454	.102	.075	.169
GSH-Px (ng/ml)	881.8	869.7	887.2	773.9	830.7	838.0	914.1	816.9	997.6	42.31	.150	.037	.215
MDA (nmol/ml)	18.42	19.25	21.58	18.59	19.16	20.34	21.48	20.10	19.47	1.872	.445	.482	.342
SOD (U/ml)	11.71	13.43	13.51	11.21	11.43	11.17	11.42[t]	9.99[u]	11.79[t]	0.502	.060	<.001	.005
CAT (U/ml)	1.69	1.85	1.56	1.73	1.75	1.94	1.63	1.90	2.00	0.292	.491	.753	.823
OSI	99.38	101.94	105.92	91.57	98.06	98.07	97.00	85.38	101.04	5.285	.112	.359	.227

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; HE, high energy; 20, 23 and 26 represent the ages were 20, 23 and 26 months, respectively; LE, low energy; MDA, malonyl dialdehyde; ME, medium energy; OSI, oxidative stress index, the ratio of ROS to TAC; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity.

[a]Different letters within age group ([abc]20 months, [mno]23 months, [tuv]26 months) means differ at $p < .05$.

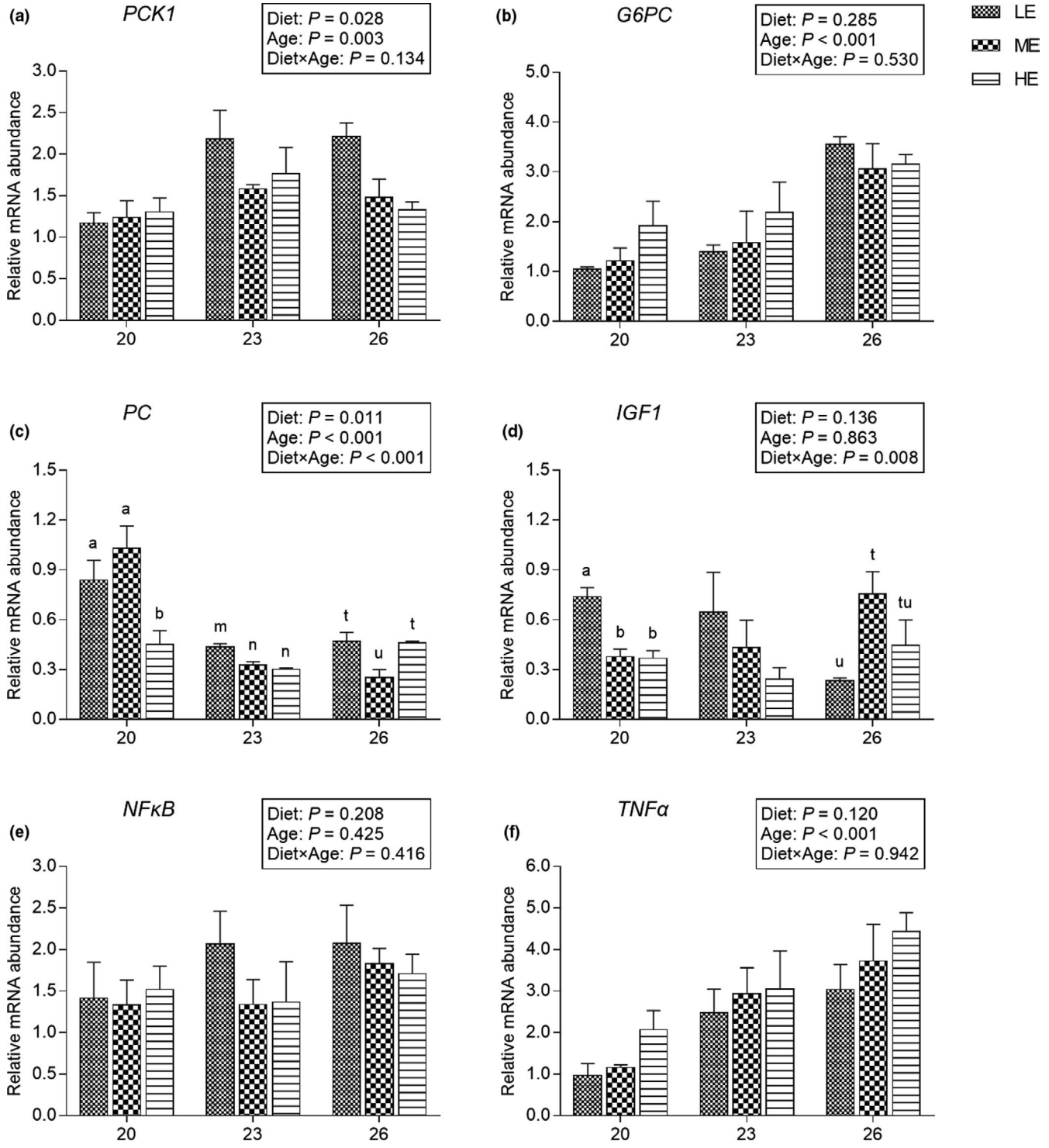

FIGURE 1 Expressions of liver gluconeogenesis and inflammation-related genes. LE, low energy; ME, medium energy; HE, high energy; *PCK1*, phosphoenolpyruvate carboxykinase 1; *G6PC*, glucose-6-phosphatase catalytic; *PC*, pyruvate carboxylase; *IGF1*, insulin-like growth factor 1; *NFκB*, nuclear factor kappa B; *TNFα*, tumour necrosis factor alpha. Different letters within age group (^{abc}20 months, ^{mno}23 months, ^{tuv}26 months) means difference at $p < .05$

At the age of 20 and 26 months, bulls in the LE group had higher expressions of *CPT1α* than those in the ME and HE groups; at the age of 23 months, the expression of *CPT1α* was similar with the increasing dietary energy. The increase of age increased the expression of sterol regulatory element binding protein 1 (*SREBP1*) ($p < .05$).

3.4 | Meat fatty acid profile

The proportions of C18:1*cis*-9, C18:2n-6*trans* and MUFA increased, while the proportion of C20:0 decreased with the increasing dietary energy ($p < .05$) (Table 4). The increase of age

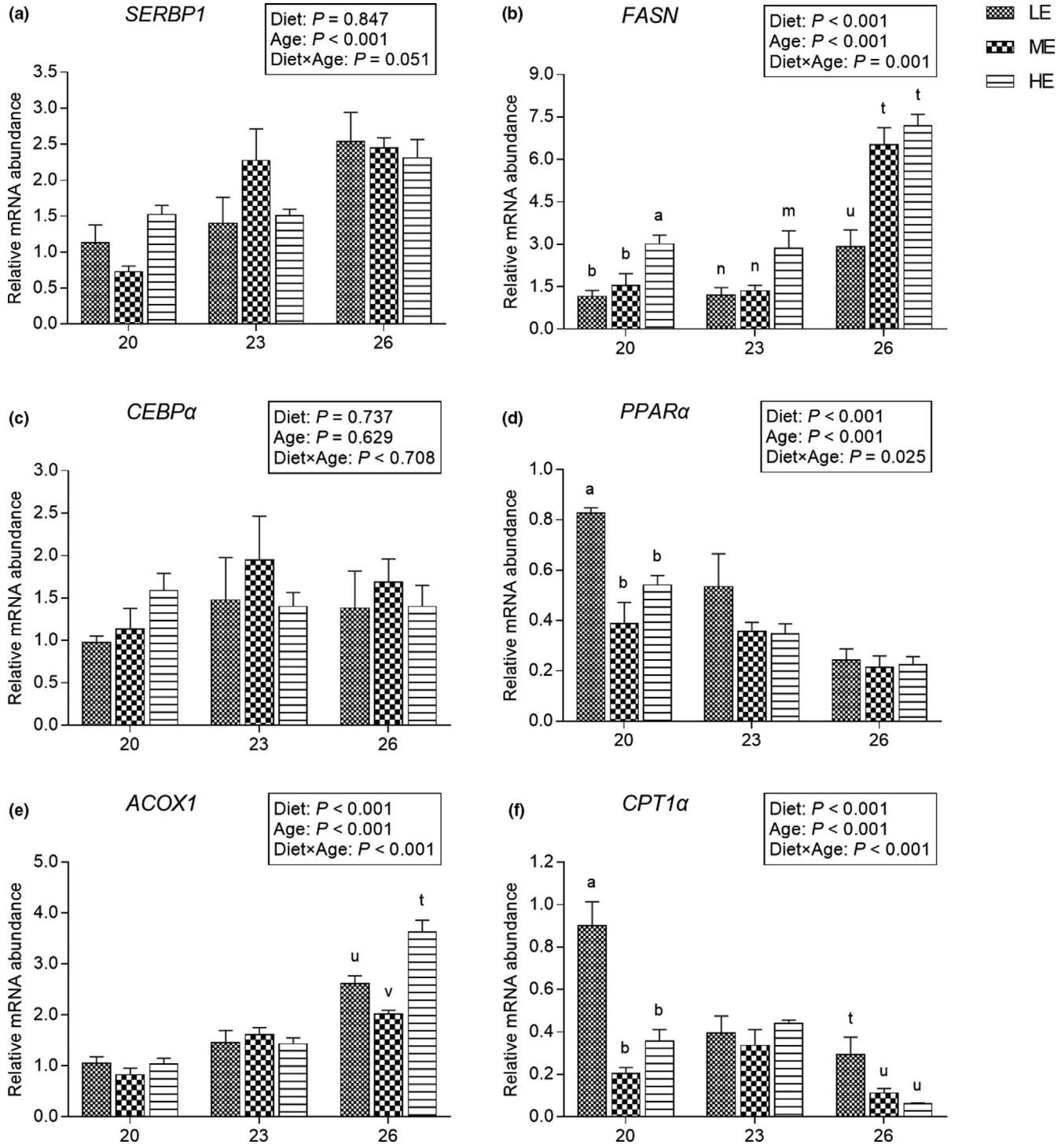

FIGURE 2 Expressions of liver lipid synthesis and metabolism-related genes. LE, low energy; ME, medium energy; HE, high energy; *SREBP1*, sterol regulatory element binding protein 1; *FASN*, fatty acid synthase; *C/EBPα*, CCAAT/enhancer-binding protein alpha; *PPARα*, peroxisome proliferator-activated receptor alpha; *ACOX1*, acyl-CoA oxidase; *CPT1α*, carnitine palmitoyl-transferase 1 alpha. Different letters within age group (^{abc}20 months, ^{mno}23 months, ^{tuv}26 months) means difference at $p < .05$

decreased the proportions of C15:0, C18:0, C20:0 and SFA, but increased the proportions of C14:1, C16:1, C18:1*cis*-9, C18:2n-6*trans* and MUFA ($p < .05$). There were no interaction effects between slaughter age and dietary energy on meat fatty acid profile ($p > .05$).

4 | DISCUSSION

Evaluation of variations on blood metabolism is often used to identify nutritional deficiencies and metabolic disorders of animal organism. For ruminants, the primary substrate of hepatic

TABLE 4 Effects of dietary energy and slaughter age on *longissimus thoracis* fatty acid profiles (% of total) of Holstein bulls

	20			23			26				*p*-value		
Item	LE	ME	HE	LE	ME	HE	LE	ME	HE	*SEM*	Diet	Age	Diet × Age
SFA													
C14:0	2.63	2.76	3.09	2.43	2.52	2.86	2.76	2.66	2.86	0.236	.237	.548	.960
C15:0	0.39	0.44	0.37	0.40	0.35	0.32	0.28	0.35	0.23	0.033	.068	.005	.423
C16:0	24.8	26.0	27.2	25.3	25.1	26.1	27.8	26.4	25.5	1.016	.904	.494	.305
C18:0	23.9	23.6	22.3	21.5	18.8	19.0	16.4	16.6	13.8	1.123	.068	<.001	.641
C20:0	1.26	0.94	0.96	1.00	0.87	0.77	1.07	0.69	0.67	0.104	.006	.044	.872
C21:0	0.02	0.01	0.01	0.01	0.01	0.03	0.00	0.02	0.02	0.009	.543	.799	.109
C22:0	0.05	0.06	0.07	0.07	0.07	0.08	0.07	0.05	0.04	0.010	.892	.198	.370
MUFA													
C14:1	0.39	0.44	0.39	0.35	0.52	0.57	0.71	0.85	1.00	0.139	.362	.003	.876
C15:1	0.21	0.32	0.37	0.38	0.39	0.29	0.40	0.24	0.21	0.072	.851	.543	.212
C16:1	2.29	2.15	2.70	2.27	3.16	3.05	3.79	3.27	4.53	0.432	.172	.002	.501
C17:1	0.49	0.39	0.38	0.48	0.50	0.48	0.47	0.52	0.54	0.043	.931	.073	.366
C18:1*trans*	4.12	3.62	2.44	3.45	3.73	3.08	2.10	3.34	2.32	0.524	.130	.158	.443
C18:1*cis*−*9*	32.7	31.9	32.7	34.1	35.4	36.6	36.5	38.5	41.9	0.911	.009	<.001	.119
C20:1	0.36	0.39	0.27	0.33	0.36	0.37	0.38	0.31	0.30	0.032	.214	.669	.159
C24:1	0.07	0.09	0.12	0.16	0.15	0.12	0.14	0.10	0.09	0.024	.741	.079	.331
PUFA													
C18:2n−6*trans*	0.08	0.08	0.09	0.09	0.09	0.11	0.12	0.12	0.16	0.010	.018	<.001	.393
C18:2n−6	4.39	4.83	4.54	5.41	5.57	4.50	4.58	4.20	4.15	0.508	.548	.195	.792
C18:3n−6	0.01	0.00	0.01	0.02	0.00	0.02	0.06	0.00	0.03	0.021	.231	.420	.811
C18:3n−3	0.26	0.26	0.25	0.23	0.25	0.29	0.24	0.27	0.26	0.020	.382	.994	.395
C20:2n−6	0.06	0.06	0.05	0.07	0.07	0.06	0.05	0.09	0.09	0.014	.616	.368	.504
C20:3n−6	0.22	0.18	0.20	0.22	0.19	0.16	0.19	0.18	0.15	0.036	.471	.655	.966
C20:4n−6	1.01	0.89	0.85	0.92	0.96	0.64	0.76	0.58	0.63	0.164	.456	.240	.872
C20:5n−3	0.05	0.04	0.04	0.06	0.05	0.05	0.05	0.05	0.03	0.007	.241	.344	.904
C22:6n−3	0.09	0.19	0.19	0.16	0.14	0.12	0.14	0.13	0.11	0.047	.883	.757	.568
SFA	53.1	53.8	54.0	50.8	47.8	49.1	48.4	46.8	43.1	1.612	.329	<.001	.301
MUFA	40.7	39.3	39.3	41.5	44.2	44.5	44.5	47.1	50.8	1.191	.042	<.001	.052
PUFA	6.19	6.52	6.24	7.17	7.33	5.96	6.20	5.62	5.63	0.728	.613	.338	.856
PUFA/SFA	0.12	0.12	0.12	0.14	0.15	0.12	0.13	0.12	0.13	0.019	.875	.401	.847
n−6 PUFA	5.71	5.97	5.70	6.65	6.81	5.43	5.71	5.08	5.13	0.693	.568	.312	.835
n−3 PUFA	0.42	0.49	0.50	0.45	0.45	0.47	0.44	0.45	0.42	0.052	.803	.791	.881
n−6/*n*−3 PUFA	13.8	13.1	11.2	14.9	15.5	11.6	13.0	11.3	12.1	1.332	.170	.284	.613

Abbreviations: HE, high energy; 20, 23 and 26 represent the ages were 20, 23 and 26 months, respectively; LE, low energy; ME, medium energy; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

gluconeogenesis is propionate from rumen fermentation (Walsh et al., 2009). Several rate-limited enzymes including PCK1, G6PC and PC are key factors that determine the rate of liver gluconeogenesis (Zhang et al., 2015). In this study, the increase of age increased the concentration of glucose, which may due to the increased expressions of gluconeogenesis-related genes *PCK1* and *G6PC*. The expressions of *PC* of the LE group were higher during the trial period, the reason maybe that bulls fed with LE diet produced lower propionate concentration, further induced higher gluconeogenesis expression to maintain the balances of glucose. The IGF1, primarily produced in liver, plays a vital role in growth regulation, development and metabolism in cattle (Mullen et al., 2011). In this study, at the age of 20 months, bulls in the LE group had higher *IGF1* expressions than those in the ME and HE groups; at the age of 26 months, bull in the LE group had lower *IGF1* expressions than those in the ME group. Consistent with those results, we previously observed that the average daily gains were similar at the age of 20 months, whereas increased at the age

of 23 and 26 months (Wang, Li et al., 2019). Serum cholesterol is a predictor of energy balance status in cattle (Kim & Lee, 2003). In this study, the concentrations of the total triglyceride, cholesterol, HDL-C and LDL-C increased with the increasing age. Similarly, research has reported that the blood chemical parameters including total cholesterol, HDL-C and triglyceride levels in female *Bos frontalis* increased with the increasing age (Sangpuii et al., 2018). High-concentrate diet could promote LPS release in rumen, and the translocation of LPS into blood will induce body inflammatory responses (Tian et al., 2019). In addition, the activity of ALP is related to metabolic syndrome and cardiovascular disease (Webber et al., 2010). In this study, the activity of serum ALP and the expression of liver *TNFα* increased with the increasing age, and the expression of liver *TNFα* numerically increased with the increasing dietary energy, those results suggesting that the increase of age and dietary energy enhanced the intensity of metabolism, further induced body inflammatory responses. In cattle, the increase of ALT activity is associated with liver damage, while the increase of AST activity could indicate a growing intensity of metabolic changes (Sakowski et al., 2012). In this study, the AST activity increased with the increasing age suggesting that bulls had high intensity of metabolism and inflammatory responses with the increasing age.

Under an inflammatory response, macrophages and neutrophils will participate in phagocytosis and produce ROS and oxylipid (Sordillo, 2016). An imbalance between ROS production and antioxidant defences availability may expose bulls to oxidative stress. The GSH-Px plays a crucial role in preventing peroxide damage to membranes induced by lipid peroxides. Blood MDA, as a product of oxidative stress, is an indicator of lipid peroxidation intensity (Castillo et al., 2006). In this study, at the age of 26 months, the concentration of serum MDA was higher in the LE group than those in the ME and HE groups; whereas at the age of 20 months, the concentrations of MDA were numerically higher in the HE group. Similarly, study has reported that compared to low energy density diet, high energy density diet makes cows more prone to oxidative stress (Wang et al., 2010). Whereas, cattle under negative energy balance is associated with dysfunctional responses and associated health disorders (Sordillo, 2016). Therefore, dietary energy and age may have interaction effects on the oxidative stress, that is, at the age of 20 months, Holstein bulls should feed with LE diet, while at the age of 26 months, Holstein bulls should fed with HE diet. Our results showed that the activity of SOD was similar with the change of the concentrations of MDA, and this could be explained that the balance between ROS production and antioxidant defences availability prevented bulls form suffering oxidative stress. Therefore, the OSI could be used to evaluate risk of oxidative stress (Celi, 2011). We found that dietary energy and age had no significant effect on OSI, indicating that the bulls had a similar health condition.

Liver plays a vital role in ruminant lipid metabolism (Bauchart et al., 1996). The genes *SREBP1*, *FASN* and *C/EBPα* are related to adipogenesis, and our results showed that the expressions of *SREBP1* increased with the increasing age. In addition, at the age of 20 and 23 months, bulls in the HE group had higher *FASN* expressions than those in the LE and ME groups; at the age of 26 months, bulls in the HE and ME groups had higher *FASN* expressions than those in the LE group, suggesting that high dietary energy and age induced the synthesis of fatty acid. Liver is the main tissue in ruminant lipid metabolism during periods of several nutritional and physiological situations (Bauchart et al., 1996). In cattle, the expression of *PPARα* is higher in liver where long-chain fatty acid oxidation is generally higher (Bionaz et al., 2013). The *ACOX1* and *CPT-1α* are target genes of *PPARα*, whose function is involved in liver lipid metabolism (Loor et al., 2005). In this study, at the age of 20 months, bulls in the LE group had higher *PPARα* expressions than those in the ME and HE groups; while at the age of 23 and 26 months, the expression of *PPARα* was similar with the increasing dietary energy. Similarly, research has reported that high energy diet is associated with the lower expression of liver *PPARα* (Janovick-Guretzky et al., 2007). In addition, we found that at the age of 20 months, bulls in the LE group had higher MDA concentration than those in the ME and HE groups, indicating that the bulls in the LE group had a higher oxidation, and both high energy diet and age-reduced lipolysis metabolism. In addition, the result that the concentration of serum total triglyceride increased with the increasing age and dietary energy verified the report that a low rate of fatty acid oxidation resulting in an accumulation of triglyceride (Hocquette & Bauchart, 1999). The CPT1α is responsible for the hydrolysis of triglycerides and importing esters of fatty acid to mitochondria for β-oxidation (De Berardinis et al., 2006). In this study, at the age of 20 and 26 months, bulls in the LE group had higher *CPT1α* expressions than those in the ME and HE groups, suggesting that fatty acid oxidation decreased with the increasing age and dietary energy. The ACOX1 is the first rate-limiting enzyme of fatty acid β-oxidation with the function involved in fatty acid degradation (Li et al., 2000). However, we observed that bulls in the HE group had the highest expressions of *ACOX1* at the age of 26 months; this may due to the relative higher fat deposition in the HE group under a long fattening time. Similarly, compared with a low-fat diet, mice fed a long-term high-fat diet had higher *ACOX1* expressions (Kim et al., 2004).

Beef contains many long-chain PUFA that are not contained in diet, suggesting that some beneficial fatty acid in beef could be de novo synthesized by the related genes expression and desaturase activity (Guil-Guerrero, 2014). In this study, the proportions of C18:1*cis*-9 and MUFA increased, while the proportion of C18:0 decreased with the increasing of slaughter age and dietary energy. We found that the increase of age decreased the proportions of C15:0, C18:0 and SFA, and increased the proportions of C14:1 and C16:1. Researchers have reported that when cattle fed a concentrate-based diet, the expression and concomitant catalytic activity of stearoyl-COA desaturase increased with the increasing age (Smith et al., 2009). Therefore, the change of beef fatty acids could be associated with the increased expressions of adipogenesis-related genes *SREBP1* and *FASN*, and the decreased expressions of lipid metabolism-related genes *PPARα* and *CPT1α* with the increasing age. Research showed that the percentage of beef PUFA

decreased while the percentages of beef SFA and MUFA increased for bull ranging in age from 14 to 24 months (Warren et al., 2008). Whereas, we observed that slaughter age and dietary energy had no effect on PUFA proportion, suggesting that the proportions of PUFA were relatively stable after 20 months of age. High n-6 to n-3 ratio has been highlighted as risk factors for certain cancers and coronary heart diseases, and the value is recommended 4.0 or less for food (Department of Health, 1994). In this study, the n-6 to n-3 ratio ranged from 11.2 to 15.5, and the results are in consistent with the report that when steers fed a concentrate based diet, the ratio ranges from 9 to 16 (Warren et al., 2008). In this study, the ratio of PUFA to SFA ranges from 0.12 to 0.15. The minimum PUFA to SFA ratio for human nutrition is recommended 0.45, which can only be found in animal with less than 1% total fat (Simopoulos, 2008). Although the Holstein bulls had a high IMF at the later fattening period (Wang, Li et al., 2019), the proportion of PUFA, especially n-3 PUFA, still needs to be improved.

5 | CONCLUSIONS

The increase of age and dietary energy enhanced the intensity of metabolism and inflammatory responses, increased the expressions of adipogenesis-related genes *SREBP1* and *FASN*, and decreased the expressions of lipid metabolism-related genes *PPARα* and *CPT1α*. Advancing age and diet energy content further altered the meat fatty acid profile indicated by the decrease of C15:0, C18:0, C20:0 and SFA proportions and increase of C18:1*cis*-9, C18:2n-6*trans* and MUFA proportions.

ACKNOWLEDGMENTS

This study was supported by the National Key R&D Program of China (2018YFD05018000), Key Technology R&D Program of Ningxia (2017BY078, 2018BFF02001), National Natural Science Foundation of China (31802084), China Agriculture Research System (CARS-37).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the animals care and experimental procedures were approved by the China Agricultural University Animal Care and Use Committee (Permit No. AW21109102-1).

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How to cite this article: Wang H, Niu W, Wu F, et al. Effects of dietary energy on antioxidant capacity, glucose–lipid metabolism and meat fatty acid profile of Holstein bulls at different ages. *J Anim Physiol Anim Nutr* 2021;105:199–209. https://doi.org/10.1111/jpn.13457

APPENDIX

TABLE A1 Specific primers used for PCR

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Product length (bp)	Accession number
PCK1	AAGATTGGCATCGAGCTGACA	GTGGAGGCACTTGACGAACTC	120	NM_174737.2
G6PC	GGATTCTGGGTCGTGCAACT	CCGCAATGCCTGACAAGACT	100	NM_001076124.2
PC	GCAAGGTCCACGTGACTAAGG	GGCAGCACAGTGTCCTGAAG	124	NM_177946.4
IGF1	GATGCTCTCCAGTTCGTGTG	CTCCAGCCTCCTCAGATCAC	141	NM_001077828.1
SREBP1	GTGCTGAGGGCAGAGATGGT	ACAAAGAGAAGTGCCAAGGAGAA	106	NM_001113302.1
FASN	ATGGCGTTCCACTCCTACTTCA	CTCTCCTGCCACTGGGTCTC	137	NM_001012669.1
C/EBPα	AGCGCCGCTTTCGGCTTTC	GCTGATGTCGATGGACGTCT	114	NM_176784.2
PPARα	CATAACGCGATTCGTTTTGGA	CGCGGTTTCGGAATCTTCT	102	NM_001034036.1
ACOX1	ACCCAGACTTCCAGCATGAGA	TTCCTCATCTTCTGCACCATGA	100	NM_001035289.3
CPT1A	TCGCGATGGACTTGCTGTATA	CGGTCCAGTTTGCGTCTGTA	100	NM_001304989.1
NFκB1	GTCAAACTCCAGAATGGCAGA	GAAATCCTCTCTGTTTAGGTTGCTC	179	NM_001076409.1
TNFα	CCAGAGGGAAGAGCAGTCCC	TCGGCTACAACGTGGGCTAC	114	NM_173966.3
GAPDH	AGATGGTGAAGGTCGGAGTG	GAAGGTCAATGAAGGGGTCA	117	NM_001034034.2

Abbreviations: ACOX1, Acyl coenzyme A oxidase 1; C/EBPα, CCAAT/enhancer-binding protein alpha; CPT1α, carnitine palmitoyl-transferase 1 alpha; FASN, fatty acid synthase; G6PC, glucose-6-phosphatase catalytic; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IGF1, insulin-like growth factor 1; NFκB, nuclear factor kappa B; PC, pyruvate carboxylase; PCK1, phosphoenolpyruvate carboxykinase 1; PPARα, peroxisome proliferator activated receptor alpha; SREBP1, sterol regulatory element binding protein 1; TNFα, tumour necrosis factor alpha.