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RESEARCH ARTICLE

Discovery of the Enrichment Pathways and Biomarkers using Metabolomics Techniques in Unilateral and Bilateral Castration in Yellow Cattle

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A B S T R A C T

In the present study, the effect of unilateral (ULC) and bilateral castration (BLC) on metabolomics in yellow cattle was conducted. The current trial was comprised of 120 days of feeding following castration, after completion of the trial the blood samples were collected from all groups for metabolomics analysis. The results displayed 46 important metabolites were found when BLC compared to the CON group (VIP >1P < 0.05), such as GPCho (22:6/18:2), 7,8-Dihydrobiopterin, phosphocholine, phosphatidylcholine (PC), Butyryl-L-carnitine and 5-Hydroxyindoleacetic acid etc. The KEGG pathway analysis showed that BLC affects the 9 metabolic pathways in the yellow cattle serum when compared with the CON group. Biomarker analysis showed that the metabolites PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z)),PC(20:4(8Z,11Z,14Z,17Z)/P-18:0),PC(18:0/22:5(4E,7E,10E,13E,16E))[U],PC(17:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))and butyryl-L-carnitine etc. were the serum biomarkers between BLC and CON groups. In addition, when we compared the differential metabolites between ULC and BLC groups, 58 differential metabolites were found. KEGG pathway analysis showed that the metabolome pathway between ULC and BLC cattle affects 12 pathways in the serum of the yellow cattle. The BLC group cattle had a higher serum concentration of phosphatidylcholine and LysoPC enriched in the Glycerophospholipid metabolism pathway and a higher serum concentration of PC(16:0/20:4(5Z,8Z,11Z,14Z)) enriched in the Arachidonic acid and Linoleic acid metabolisms pathway, respectively. The Phospholipid Metabolites were the biomarkers in the serum of BLC cattle. Therefore, Castration enhanced the synthesis metabolism of unsaturated fatty acids and increased concentrations of unsaturated fatty acids, including linoleic acid (C18:2n6c), linolenic acid (C18:3n3), arachidonic acid (C20:2), docosahexaenoic acid (C22:6n3), in the fat tissue of cattle.

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INTRODUCTION

The growth performance and metabolism are influenced by the various hormones levels. The growth, body development, meat quality and fat in animals are affected by the testosterone hormone (Bender *et al.*, 2006; Shah *et al.*, 2023). The fat percentage, meat quality and growth of animals are increased through castration because castration changes the levels of sex hormones and their pattern inside the body of animals (Baik *et al.*, 2014). The meat quality depends on the fat deposition in the body of animals and this deposition of fat in the body of animals depends on anabolism and catabolism in adipose tissue. Each procedure is separately controlled by hormonal and non-hormonal features (Kersten, 2001). The energy is stored in the lipid droplets in the form of triglycerides (TG). The de novo lipogenesis largely takes place in the adipose tissue of the beef (Bergen and Mersmann, 2005) and this depends on notorious enzyme-regulated mechanism. In fatty acid synthesis, the fatty acid synthase and acetyl-CoA carboxylase play a crucial role. Fatty acid synthase is a critical factor in the greater

capacity of tissue to synthesize a fatty acid, catalyzes nearly all of the phases in the alteration of malonyl-CoA into palmitate (Joseph *et al.*, 2002).

Metabolomics is a post-genomic technology which tries to deliver an inclusive outline of all low-molecular-weight metabolites. Metabolites can be observed as the ultimate response of biological systems to genetic, environmental or dietary changes (Whitfield *et al.*, 2004; Wishart, 2019). So, metabolomics has been used in numerous research areas, e.g. disease diagnosis (Zhang *et al.*, 2012), environmental science (Lankadurai *et al.*, 2013), nutrition (Jones *et al.*, 2012; Rist *et al.*, 2017) and food science (Herrero *et al.*, 2012; Consonni and Cagliani, 2019).

The metabolomics technique was first used by Nicholson *et al.* (1999) (Nicholson *et al.*, 1999; Luo *et al.*, 2019) and divided into untargeted and targeted approaches. The untargeted approach is used to examine the metabolite differentiation among the control and treated groups which plays a crucial role in marker screening of disease. In contrast, the targeted metabolomics approach is used to quantitatively determine the metabolites in the metabolic pathway of interest (Patti *et al.*, 2012; Li *et al.*, 2017). In the present research, we used the untargeted metabolomics approach in cattle and determined the differential metabolites in control, Unilateral and bilateral castrated cattle. The identification of differential metabolites may deliver important information for investigating physiological and characterizing new pathways that may begin under ULC and BLC in cattle.

MATERIALS AND METHODS

Animal experimental design: The current research study was conducted (R-2017 care of laboratory animals, approved by the Sichuan Agricultural University Chengdu P.R China) at the Animal experimental station Ya'an campus Sichuan Agricultural University P.R China. In current research 18 healthy male yellow cattle having body weight 184.43±4.08 were chosen and divided into three groups; each group comprised of 6 cattle per pen. The groups were control (CON: no castration/normal), unilateral castrated (ULC: one testes removed) and bilateral (BLC: two testes removed). The castration of the cattle was done through an open surgical method after using anaesthesia. To avoid the effect of the surgical castration, after one month after the castration, a formal experiment was started. During this month, the post-operative care of the surgically castrated cattle was done, and different antibiotics and analgesic drugs were used routinely. The duration of the formal experiment was 120 days. The total mixed ration was provided to animals per the Chinese Beef Cattle Raising Standard (NY/y815-2004); the diet composition is mentioned in supplementary Table S1.

Blood sample collection and analysis: On the last day of the research trial blood samples from all the cattle were collected through the jugular vein and centrifuged for 15 min at 2000x to get serum and the serum was stored at -80^0C till analysis.

Metabolomics analysis through GC-MS

Preparation of the samples: The blood samples from the different groups were prepared according to the previous research (Luo *et al*., 2022).

Metabolomics analysis: Metabolomics analysis technique was used through an Agilent 7890A/5975C GC-MS system. The raw GC-MS data was analyzed for peak picking, alignment, and deconvolution as followed as mentioned in Gao *et al*., 2010. While, the PLS-DA, Enrichment analysis of metabolic pathways, Biomarker Analysis were carried out in MetaboAnalyst v5.0. (McGill University, Montreal, QC Canada).

RESULTS

PLS-DA analysis: The effect of ULC and BLC on serum metabolomics data of cattle was studied. The results of partial least square discriminant analysis (PLS-DA) are shown in Fig. 1. According to the results, the degree of separation of the metabolic profiling between each group is higher, positive ions mode. ULC vs CON R^2Y=0.8161, Q^2 =0.5213, BLC vs. CON= R^2Y= 0.876=0.6485, ULC vs. BLC R^2Y= 0.9405, Q^2= 0.7055. There is a significant difference observed among the groups. The PLS-DA permutation test showed that the present model is valid.

Different metabolites identification in groups (VIP): The results of the VIP analysis of differential metabolites between the BLC vs CONT groups are presented in Table 1. The results showed that 46 important differential metabolites were found in the BLC compared to CONT VIP >1 and *P <0.05*. When compared to BLC and CONT, there was a significant difference was observed in the metabolites 7,8-Dihydrobiopterin, 5-Hydroxyindoleacetic acid, 2-Methoxyacetaminophen glucuronide, P-Toluenesulfonic acid, Butyryl-L-carnitine, 2-hydroxyhexadecanoic acid, Dimethyl sulfoxide, Ala-Leu, Vasicinone, Hexyl glucoside, Tyrosol 4-sulfate, Pyrroline hydroxycarboxylic acid, Threoninyl-Serine, Alline and other metabolites.

In addition, when we compared ULC and BLC groups, 58 differential metabolites were found between the groups. The significant differences VIP> 1 and *P <0.05* of differential metabolites are presented in Table 2. Lastly, the results of the differential metabolites between the ULC and CONT groups showed that 8 differential metabolites were found between the groups. The significant differences VIP >1 and *P<0.05* of differential metabolites are mentioned in Table 3.

Enrichment analysis of metabolic pathways of differential metabolism: In the present research, a total of nine pathways were found in BLC vs CONT groups, and twelve pathways were found in ULC vs BLC groups; however, there is no pathway found in ULC and CONT groups (Fig. 2).

Biomarker analysis: Further, on the differential metabolites, ROC curve analysis was conducted to obtain the corresponding AUC value, which was between 0.5 and 1.0, can be used as a variable to predict the accuracy of diagnostic ability, the closer its value can be used as a

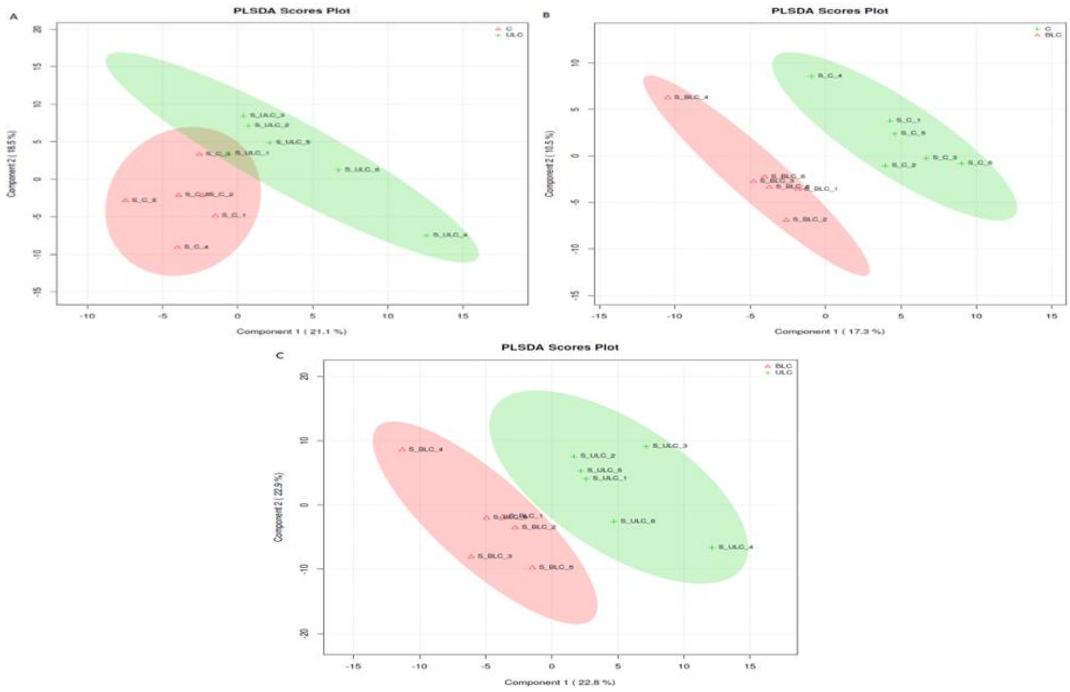


Fig. 1: Serum metabolomics PLS-DA analysis of cattle. A= ULC vs. BLC, B= BLC vs. CON, C=ULC vs. BLC

Table 1: Differential metabolites found in BLC vs CONT groups.

Metabolite Name	m/z	Retention time	VIP	Fold change	*P-value*
GPCho(22:6/18:2)	830.6	7.03	1.98	1.05	0.002
7,8-Dihydrobiopterin	272.1	1.82	2.16	0.92	0.005
1-eicsoatetraenoyl-2-(1-enyl-stearoyl)-sn-glycero-3-phosphocholine	832.6	7.30	2.05	1.05	0.005
PC(18:0/22:5(4E,7E,10E,13E,16E))[U]	836.6	7.45	2.08	1.07	0.010
Phosphatidylcholine(16:1/22:6)	804.6	7.03	1.53	1.03	0.013
5-Hydroxyindoleacetic acid	209.1	1.37	1.50	0.97	0.013
2-Methoxyacetaminophen glucuronide	340.1	2.86	1.72	0.95	0.015
P-Toluenesulfonic acid	217.0	4.45	1.98	0.94	0.015
Butyryl-L-carnitine	232.2	2.16	1.70	0.95	0.017
2-hydroxyhexadecanoic acid	271.2	6.37	1.48	0.97	0.018
Dimethyl sulfoxide	79.0	0.63	2.77	0.83	0.019
Phosphatidylcholine(15:0/22:5n3)	776.6	7.14	1.90	1.05	0.020
5-(3',4'-Dihydroxyphenyl)-gamma-valerolactone	229.1	5.83	1.84	0.93	0.021
(3-phenylpropoxy)sulfonic acid	215.0	5.76	1.68	0.95	0.024
Phosphatidylcholine(18:0/22:6)	856.6	7.17	1.78	1.06	0.025
CL(a-13:0/i-24:0/18:2(9Z,11Z)/i-24:0)[rac]	802.6	7.26	1.51	1.04	0.025
2-Methoxyacetaminophen sulfate	242.0	2.87	1.87	0.94	0.025
GPCho(18:3w3/22:1w9)	860.6	7.51	2.11	1.07	0.025
Ala-Leu	185.1	1.31	1.45	0.95	0.026
3-(2,4-Cyclopentadien-1-ylidene)-5alpha-androstan-17beta-ol	339.3	6.06	2.01	1.08	0.026
6-(Allylthio)purine	193.1	4.78	2.45	0.89	0.029
4-Vinylphenol sulfate	199.0	4.26	2.12	0.91	0.031
7-Chloro-L-tryptophan	239.1	4.78	2.42	0.89	0.033
1,2-di-(2E,4E-octadecadienoyl)-sn-glycero-3-phosphocholine	782.6	7.03	1.77	1.05	0.033
Vasicinone	244.1	3.37	2.47	0.87	0.034
5-Hydroxy-L-tryptophan	221.1	1.06	1.45	0.96	0.035
Hexyl glucoside	245.1	4.96	1.32	0.96	0.036
1,2,3,4,5,6-Hexahydro-5-(1-hydroxyethylidene)-7H-cyclopenta[b]pyridin-7-one	202.1	2.45	2.46	0.88	0.036
Deoxycholic acid glycine conjugate	414.3	6.06	1.70	1.04	0.037
PC(17:0/22:6)	820.6	7.30	1.88	1.07	0.037
L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid	217.1	3.37	2.64	0.84	0.039
1-vaccenoyl-2-arachidonoyl-sn-glycero-3-phosphocholine	852.6	7.04	1.76	1.06	0.040
Tyrosol 4-sulfate	217.0	3.39	1.61	0.95	0.040
Pyrroline hydroxycarboxylic acid	171.1	0.85	1.51	0.94	0.041
2-Propenyl 1-(propylsulfinyl)propyl disulfide	237.0	5.98	1.91	0.91	0.041
N-(tetradecanoyl)-sphing-4-enine-1-phosphocholine	697.5	6.96	1.79	1.05	0.043
1-eicsoatetraenoyl-2-(1-enyl-vaccenoyl)-sn-glycero-3-phosphocholine	833.6	8.00	2.10	1.08	0.047
1-palmitoyl-2-docosadienoyl-sn-glycero-3-phosphocholine	858.6	7.97	2.23	1.09	0.048
1-stearidonoyl-2-(1-enyl-vaccenoyl)-sn-glycero-3-phosphocholine	786.5	6.96	1.66	1.05	0.048
Threoninyl-Serine	171.1	1.01	1.50	0.95	0.048
PC(15:0/P-18:0)	754.6	7.14	1.95	1.07	0.048
3-O-Sulfogalactosylceramide (d18:1/22:0)	902.6	7.47	2.14	1.09	0.048
Citrulline	174.1	0.50	1.35	1.04	0.049
Alline	171.1	3.36	2.34	0.85	0.049
sulfonic acid	213.0	5.19	1.36	0.96	0.051
3-Indolepropionic acid	190.1	5.39	1.64	0.94	0.054

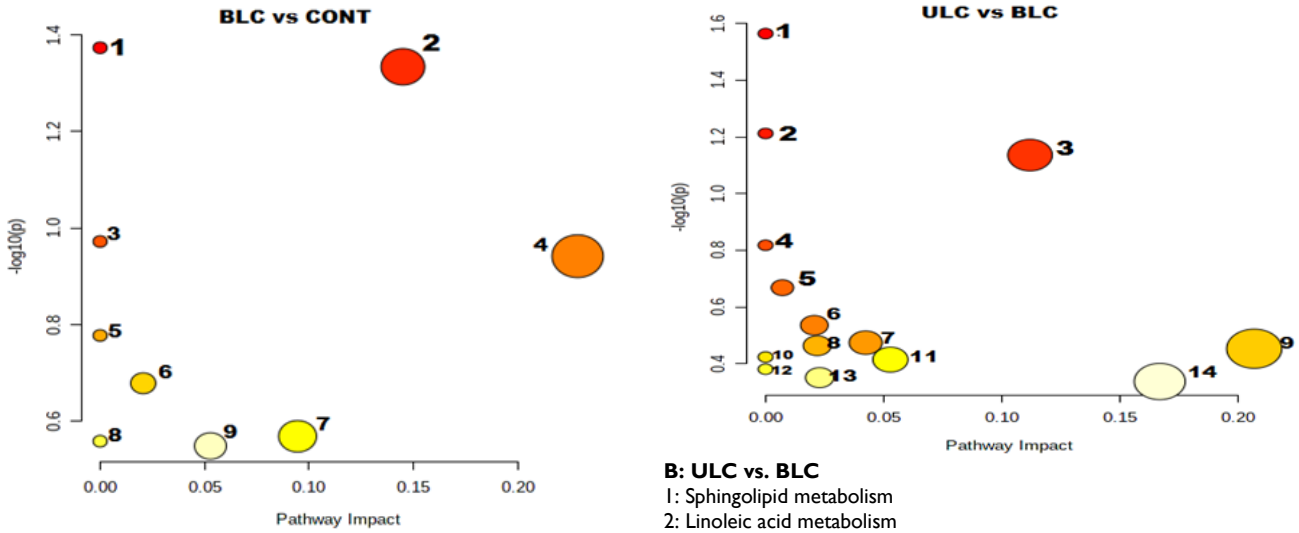
Table 2: Differential metabolites found in ULC vs. BLC groups

Metabolite Name	m/z	Retention time	VIP	Fold change	*P-value*
Dimethyl sulfoxide	79.0	0.63	3.09	1.26	0.001
GPCho(22:6n3/18:2n6)	830.6	7.03	1.90	0.95	0.002
2-Hydroxycinnamic acid	182.1	1.23	1.51	1.03	0.003
1-vaccenoyl-2-arachidonoyl-sn-glycero-3-phosphocholine	852.6	7.04	2.01	0.94	0.004
Phosphatidylcholine(16:1/22:6)	804.6	7.03	1.54	0.97	0.006
Phosphatidylcholine(18:0/22:6)	856.6	7.17	1.97	0.93	0.008
1-eicsoatetraenoyl-2-(1-enyl-stearoyl)-sn-glycero-3-phosphocholine	832.6	7.30	1.86	0.95	0.011
LysoPE(0:0/20:1(11Z))	530.3	6.59	1.32	1.03	0.012
N-Carboxytocainide	237.1	1.79	1.64	1.07	0.012
P-Cresol glucuronide	283.1	3.40	2.02	1.08	0.013
Pyrroline hydroxycarboxylic acid	171.1	0.85	1.70	1.08	0.014
lysophosphatidylcholine (17:2/0:0)	506.3	6.14	1.69	1.06	0.014
2-Methoxyacetaminophen glucuronide	340.1	2.86	1.67	1.07	0.016
5-(3',4'-Dihydroxyphenyl)-gamma-valerolactone	229.1	5.83	1.71	1.09	0.016
Phosphatidylcholine(38:4)	854.6	7.30	1.76	0.95	0.017
Lucidenic acid M	507.3	6.53	2.09	0.90	0.017
Citreovirenone	266.1	2.34	1.67	1.08	0.018
5,7-Dihydroxy-4'-methoxy-8-methylflavanone	318.1	5.57	1.74	1.09	0.018
3-O-Sulfogalactosylceramide (d18:1/22:0)	902.6	7.47	2.52	0.88	0.019
Alanyl-dl-phenylalanine	237.1	2.14	2.26	1.15	0.019
Arachidonoyl Serotonin	463.3	5.90	2.73	1.22	0.020
3-hydroxyhexadecanoyl carnitine	450.3	6.22	1.57	1.05	0.021
2(3H)-Benzothiazolethione	168.0	5.81	1.61	1.08	0.023
7-Chloro-L-tryptophan	239.1	4.78	2.43	1.15	0.023
Phosphatidylcholine(16:0/20:4)	826.6	7.04	1.29	0.97	0.026
6-(Allylthio)purine	193.1	4.78	2.42	1.15	0.026
7,8-Dihydrobiopterin	272.1	1.82	1.89	1.09	0.028
Chlorogenoquinone	351.1	3.40	2.12	1.15	0.028
Hordenine	372.3	2.37	1.66	1.06	0.029
5'-(3',4'-Dihydroxyphenyl)-gamma-valerolactone sulfate	269.0	3.14	1.99	1.14	0.031
Se-Methylselenomethionine	212.0	6.32	1.44	1.04	0.031
4'-Hydroxydiclofenac	292.0	2.98	1.26	1.05	0.031
Vasicinone	244.1	3.37	2.25	1.16	0.031
Threoninyl-Serine	171.1	1.01	1.61	1.07	0.034
P-Tolyl Sulfate	187.0	3.45	1.29	1.03	0.034
N6-cis-p-Coumaroylserotonin	355.2	4.88	2.57	1.23	0.037
LysoPC(20:3(5Z,8Z,11Z))	590.3	6.21	1.50	0.95	0.038
Phosphatidylcholine(18:0/20:3n9)	856.6	7.46	2.58	0.86	0.039
Lotaustralin	325.1	2.99	1.82	1.10	0.040
Androsterone sulfate	353.2	2.30	2.08	1.14	0.041
Pantothenic Acid	220.1	1.65	1.22	1.04	0.042
L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid	217.1	3.37	2.26	1.19	0.043
1-palmitoyl-2-docosadienoyl-sn-glycero-3-phosphocholine	858.6	7.97	2.16	0.89	0.044
Glycocholic Acid	464.3	5.98	1.81	0.95	0.044
4-Hydroxy-4-(3-pyridyl)-butanoic acid	204.1	1.05	1.46	1.07	0.045
1-docosahexaenoyl-glycero-3-phosphocholine	612.3	6.14	1.69	0.93	0.045
N-myristoylsphingosine-1-phosphocholine	697.5	6.96	1.55	0.95	0.045
Thyroxine	777.7	5.88	1.42	1.04	0.046
Plantagonine	176.1	3.21	1.99	1.15	0.047
Antibiotic SB 202742	412.3	5.98	1.74	0.94	0.049
2-Methoxyacetaminophen sulfate	242.0	2.87	1.45	1.06	0.049
L-Serine	88.0	3.15	1.52	1.07	0.050
Ethyl-2-amino-4-methyl-Thiazole-5-Carboxylate	187.1	1.83	2.29	1.19	0.050
Deoxycholic acid glycine conjugate	414.3	6.06	1.31	0.97	0.051
3-Oxo-2-(2-pentenyl)-1-cyclopentene-1-acetic acid	250.1	5.56	1.53	1.07	0.051
6-[2-(2H-1,3-benzodioxol-5-yl)ethyl]-4-methoxyoxan-2-one	296.1	5.58	1.50	1.06	0.052
LysoPC(18:3(9Z,12Z,15Z))	500.3	6.58	1.57	1.07	0.052
LysoPC(16:1(9Z)/0:0)	492.3	6.21	1.34	1.05	0.053

Table 3: Differential metabolites found in ULC vs CONT groups.

Metabolite Name	m/z	Retention time	VIP	Fold change	*P-value*
Octadecanamide	325.3	5.95	3.53	1.28	0.005
4-Vinylphenol sulfate	199.0	4.26	3.11	0.86	0.011
9,10,13-TriHOME	329.2	6.36	1.47	1.02	0.018
Arachidonoyl Serotonin	463.3	5.90	2.97	1.22	0.018
Ethylene brassylate	235.2	6.11	2.13	1.07	0.020
4-ethylphenylsulfate	201.0	4.76	2.44	0.94	0.031
Corchorifatty acid F	327.2	6.00	1.42	1.02	0.041
Butyryl-L-carnitine	232.2	2.16	1.29	0.97	0.043

variable to predict the accuracy of diagnostic ability, the closer its value is to 1.0, the variable predicts diagnostic ability. Therefore, in this study, we used AUC 0.95 1.0 was used as a biomarker. The Biomarker analysis of the CONT vs BLC groups is present in Fig. 3. 6 metabolites were differentiated as mentioned in Fig. 4. These can be used as a potential biomarker for the effect of Different castration on the metabolism in cattle.



A: BLC vs. CONT
1: Linoleic acid metabolism
2: Tryptophan metabolism
3: alpha-Linolenic acid metabolism
4: Arginine biosynthesis
5: Sphingolipid metabolism
6: Folate biosynthesis
7: Glycerophospholipid metabolism
8: Arachidonic acid metabolism
9: Arginine and proline metabolism

B: ULC vs. BLC
1: Sphingolipid metabolism
2: Linoleic acid metabolism
3: Glycerophospholipid metabolism
4: alpha-Linolenic acid metabolism
5: Pantothenate and CoA biosynthesis
6: Folate biosynthesis
7: Glyoxylate and dicarboxylate metabolism
8: Cysteine and methionine metabolism
9: Glycine, serine and threonine metabolism
10: Arachidonic acid metabolism
11: Arginine and proline metabolism
12: Tyrosine metabolism

Fig. 2: The Metabolome pathway enrichment analysis for serum differential metabolites of **(A)** BLC vs CONT, **(B)** ULC vs BLC in cattle: Note: White to red color intensity shows upsurge statistical significance, and diameter of circle change with impact of the pathway.

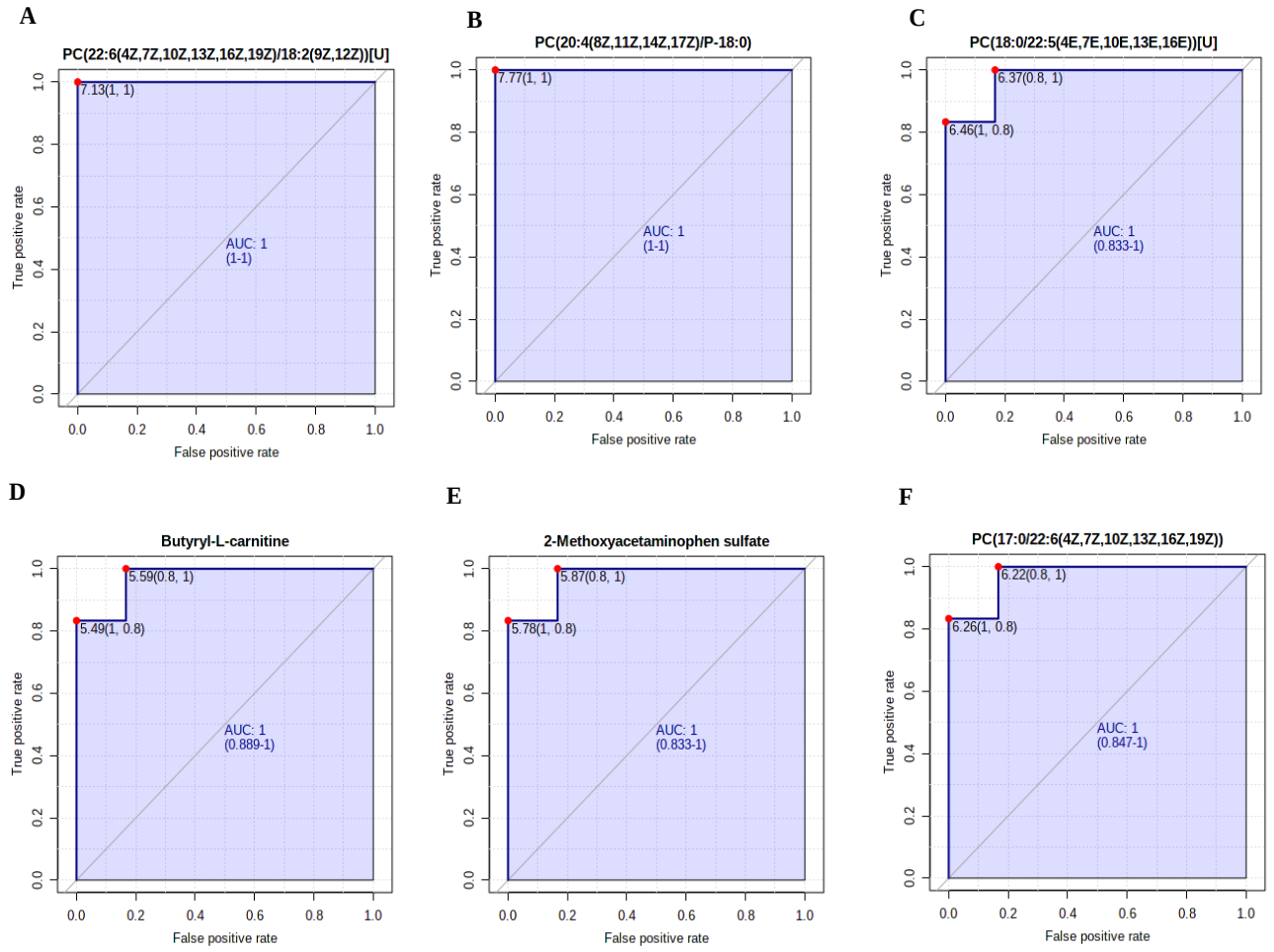


Fig. 3: Biomarker analysis for serum differential metabolites of BLC vs CONT groups of cattle.

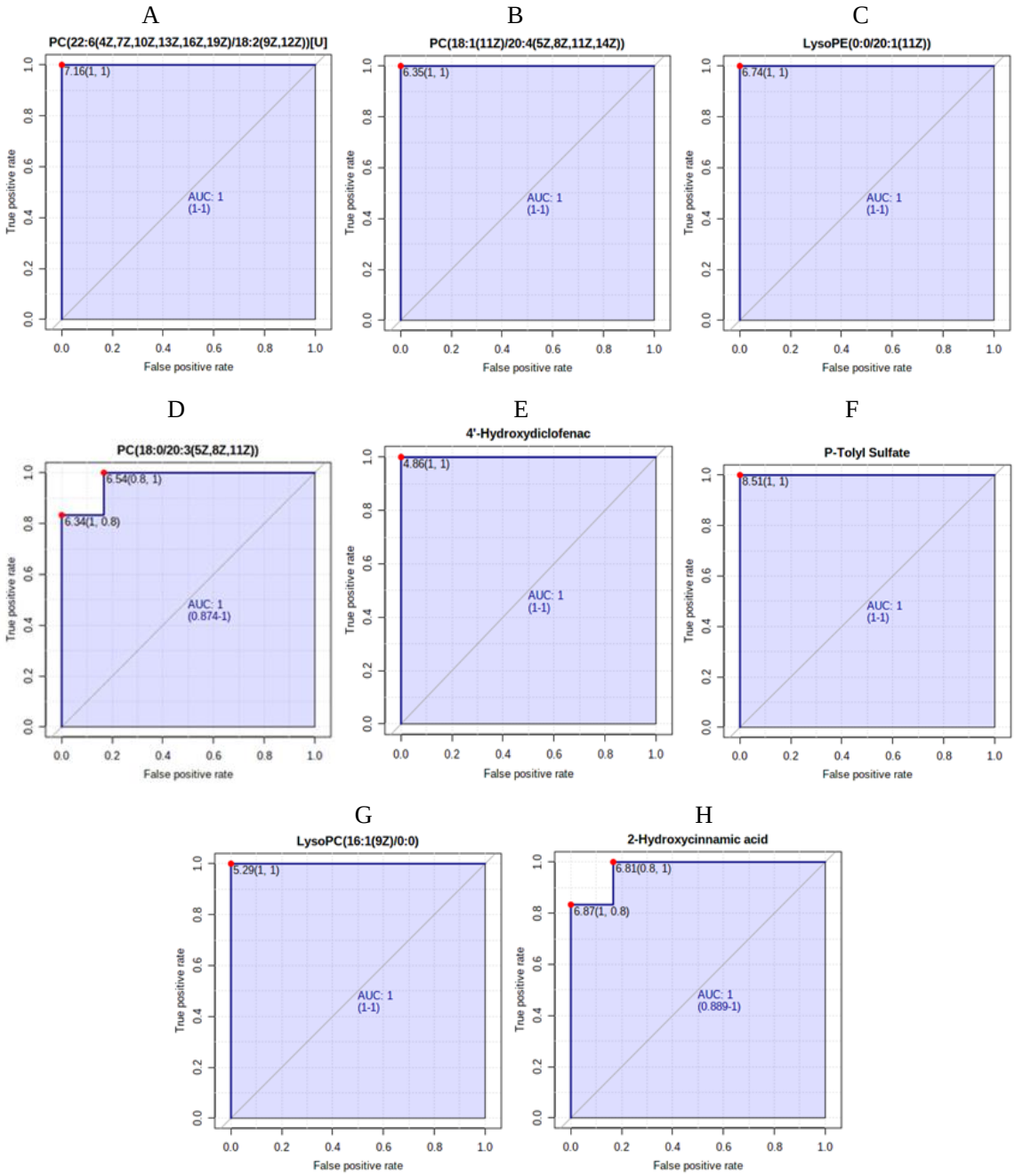


Fig. 4: Biomarker analysis for serum differential metabolites of ULC vs CONT groups cattle. (ULC vs. BLC.

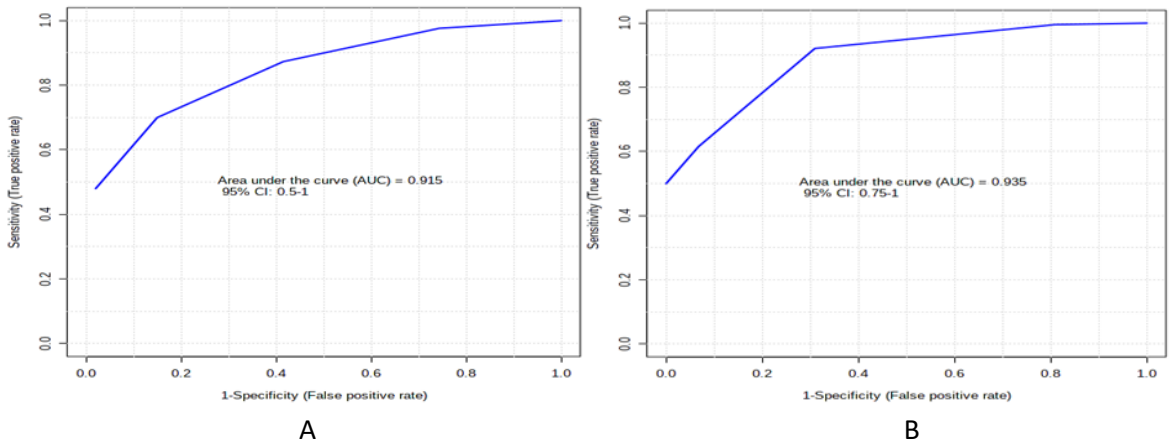


Fig. 5: ROC curves of biomarker predicted model A,BLC vs CONT ;B, ULC vs BLC.

Table S1. Chemical composition of feed ingredients used in experimental diet.

Ingredients (kg/1000kg DM)	
Corn grain	174.0
Wheat bran	54.0
Soybean meal	24.0
Rapeseed meal	30.0
Sorghum distiller's grains	400.0
Rice straw	300.0
Salt	4.5
Sodium bicarbonate	3.6
Calcium carbonate	0.3
Calcium hydrophosphate	6.6
Premix*	3.0
Nutrient levels	
NEg (MJ/kg)	3.4
OM (%)	91.7
CP (%)	12.6
EE (%)	2.3
NDF (%)	54.1
ADF (%)	41.1
Ca (%)	0.8
P (%)	0.5

*The premix delivers per kg diet with: Fe in the form of sulfate 50 mg, Cu in the form of sulfate 8 mg, Zn in the form of sulfate 40 mg, Mn in the form of sulfate 50 mg, Co in the form of chloride 0.2 mg, I in the form of iodate 0.5 mg, Se in the form of selenite 0.2 mg and, Vitamin A 8800 IU, Vitamin D 1000 IU, and Vitamin E 100 IU. OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.

In addition, the Biomarker analysis of the ULC vs BLC groups are presented in Fig. 4. 9 metabolites were differentiated, which have AUC 0.95-1 respectively metabolism. It can be used as a potential biomarker for the effect of different castration on the metabolism in cattle. However, when we compared the ULC vs CONT there were no any metabolites were found in the AUC 0.95-1.

DISCUSSION

As Metabolomics can deliver important information at a global system level, in the current study, the effect of ULC and BLC on metabolomics was studied. The results of the VIP analysis of the different metabolites showed that 46 significant metabolites were found between the BLC and CONT groups. These metabolites were involved in 9 metabolic pathways in the serum between the BLC and CONT. In the Glycerophospholipid metabolism pathway, LysoPC is unregulated, and LysoPC can increase fat transport in the form of phosphatidylcholine and improve the transport of fatty acids in the form of phosphatidylcholine in liver fat metabolism. Oxidative utilization alleviates lipid deposition and ketone body formation in the liver. Phosphoserine is used in the liver to synthesize phosphatidylcholine through a series of enzymes catalyzed by the diacylglycerol lipid metabolic pathway. The synthesis of phosphatidylcholine with diacylglycerol in the liver is catalyzed by a series of enzymes. Glycerophospholipid metabolism (Glycerophospholipid) phosphatidylcholine is transported by VLDL in the liver (Ridgway, 2013). The fatty acyl chain is removed by phospholipase A2 and lysophospholipase to form Glycerophosphocholine (Milkevitch *et al.*, 2005). The results showed that the LysoPC in the BLC cattle was significantly higher than in the CONT and ULC cattle. A higher concentration of the LysoPC indicates that the treatment promoted the glycerophospholipid metabolism, enhanced the turnover of the LysoPC and promoted the transport of fat to the muscle or subcutaneous fat from the liver. The final dealiphatic acyl is converted to glycerophosylcholine, which alleviates liver fat deposition and improves liver health, as well as increases the fat deposition in fat tissues. In this study, the BLC significantly affected the Pantothenic acid and coenzyme A biosynthesis pathway (Pantothenate and CoA biosynthesis). The catabolites of pantothenic acid, uracil and N-carbamyl, were increased β-alanine and valine levels and pantothenic acid in castrated cattle compared to non-castrated cattle. The biosynthesis of pantothenic acid decreased significantly in the control group. Pantothenic acid is a precursor for the synthesis of activated molecular coenzyme A, which is involved in the activation of lipid-fat acid to lipoyl coenzyme A. The increase in the number of staff increases the body's need for coenzyme A significantly (Luís, 2011). Pantothenic acid plays a key part in the synthesis of coenzyme A (CoA). This CoA is important for the synthesis of fatty acid and degradation transfer of acetyl and acyl groups and a multitude of other anabolic and catabolic processes. The serum pantothenic acid concentration of non-castrated cattle was reduced. The castration increased the pantothenic acid concentration which alternatively increased the synthesis of coenzyme A (CoA) in the metabolites of cattle. The contribution of pantothenic acid derivative coenzyme A to the oxidation of fatty acids was decreased by the influence of the pathway of synthesis and metabolism. In response, the fatty acid activation phase weakened the oxidative decomposition of fat (Yuan *et al.*, 2012). In addition, a greater amount of the LysoPCs is synthesized by lecithin cholesterol acyltransferase in plasma which catalyzes the transfer of the fatty acids of position sn-2 of PC to the free cholesterol (Hishikawa *et al.*, 2014; Wang *et al.*, 2020). There are numerous categories of LysoPCs because LysoPCs have numerous combinations of fatty acids with different saturation and length. In the current research, the different categories of the LysoPCs in plasma concentrations decreased (saturated and unsaturated) in the CONT group, which is inconsistent with the findings in humans (Del Bas *et al.*, 2016) and mice (Barber *et al.*, 2012). The results of metabolic pathway enrichment displayed that the arginine biosynthesis was increased in the bilateral castrated cattle compared to non-castrated cattle. Arginine is an α-amino acid which is used for the biosynthesis of the protein the results displayed that the castration increases the protein biosynthesis in castrated cattle compared to the control group. Furthermore, Arginine and citrulline, ornithine participate in urea cycle and play a key role in removing ammonia generated by protein decomposition and deamination. Acetylornithine, one of the intermediate products, free ammonia, through the action of a series of enzymes, synthesizes sperminosuccinic acid and then breaks down into ornithine. The acid returns to circulation, and the urea is excreted in the urine to reduce the toxic effects of ammonia. Arginine biosynthesis: At the same time, the metabolic intermediates N, N dimethylglycine and tryptophan. The indole acetic acid also decreased significantly, which also indicated the low catabolism level of amino acid. The above results indicate the

experimental design in cattle, the body protein mobilization and decomposition and utilization of amino acids in blood metabolites are relatively low, and the metabolism of the urea cycle pathway involved in ammonia removal is also low. In addition, compared to non-castrated cattle, the concentration of glutamine and the glutamate derivative pyrrolidonecarboxylic acid in cattle was significantly increased, possibly related to the increase of TCA substrates alpha-ketoglutarate can be directly synthesized into glutamic acid by transamination (Mason *et al.*, 1995). The metabolite 3-O-sulfogalactosylceramide from the present study result we concluded that bilateral castration increased the 3-O-Sulfogalactosylceramide Sphingolipid metabolism because the sphingolipid with cholesterol and phospholipid major constituents of the lipid bilayers and pay to particular membrane domains called rafts and function as signalling molecules. Sphingolipids have been known to employ a different role in the post-transcriptional regulation of the sterol-regulatory element binding proteins (SREBPs), key transcription factors of lipid synthesis. The synthesis of sphingolipid is an obligate activator of SREBP. Inhibition of sphingolipid synthesis reduces SREBP on a post-transcriptional level. With the exemption of enzymes that produce sphingolipids, SREBPs control the transcription of key enzymes that produce cholesterol, phospholipids and fatty acids. This opinion proposes a special role for sphingolipids in lipid metabolism regulation.

Conclusions: In the current research, we have concluded that 46 important differential metabolites were identified in BLC compared to the CONT group, and 58 differential metabolites were found between ULC and BLC groups. Most of the metabolites are closely related to lipid and amino acid metabolism. Compared with the CON group, BLC affects 9 metabolic pathways in the cattle serum, including linoleic acid, tryptophan, alpha-linolenic acid metabolisms, etc. Castration increased the serum concentrations of phospholipid metabolites PC and LysoPC in the Glycerophospholipid, arachidonic and linoleic acids metabolisms pathway. The phospholipid metabolites were the biomarkers in the serum of castrated cattle.

Declaration of competing interest: The authors declare no conflict of interest.

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