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Associations between rumen microbiota, rumen fermentation parameters and serum biochemical indexes during the perinatal period in sheep

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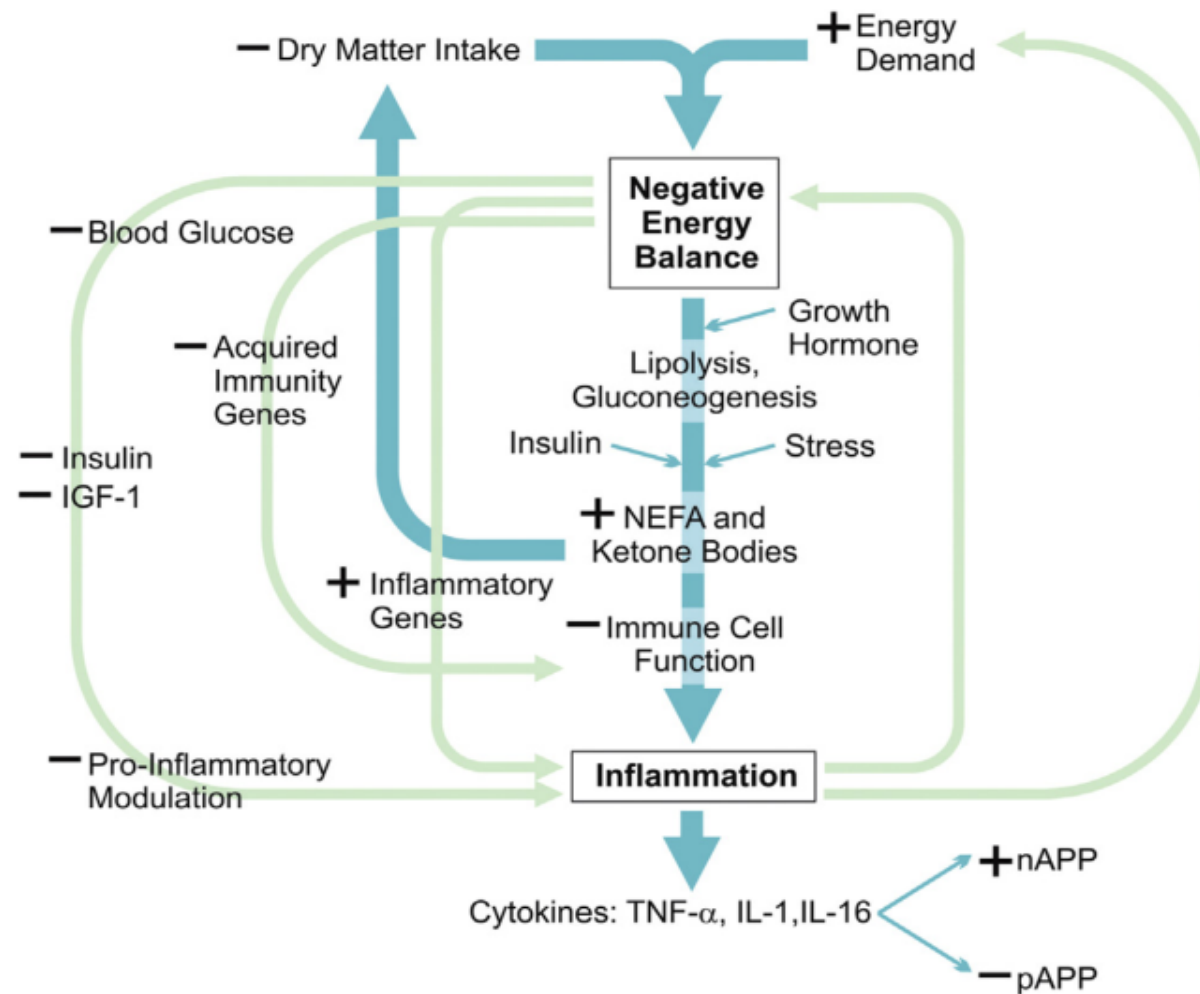
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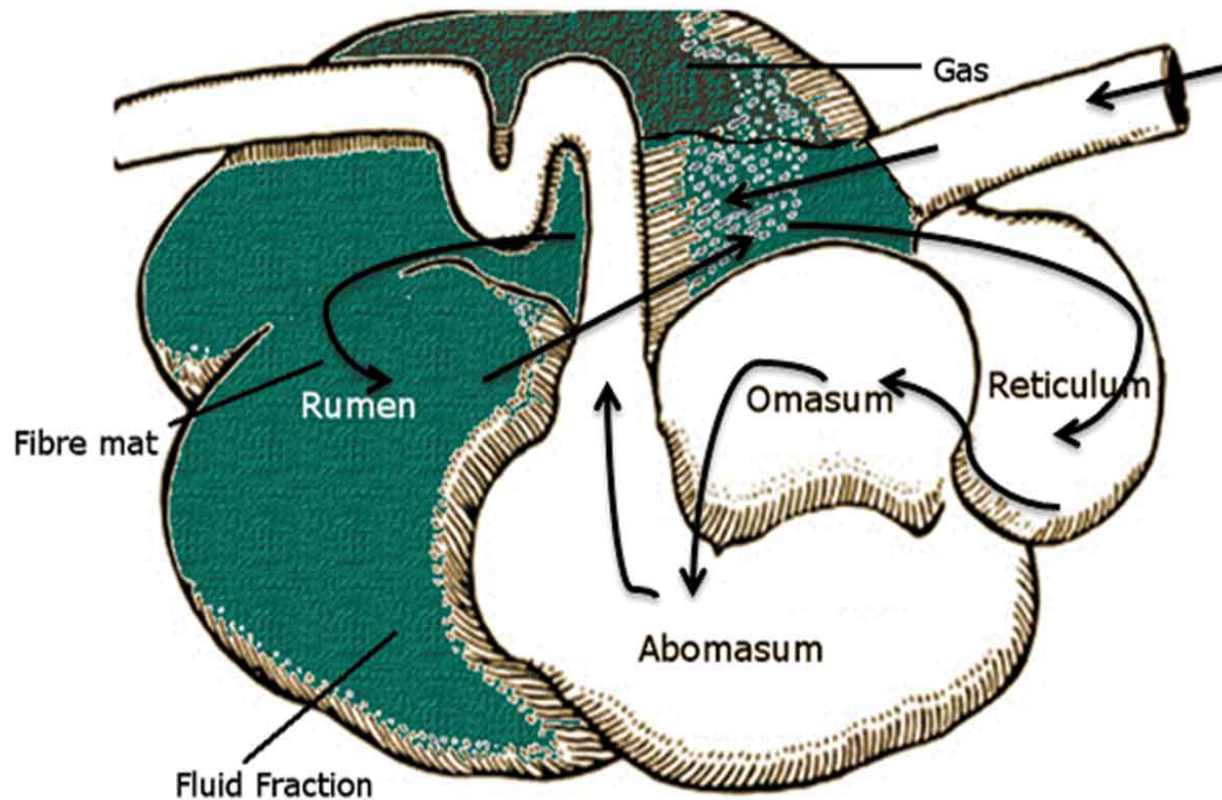
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1. Introduction



- ◆ The perinatal period refers to the last three weeks of pregnancy and the first three weeks of lactation.
- ◆ The decrease of dry matter intake (DMI) and the increase of energy demand induce negative energy balance (NEB).
- ◆ The increased levels of circulated non-esterified fatty acid (NEFA) and ketone bodies have been reported to induce immunosuppression and increase the release of acute phase proteins.

1. Introduction



- ◆ The energy source of ruminants mainly depends on the digestion and absorption of nutrients in rumen microbiota fermented feed.
- ◆ The ratio of acetate, propionate and butyrate is related to the types and quantity of microbiota in rumen.
- ◆ Therefore, it is of great significance to study the variation of rumen microbiota in ruminants during perinatal period and the relationship between rumen microbiota and rumen fermentation parameters as well as serum biochemical indexes in order to preventing negative energy balance (NEB) diseases.

2. Materials and Methods



The experimental animal is Hu sheep, is a world-famous multi-lamb sheep, which has the characteristics of fast growth rate and strong adaptability.

2. Materials and Methods

We selected ten ewes (n=10, average parity: 2-3, BW =55.8±5.09 kg) to track the body dynamic development from 120 days of gestation to 14 days of lactation

Blood, rumen fluid, feed and fecal samples were collected on day twenty-first, fourteen, seven prepartum (Q21, Q14 Q7), three, seven and fourteen postpartum (H3, H7 and H14)

Rumen fermentation parameters and serum biochemical indexes were determined

High-throughput 16S rRNA gene sequencing was performed on rumen fluid samples and a random forest model was used to predict rumen bacteria changes during the perinatal period

3. Results

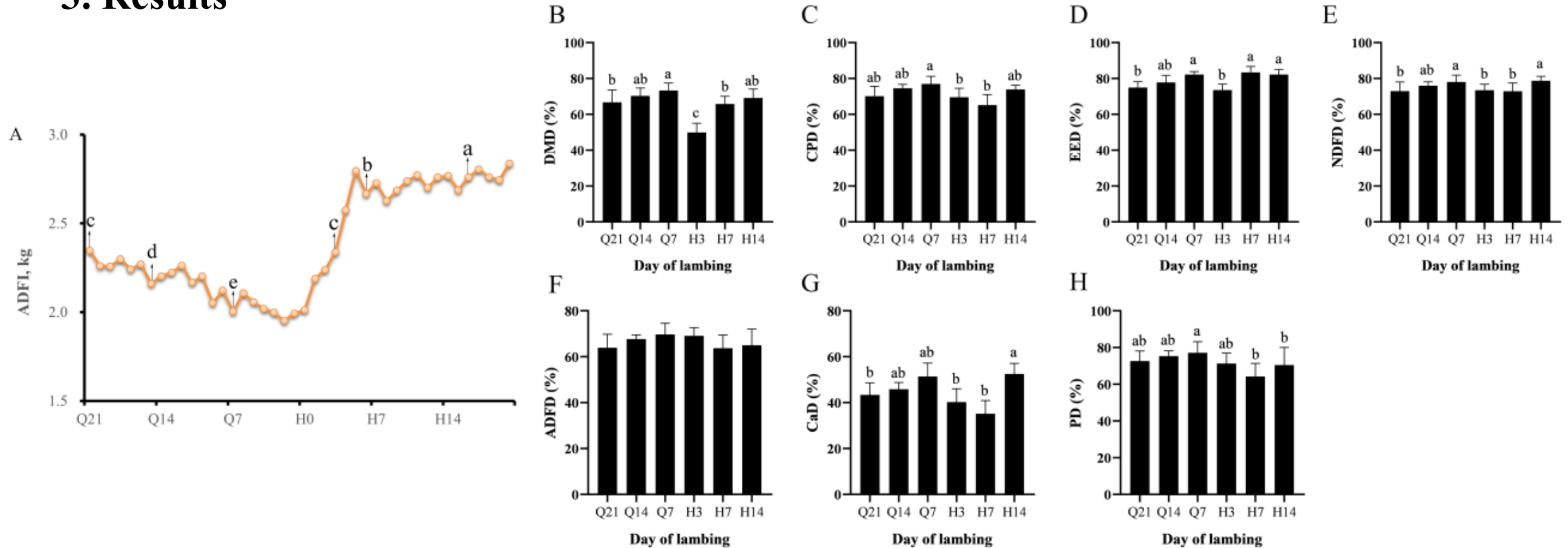


Fig.1. Changes of the ADFI (A), DMD (B), CPD (C), EED (D), NDFD (E), ADFD (F), CaD (G) and PD (H) during the perinatal period in ewes.

- Average daily feed intake (ADFI) decreased before and increased after lambing.
- The digestibility of dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) increased on day seven prepartum (Q7), then decreased on day three (H3) or seven postpartum (H7).

3. Results

Table 1 Changes of rumen fermentation parameters during perinatal period in sheep

Item ¹	prepartum			postpartum			SEM	P-value
	Q21	Q14	Q7	H3	H7	H14		
pH	7.12 ^a	6.79 ^b	6.99 ^{ab}	6.54 ^c	6.49 ^c	6.53 ^c	0.45	<0.001
Ammonia N/(mg/dL)	6.40	8.53	6.75	7.78	6.42	7.86	0.41	0.571
MCP/(mg/mL) ¹	0.42 ^{ab}	0.39 ^b	0.42 ^{ab}	0.41 ^{ab}	0.42 ^{ab}	0.43 ^a	0.004	0.354
Acetate/(mmol/L)	53.20 ^c	65.68 ^a	59.04 ^b	53.02 ^c	50.09 ^{cd}	47.33 ^d	1.62	<0.001
Propionate/(mmol/L)	16.55 ^c	10.90 ^d	17.72 ^{bc}	23.87 ^b	30.78 ^a	31.02 ^a	1.60	<0.001
Butyrate/(mmol/L)	3.22 ^{ab}	1.56 ^b	2.23 ^b	4.51 ^{ab}	5.56 ^a	4.42 ^{ab}	0.48	0.039
T-VFA/(mmol/L) ¹	72.96 ^b	79.14 ^{ab}	78.99 ^b	82.36 ^{ab}	86.43 ^a	82.77 ^a	1.18	0.077
A/P ²	3.23 ^b	6.45 ^a	3.32 ^b	2.44 ^{bc}	1.67 ^c	1.73 ^c	0.38	<0.001

¹ MCP: microbial crude protein; T-VFA: Total volatile fatty acids. ² A/P: acetate to propionate ratio.

- Rumen pH was the highest on day twenty-first prepartum (Q21), and acetate to propionate ratio (A/P) was on day seven prepartum (Q14).
- The propionate and total volatile fatty acid (T-VFA) were significantly higher on day seven postpartum (H7) than other time points.

3. Results

Table 2. Changes of serum biochemical indexes during perinatal period of ewes

Item ¹	prepartum			postpartum			SEM	P-value
	Q21	Q14	Q7	H3	H7	H14		
Energy metabolism								
GLU, mmol/L	1.90 ^b	1.09 ^c	1.83 ^b	1.62 ^{bc}	2.63 ^a	3.01 ^a	0.79	<0.001
BHBA, mmol/L	0.56 ^b	0.69 ^{ab}	0.77 ^{ab}	0.66 ^{ab}	0.67 ^{ab}	0.93 ^a	0.25	0.163
NEFA, mmol/L	0.28 ^b	0.53 ^{ab}	0.59 ^a	0.60 ^a	0.59 ^a	0.81 ^a	0.28	0.041
TG, mmol/L	0.42 ^b	0.33 ^b	0.53 ^a	0.44 ^{ab}	0.36 ^b	0.37 ^b	0.11	0.009
TC, mmol/L	2.28 ^a	2.18 ^a	1.74 ^b	1.49 ^{bc}	1.17 ^c	1.17 ^c	0.56	<0.001
HDL-C, mmol/L	1.01 ^a	0.84 ^{ab}	0.63 ^b	0.63 ^b	0.55 ^b	0.62 ^b	0.23	0.001
LDL-C, mmol/L	0.73 ^a	0.75 ^a	0.77 ^a	0.59 ^{ab}	0.38 ^b	0.36 ^b	0.28	0.011
Liver function								
ALT, U/L	3.18 ^a	2.79 ^{ab}	1.90 ^b	1.84 ^b	2.32 ^{ab}	1.65 ^b	0.96	0.027
AST, U/L	11.59 ^b	11.49 ^b	11.68 ^b	13.76 ^{ab}	15.51 ^a	11.97 ^b	2.50	0.049
ALP, U/L	9.42	7.57	8.52	7.90	9.03	8.55	3.21	0.939
BUN, mmol/L	2.96 ^{ab}	2.28 ^b	2.79 ^{ab}	3.47 ^a	2.67 ^{ab}	3.27 ^a	0.85	0.170
TBIL, μmol/L	5.58 ^b	3.80 ^c	5.58 ^b	3.06 ^c	7.65 ^a	5.73 ^b	1.81	<0.001
TP, g/L	65.30 ^{ab}	61.46 ^b	69.19 ^{ab}	69.07 ^{ab}	69.28 ^{ab}	71.40 ^a	7.25	0.187
ALB, g/L	31.65 ^{ab}	33.63 ^a	30.44 ^{ab}	30.48 ^{ab}	29.26 ^b	30.29 ^{ab}	3.55	0.375
Micronutrient								
Calcium, mmol/L	2.28 ^b	2.25 ^b	2.51 ^a	2.50 ^a	2.51 ^a	2.38 ^{ab}	0.15	<0.001
Phosphorus, mmol/L	2.24 ^a	1.94 ^b	2.09 ^{ab}	1.96 ^b	2.19 ^{ab}	1.84 ^b	0.26	0.045
Magnesium, mmol/L	0.83 ^b	0.80 ^b	0.92 ^{ab}	0.99 ^a	0.94 ^{ab}	0.90 ^{ab}	0.09	0.021
25HVD ₃ , ng/mL	9.31 ^c	12.84 ^b	13.80 ^b	16.26 ^{ab}	13.65 ^b	16.82 ^a	3.44	<0.001
Hormone								
INS, mIU/L	20.35 ^b	16.06 ^c	20.80 ^b	20.07 ^b	21.72 ^b	25.90 ^a	3.52	<0.001
GC, ug/L	258.55 ^a	261.96 ^a	245.32 ^a	254.04 ^a	212.32 ^b	191.99 ^b	33.87	<0.001
NPY, ng/L	161.82 ^b	180.85 ^b	209.44 ^{ab}	228.31 ^a	197.13 ^{ab}	212.89 ^{ab}	36.25	0.011
IGF-I, ng/mL	105.79 ^b	83.06 ^c	111.87 ^b	114.25 ^b	127.74 ^{ab}	144.130 ^a	25.11	<0.001
LEP, ng/mL	3.58 ^a	3.94 ^a	3.51 ^{ab}	3.53 ^{ab}	2.781 ^b	2.391 ^b	0.82	0.004

- Compare with the day twenty-first prepartum (Q21), the concentrations of glucose (GLU), insulin (INS), total protein (TP), non-esterified fatty acid (NEFA), β-hydroxybutyric acid (BHBA), blood urea nitrogen (BUN), 25-hydroxy vitamin D3 (25HVD3), and neuropeptide γ (NPY) were significantly increased on day fourteen postpartum (H14).
- The concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucagon (GC), leptin (LEP) and Phosphorus were the highest on day twenty-first prepartum (Q21) and gradually decreased after lambing.

3. Results

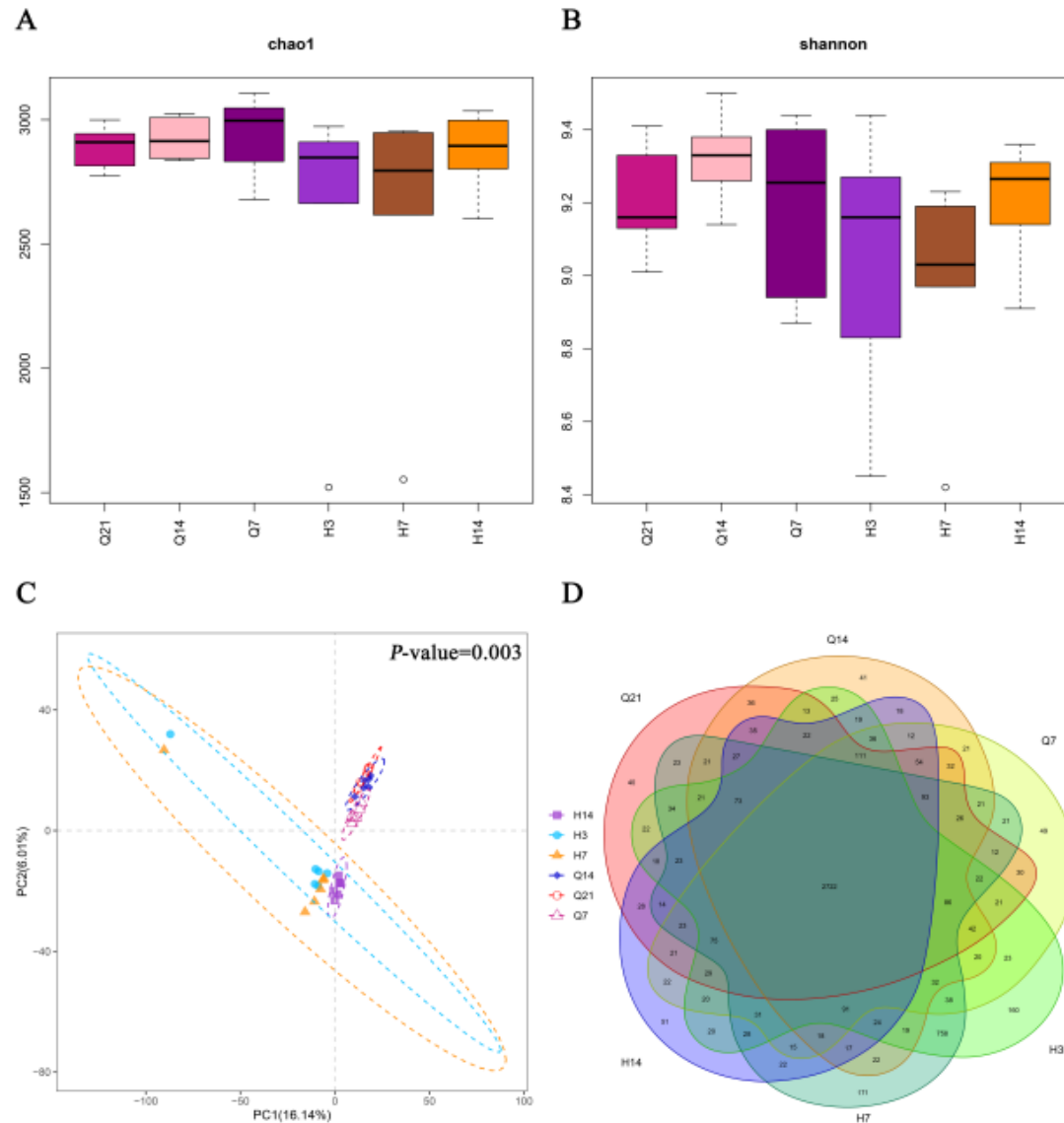


Fig 2 Changes of rumen bacterial during perinatal period in sheep.

(A) Species richness estimates (Chao1 Index) and (B) diversity (Shannon Index) at six sampling time points; (C) Partial least squares Discriminant analysis based on OTU for rumen bacterial in perinatal sheep (PLS-DA); (D) Venn diagram of rumen bacterial OTUs at six sampling time points.

- The 16S rRNA gene sequencing revealed that Chao1 Index and Shannon Index no difference during perinatal period.
- Figure 2C showed that rumen microbiota composition also differed before and after lambing.

3. Results

Table 3 Changes of rumen bacterial communities at phylum level in sheep during perinatal period

Item%	prepartum			postpartum			SEM	<i>P</i> -value
	Q21	Q14	Q7	H3	H7	H14		
Bacteroidota	57.67 ^a	53.78 ^{ab}	48.98 ^{ab}	44.79 ^b	45.29 ^b	52.97 ^{ab}	0.015	0.061
Firmicutes	36.59 ^b	39.37 ^{ab}	44.69 ^{ab}	47.55 ^a	47.86 ^a	40.93 ^{ab}	0.014	0.083
Spirochaetota	1.00	0.87	0.79	0.96	1.66	0.77	0.002	0.758
Patescibacteria	1.92 ^b	2.54 ^a	2.97 ^a	1.85 ^b	1.88 ^b	2.57 ^a	0.001	0.077
Proteobacteria	0.57	1.25	0.67	2.2	0.69	0.93	0.003	0.477

- At phylum level, Bacteroidetes and Firmicutes were the main dominant bacterial phyla, which accounting for more than ninety percent of the bacteria in rumen.

3. Results

Table 4 Changes of rumen bacterial communities at genus level in perinatal sheep

Item%	prepartum			postpartum			SEM	P-value
	Q21	Q14	Q7	H3	H7	H14		
<i>Prevotella</i>	26.6 ^a	21.41 ^{ab}	21.05 ^{ab}	17.26 ^b	17.01 ^b	20.00 ^{ab}	0.013	0.342
<i>uncultured_rumen_bacterium</i>	11.35 ^{ab}	11.83 ^a	10.38 ^{ab}	7.86 ^b	8.64 ^b	12.34 ^a	0.005	0.036
<i>Rikenellaceae_RC9_gut_group</i>	8.14 ^{ab}	8.89 ^a	6.30 ^b	6.60 ^b	6.46 ^b	8.01 ^{ab}	0.003	0.069
<i>uncultured_bacterium</i>	7.87 ^{ab}	6.64 ^{ab}	5.93 ^b	8.74 ^a	9.35 ^a	7.93 ^{ab}	0.004	0.149
<i>unidentified</i>	6.75 ^b	9.37 ^{ab}	8.81 ^{ab}	8.82 ^{ab}	7.23 ^{ab}	9.44 ^a	0.004	0.151
<i>uncultured</i>	3.08	2.97	3.10	3.97	3.77	3.42	0.002	0.309
<i>Succiniclasticum</i>	3.63 ^b	3.71 ^b	6.86 ^a	4.27 ^{ab}	5.86 ^{ab}	3.89 ^b	0.004	0.147
<i>Butyrivibrio</i>	2.94	2.13	2.59	2.16	3.11	2.56	0.002	0.647
<i>Prevotellaceae_UCG_001</i>	2.47	3.00	2.7	2.06	2.12	2.86	0.001	0.333
<i>Prevotellaceae_UCG_003</i>	2.23	2.61	2.53	2.15	2.22	2.34	0.001	0.924
<i>Ruminococcus</i>	1.86 ^b	2.77 ^a	2.98 ^a	3.01 ^a	3.22 ^a	2.69 ^a	0.001	0.027
<i>NK4A214_group</i>	1.53 ^b	1.47 ^b	1.92 ^{ab}	2.32 ^a	2.05 ^{ab}	1.77 ^{ab}	0.001	0.122
<i>UCG005</i>	0.45	0.27	0.35	2.21	2.37	0.71	0.004	0.421
<i>Veillonellaceae_UCG_001</i>	1.35	0.73	0.96	1.30	1.30	1.20	0.001	0.409
<i>Lachnospiraceae_AC2044_group</i>	0.91 ^b	1.79 ^a	1.68 ^{ab}	1.31 ^b	1.47 ^{ab}	1.33 ^{ab}	0.001	0.010
<i>Lachnospiraceae_XPB1014_group</i>	0.84	1.01	1.47	1.14	1.06	0.85	0.001	0.421
<i>Treponema</i>	0.96	0.83	0.77	0.89	1.62	0.75	0.002	0.749
<i>Candidatus_Saccharimonas</i>	0.76	0.90	1.04	0.82	0.90	0.98	0.001	0.683
<i>Prevotellaceae_NK3B31_group</i>	0.67 ^b	0.83 ^b	1.39 ^a	0.84 ^b	0.64 ^b	0.87 ^{ab}	0.001	0.115
<i>Lachnospiraceae_NK4A136_group</i>	0.51 ^b	1.08 ^{ab}	1.15 ^a	0.75 ^b	0.73 ^b	0.55 ^b	0.001	0.002
<i>Succinivibrionaceae_UCG_002</i>	0.10	0.61	0.12	1.57	0.29	0.50	0.002	0.501

At genus level, *Prevotella* and *uncultured_rumen_bacterium* were the main dominant bacterial genera during the perinatal period in sheep.

3. Results

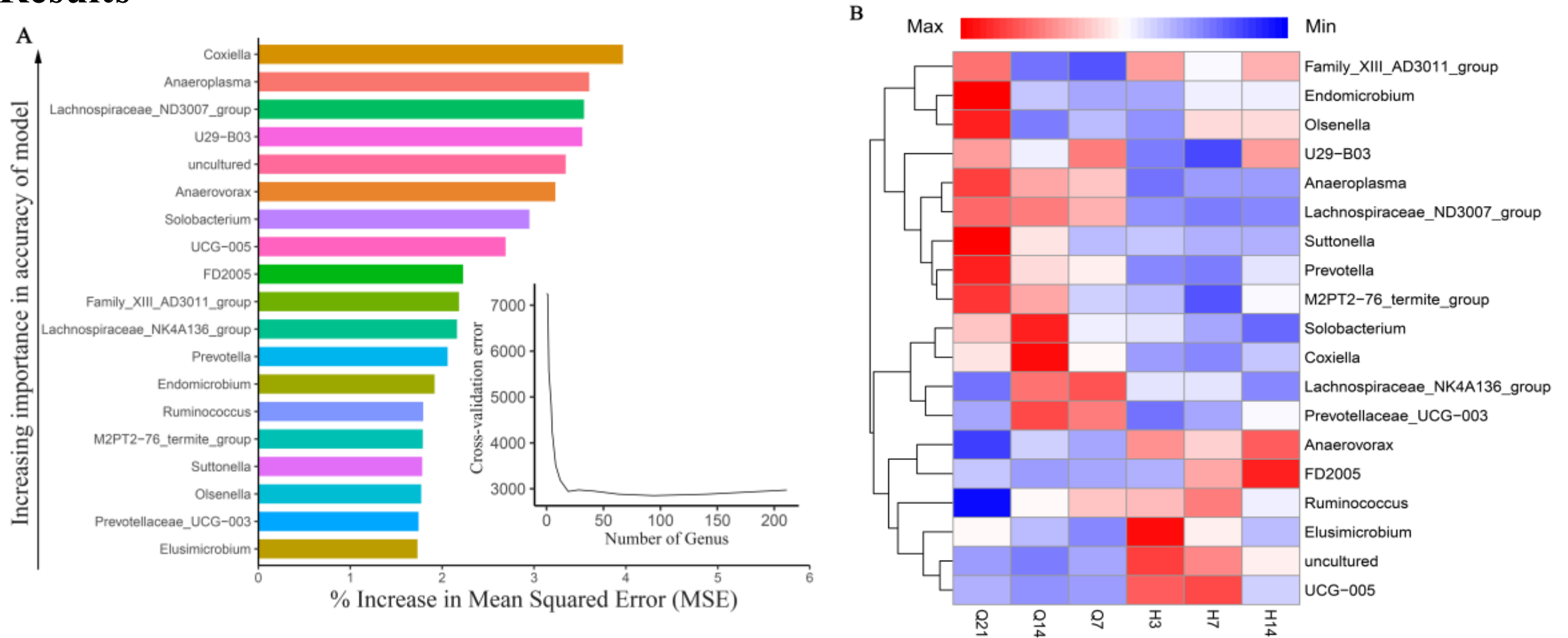
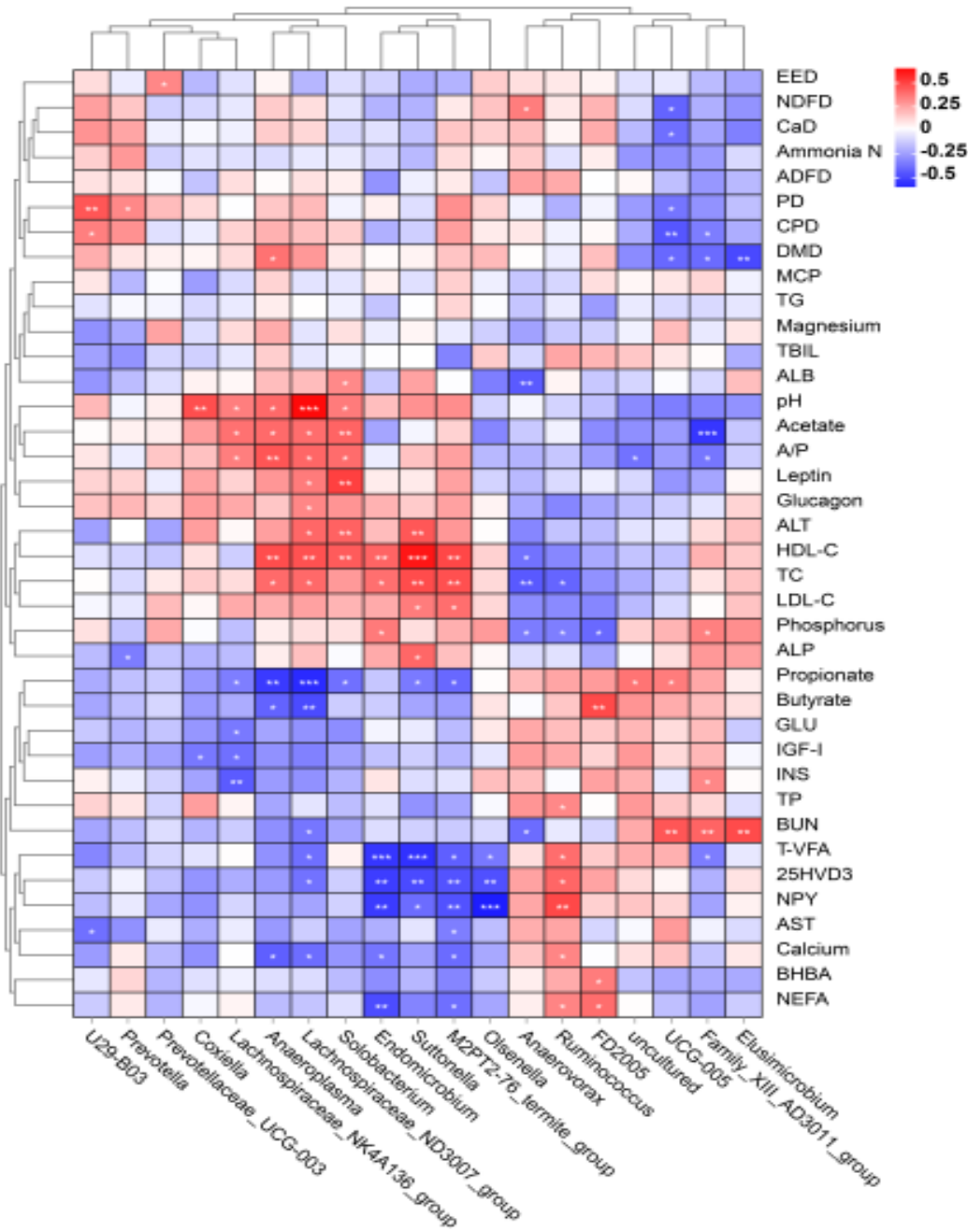


Fig. 3 Perinatal time-related bacterial taxonomic biomarkers and rumen bacterial genera of ewes.

- We used the random forest model to analyze the relative abundance of bacterial genera at six sampling time points. When the cross-validation error curve was stable, a total of nineteen biomarkers of bacterial genera were identified.
- A heat map showed the relative abundance of the top nineteen predicted biomarkers for bacterial genera at six time points.

3. Results



In figure four, we found that ten bacterial genera were positively correlated with the most rumen fermentation parameters and serum biochemical indexes, such as rumen pH, acetate, acetate to propionate ratio, total volatile fatty acid (T-VFA), high-density lipoprotein cholesterol (HDL-C), Total cholesterol (TC), 25-hydroxy vitamin D3 (25HVD3) and neuropeptide γ (NPY).

Fig. 4 A correlation analysis between the main bacterial genera with perinatal time-related changes and rumen fermentation parameters, serum biochemical indexes.

4. Conclusion

1. The major of nutrient digestibility, rumen fermentation parameters and serum biochemical indexes could alter along with the process of parturition.
2. Those bacteria genera (*Lachnospiraceae_NK4A136_group*, *Anaeroplasma*, *Lachnospiraceae_ND3007_group*, *Solobacterium*, *Endomicrobium*, *Suttonella*, *M2PT2-76_termite_group*, *Olsenella*, *Ruminococcus* and *UCG-005*) were the main bacteria that affected feed utilization and rumen fermentation patterns, which were of immense importance for shaping serum biochemical indexes.



THANKS!

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