

Supplementary materials

Table S1. The chemical compositions of the herbage.

Itmes	Values
Dry matter (DM), %	34.7
Chemical composition, % DM	
Crude protein	16.9
Acid detergent fiber	37.6
Neutral detergent fiber	63.4
Ether extract	3.3
Ash	9.6
Non-fiber carbohydrate ¹	12.7
Starch	382
Calcium	0.61
Phosphorus	0.33

¹ Non-fiber carbohydrate is calculated as $100 - (\text{crude protein} + \text{ether extract} + \text{ash} + \text{neutral detergent fiber})$.

Method:

Starter samples were analyzed for DM (method 930.15) [47], crude protein (method 976.05) [47], ether extract (method 920.39) [47], calcium (method 985.35) [47], and phosphorus (method 986.24) [47]. Starch was analyzed using a modified glucoamylase method [48]. Starch was hydrolyzed to free D-glucose, and the glucose concentration was then measured. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to methods of Van Soest et al. (1991) [49] with an Ankom 220 Fiber Analyzer (Ankom technology co., NY, USA). The NDF was analyzed with a heat stable α -amylase and sodium sulfite. Both NDF and ADF are expressed inclusive of residual ash.

Table S2. Significantly enriched predicted Level 3 KEGG pathways (relative abundance, %) on day 0 (D0), day 9 (D9), and day 12 (D12).

Pathway level1	Pathway level2	Pathway level2 Description	D0	D9	D12	SEM	<i>P</i> -value
Metabolism	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	1.3468 ^b	1.3568 ^a	1.3413 ^b	0.00268	0.0008
Metabolism	Carbohydrate metabolism	Starch and sucrose metabolism	0.9915 ^a	1.0018 ^a	0.9684 ^b	0.00522	0.0002
Metabolism	Carbohydrate metabolism	Fructose and mannose metabolism	0.8182 ^b	0.8306 ^a	0.8106 ^b	0.00414	0.0056
Metabolism	Carbohydrate metabolism	Galactose metabolism	0.6893 ^b	0.7008 ^a	0.6788 ^c	0.00266	< 0.0001
Metabolism	Carbohydrate metabolism	Butanoate metabolism	0.7025 ^a	0.6900 ^b	0.7078 ^a	0.00360	0.0038
Metabolism	Carbohydrate metabolism	Pentose and glucuronate interconversions	0.4789 ^a	0.4787 ^a	0.4722 ^b	0.00201	0.0347
Metabolism	Energy metabolism	Methane metabolism	1.3460 ^a	1.3374 ^b	1.3376 ^b	0.00256	0.0349
Metabolism	Energy metabolism	Carbon fixation pathways in prokaryotes	1.0830 ^b	1.0791 ^b	1.0976 ^a	0.00459	0.0173
Metabolism	Lipid metabolism	Glycerolipid metabolism	0.3465 ^{ab}	0.3521 ^a	0.3424 ^b	0.00208	0.0078
Metabolism	Lipid metabolism	Arachidonic acid metabolism	0.0287 ^b	0.0280 ^b	0.0304 ^a	0.00043	0.0011
Metabolism	Lipid metabolism	Steroid hormone biosynthesis	0.0082 ^b	0.0087 ^b	0.0101 ^a	0.00042	0.0080
Metabolism	Lipid metabolism	Steroid biosynthesis	0.00082 ^b	0.00118 ^b	0.00184 ^a	0.000186	0.0016
Metabolism	Lipid metabolism	Biosynthesis of unsaturated fatty acids	0.1177 ^b	0.1183 ^b	0.1219 ^a	0.00090	0.0040

Metabolism	Lipid metabolism	Secondary bile acid biosynthesis	0.0269 ^a	0.0279 ^a	0.0254 ^b	0.00049	0.0038
Metabolism	Lipid metabolism	Primary bile acid biosynthesis	0.0269 ^a	0.0280 ^a	0.0254 ^b	0.00049	0.0023
Metabolism	Xenobiotics biodegradation and metabolism	Polycyclic aromatic hydrocarbon degradation	0.0987 ^b	0.1006 ^a	0.0984 ^b	0.00054	0.0136
Metabolism	Biosynthesis of other secondary metabolites	Isoquinoline alkaloid biosynthesis	0.0577 ^{ab}	0.0565 ^b	0.0582 ^a	0.00047	0.0442
Metabolism	Xenobiotics biodegradation and metabolism	Ethylbenzene degradation	0.0493 ^{ab}	0.0485 ^b	0.0505 ^a	0.00053	0.0335
Metabolism	Xenobiotics biodegradation and metabolism	Atrazine degradation	0.0286 ^b	0.0296 ^b	0.0314 ^a	0.00059	0.0069
Metabolism	Metabolism of terpenoids and polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.0108 ^b	0.0123 ^a	0.0113 ^b	0.00031	0.0076
Metabolism	Glycan biosynthesis and metabolism	Various types of N-glycan biosynthesis	0.00029 ^b	0.00049 ^a	0.00023 ^b	0.000040	< 0.0001
Genetic Information Processing	Replication and repair	Non-homologous end-joining	0.00527 ^b	0.00607 ^{ab}	0.00660 ^a	0.000354	0.0361
Genetic Information Processing	Transcription	Basal transcription factors	0.00066 ^{ab}	0.00083 ^a	0.00047 ^b	0.00008	0.0125
Genetic Information Processing	Translation	Ribosome biogenesis in eukaryotes	0.0494 ^b	0.0497 ^a	0.0494 ^b	0.00011	0.0448

Genetic Information Processing	Folding, sorting and degradation	Proteasome	0.04784 ^b	0.04813 ^a	0.04753 ^c	0.000101	0.0007
Organismal Systems	Endocrine system	Insulin signaling pathway	0.0667 ^a	0.0683 ^a	0.0646 ^b	0.00065	0.0013
Organismal Systems	Excretory system	Proximal tubule bicarbonate reclamation	0.0234 ^{ab}	0.0231 ^b	0.0248 ^a	0.00048	0.0347
Organismal Systems	Digestive system	Carbohydrate digestion and absorption	0.0054 ^a	0.0059 ^a	0.0044 ^b	0.00030	0.0044
Organismal Systems	Environmental adaptation	Circadian rhythm - plant	0.00029 ^b	0.00044 ^{ab}	0.00063 ^a	0.000071	0.0055

SEM = standard error of the means.

^{a-c} Means without a common superscript within a row differ significantly ($P < 0.05$).

Table S3. The relationship of reproductive hormones and the biomarkers of the intestinal microbiota (uncorrelated results).

Item	GnRH	FSH	LH	PROG	E2	PRL	OT
<i>p__Cyanobacteria</i>	0.29	0.29	0.29	0.20	0.30	0.13	0.12
<i>o__unclassified_c__Clostridia</i>	0.22	0.06	0.16	0.11	0.16	0.05	0.14
<i>f__Muribaculaceae</i>	-0.08	0.03	-0.16	0.03	-0.23	0.02	0.01
<i>f__Bacteroidaceae</i>	-0.01	-0.07	-0.12	-0.16	0.11	0.18	-0.29
<i>f__F082</i>	-0.08	-0.18	-0.07	-0.29	0.12	-0.15	-0.11
<i>f__unclassified_c__Clostridia</i>	0.22	0.06	0.16	0.11	0.16	0.05	0.14
<i>g__Prevotellaceae_UCG-004</i>	-0.02	-0.07	-0.02	0.03	0.00	0.08	0.08
<i>g__Bacteroides</i>	-0.01	-0.07	-0.12	-0.16	0.11	0.18	-0.29
<i>g__norank_f__Muribaculaceae</i>	-0.09	0.16	-0.08	0.10	-0.22	-0.02	0.12
<i>g__norank_f__Oscillospiraceae</i>	-0.01	0.14	0.08	-0.26	0.12	0.11	-0.07
<i>g__Alloprevotella</i>	0.04	0.00	0.01	0.01	0.30	0.15	-0.15
<i>g__norank_f__F082</i>	-0.08	-0.18	-0.07	-0.29	0.12	-0.15	-0.11
<i>g__Agathobacter</i>	-0.05	-0.17	-0.16	-0.19	-0.21	0.00	-0.09
<i>g__unclassified_c__Clostridia</i>	0.22	0.06	0.16	0.11	0.16	0.05	0.14

<i>g__UCG-002</i>	0.08	0.18	0.21	0.08	0.22	0.24	0.16
<i>p__Actinobacteriota</i>	-0.19	-0.36*	-0.24	-0.13	-0.33*	-0.10	0.05
<i>p__Verrucomicrobiota</i>	0.25	0.36*	0.36*	0.32*	0.44**	0.30	0.26
<i>p__Patescibacteria</i>	-0.30	-0.41**	-0.20	-0.03	-0.30	-0.22	0.02
<i>o__Acidaminococcales</i>	0.23	0.27	0.22	0.08	0.38*	0.06	0.01
<i>o__Christensenellales</i>	-0.26	0.03	0.11	0.15	0.16	0.08	0.40**
<i>o__Saccharimonadales</i>	-0.30	-0.41**	-0.20	-0.03	-0.30	-0.22	0.02
<i>o__Gastranaerophilales</i>	0.29	0.29	0.29	0.20	0.31*	0.13	0.12
<i>o__Verrucomicrobiales</i>	0.19	0.34*	0.31*	0.28	0.41**	0.31*	0.27
<i>o__Coriobacteriales</i>	-0.21	-0.32*	-0.31*	-0.20	-0.27	-0.12	0.00
<i>c__Vampirivibrionia</i>	0.19	0.34*	0.31*	0.28	0.41**	0.31*	0.27
<i>c__Negativicutes</i>	0.20	0.25	0.21	0.07	0.38*	0.06	0.01
<i>c__Coriobacteriia</i>	-0.21	-0.32*	-0.31*	-0.20	-0.27	-0.12	0.00
<i>c__Verrucomicrobiae</i>	0.19	0.34*	0.31*	0.28	0.41**	0.31*	0.27
<i>c__Saccharimonadia</i>	-0.30	-0.41**	-0.20	-0.03	-0.30	-0.22	0.02
<i>f__Acidaminococcaceae</i>	0.23	0.27	0.22	0.08	0.38*	0.06	0.01

<i>f__Christensenellaceae</i>	-0.26	0.03	0.11	0.15	0.16	0.08	0.40**
<i>f__Hungateiclostridiaceae</i>	-0.08	-0.43**	-0.21	-0.21	-0.35*	-0.37*	0.07
<i>f__norank_o__Gastranaerophilales</i>	0.29	0.29	0.29	0.20	0.31*	0.13	0.12
<i>f__Akkermansiaceae</i>	0.19	0.34*	0.31*	0.28	0.41*	0.31*	0.27
<i>f__Atopobiaceae</i>	-0.23	-0.35*	-0.35*	-0.36*	-0.38*	-0.16	-0.07
<i>f__Saccharimonadaceae</i>	-0.30	-0.41**	-0.20	-0.03	-0.30	-0.22	0.02
<i>g__Ruminococcus_torques_group</i>	-0.22	-0.42**	-0.32*	-0.29	-0.51#	-0.17	-0.22
<i>g__Phascolarctobacterium</i>	0.24	0.27	0.23	0.09	0.39*	0.07	0.01
<i>g__Christensenellaceae_R-7_group</i>	-0.27	0.02	0.10	0.16	0.15	0.11	0.40**
<i>g__norank_f__Ruminococcaceae</i>	-0.19	-0.16	-0.03	-0.05	-0.31*	0.10	0.13
<i>g__Candidatus_Saccharimonas</i>	-0.30	-0.41**	-0.20	-0.03	-0.30	-0.22	0.02
<i>g__norank_f__norank_o__Gastranaerophilales</i>	0.29	0.29	0.29	0.20	0.31*	0.13	0.12
<i>g__Akkermansia</i>	0.19	0.34*	0.31*	0.28	0.41**	0.31*	0.27
<i>g__Saccharofermentans</i>	-0.14	-0.37*	-0.30	-0.30	-0.44**	-0.40**	0.06
<i>g__Olsenella</i>	-0.26	-0.34*	-0.35*	-0.37*	-0.40**	-0.20	-0.10

GnRH: gonadotropin-releasing hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PROG: progesterone, E2: Estrogen 2, PRL: prolactin, and OT: oxytocin.