Supplementary materials

Figure S1. Scrotal insulation bag (Fig. S1A) with temperature and humidity sensor (Fig. S1B).

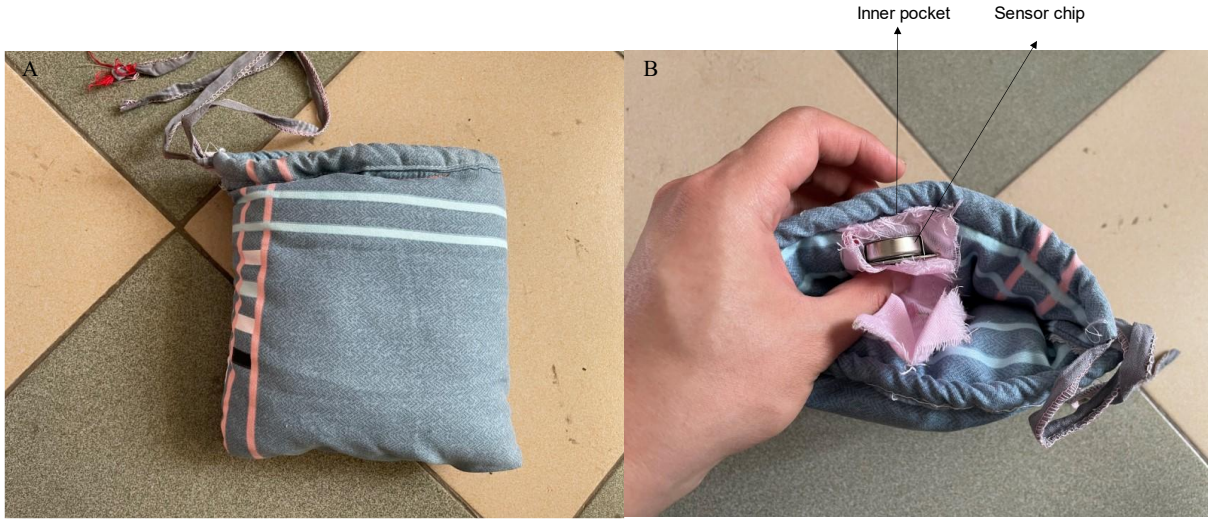


Figure S2. Scrotal surface humidity in 3 days.

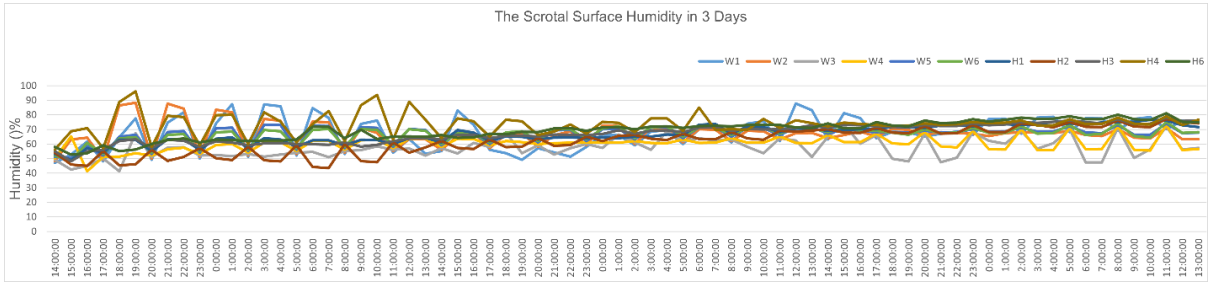


Table S1. GO analysis in BP ontology (W-SI vs W-CTR).

ID	Description	pval	Count
GO:0007283	Spermatogenesis	1.91064E-23	248
GO:0007155	Cell adhesion	3.47203E-18	256
GO:0003341	Cilium movement	1.80895E-13	39
GO:0016477	Cell migration	3.22131E-12	139
GO:0030198	Extracellular matrix organization	4.27912E-12	122
GO:0007275	Multicellular organism development	1.8517E-10	268
GO:0030317	Flagellated sperm motility	6.77671E-10	58
GO:0030154	Cell differentiation	1.10874E-09	328
GO:0006508	Proteolysis	2.35995E-08	111
GO:0007018	Microtubule-based movement	6.90458E-08	58
GO:0007160	Cell-matrix adhesion	6.82362E-08	62
GO:0007339	Binding of sperm to zona pellucida	6.58241E-08	42
GO:0050808	Synapse organization	7.28368E-08	43
GO:0060271	Cilium assembly	1.02245E-07	124
GO:0030199	Collagen fibril organization	2.09571E-07	50
GO:0007156	Homophilic cell adhesion via plasma membrane adhesion molecules	3.73165E-07	86
GO:0060285	Cilium-dependent cell motility	4.32491E-07	17
GO:0006836	Neurotransmitter transport	5.2612E-07	40
GO:0007129	Homologous chromosome pairing at meiosis	8.76699E-07	26
GO:0035082	Axoneme assembly	8.78672E-07	20
GO:0001525	Angiogenesis	1.25527E-06	119
GO:0003351	Epithelial cilium movement involved in extracellular fluid movement	2.06386E-06	13
GO:0008360	Regulation of cell shape	2.50225E-06	80
GO:0034446	Substrate adhesion-dependent cell spreading	3.85644E-06	40
GO:0098609	Cell-cell adhesion	4.00395E-06	73
GO:0007411	Axon guidance	5.51819E-06	111
GO:0034220	Ion transmembrane transport	6.27105E-06	66
GO:0007165	Signal transduction	9.3029E-06	328
GO:0048167	Regulation of synaptic plasticity	9.74674E-06	30
GO:0007507	Heart development	1.13326E-05	87
GO:0006749	Glutathione metabolic process	1.15359E-05	33
GO:0007613	Memory	1.24806E-05	40
GO:0045165	Cell fate commitment	1.24368E-05	36
GO:0051480	Regulation of cytosolic calcium ion concentration	1.25986E-05	25
GO:0007420	Brain development	1.30999E-05	107
GO:0036158	Outer dynein arm assembly	1.80191E-05	18
GO:0035725	Sodium ion transmembrane transport	2.1084E-05	41
GO:0002576	Platelet degranulation	2.15136E-05	32
GO:0051965	Positive regulation of synapse assembly	2.203E-05	33

GO:0045766	Positive regulation of angiogenesis	2.28639E-05	58
GO:0046513	Ceramide biosynthetic process	2.87513E-05	25
GO:0055085	Transmembrane transport	3.71062E-05	67
GO:0034587	Pirna processing	3.9346E-05	14
GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	3.99317E-05	66
GO:0031274	Positive regulation of pseudopodium assembly	4.23781E-05	10
GO:0060317	Cardiac epithelial to mesenchymal transition	4.23781E-05	10
GO:0018146	Keratan sulfate biosynthetic process	4.8709E-05	12
GO:0007605	Sensory perception of sound	4.93234E-05	72
GO:0016525	Negative regulation of angiogenesis	5.03113E-05	44
GO:0007099	Centriole replication	5.44021E-05	18
GO:0007229	Integrin-mediated signaling pathway	6.46498E-05	54
GO:0007286	Spermatid development	7.8726E-05	51
GO:0034765	Regulation of ion transmembrane transport	7.81339E-05	66
GO:0007413	Axonal fasciculation	9.47941E-05	13
GO:0036159	Inner dynein arm assembly	9.47941E-05	13
GO:0050982	Detection of mechanical stimulus	9.47941E-05	13
GO:0071805	Potassium ion transmembrane transport	9.43531E-05	59
GO:0008285	Negative regulation of cell population proliferation	0.000100314	158
GO:0002040	Sprouting angiogenesis	0.000111195	23
GO:0007141	Male meiosis I	0.00011767	17
GO:0007219	Notch signaling pathway	0.000117472	56
GO:0043588	Skin development	0.000118166	24
GO:0009612	Response to mechanical stimulus	0.000123565	25
GO:0016042	Lipid catabolic process	0.000123958	50
GO:0030501	Positive regulation of bone mineralization	0.000123565	25
GO:0070374	Positive regulation of ERK1 and ERK2 cascade	0.000135324	94
GO:0007338	Single fertilization	0.000183406	44
GO:0010951	Negative regulation of endopeptidase activity	0.000214925	32
GO:0035556	Intracellular signal transduction	0.000223178	168
GO:0009566	Fertilization	0.000231981	29
GO:0051497	Negative regulation of stress fiber assembly	0.000235935	25
GO:0070588	Calcium ion transmembrane transport	0.000291952	36
GO:0007130	Synaptonemal complex assembly	0.00029693	17
GO:0070831	Basement membrane assembly	0.000311389	10
GO:0032488	Cdc42 protein signal transduction	0.000317659	8
GO:0030036	Actin cytoskeleton organization	0.000331802	83
GO:0050679	Positive regulation of epithelial cell proliferation	0.000353131	32
GO:0006821	Chloride transport	0.00038189	30
GO:0009888	Tissue development	0.000429232	23
GO:0051017	Actin filament bundle assembly	0.000422436	22
GO:0001764	Neuron migration	0.000443098	56
GO:0001558	Regulation of cell growth	0.000535746	33

GO:0007340	Acrosome reaction	0.000530912	15
GO:0051321	Meiotic cell cycle	0.000534557	45
GO:0014068	Positive regulation of phosphatidylinositol 3-kinase signaling	0.000564571	32
GO:0007157	Heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	0.000593595	31
GO:1902476	Chloride transmembrane transport	0.000593595	31
GO:0006509	Membrane protein ectodomain proteolysis	0.000608599	16
GO:2001238	Positive regulation of extrinsic apoptotic signaling pathway	0.000608599	16
GO:0007517	Muscle organ development	0.000619389	43
GO:0007266	Rho protein signal transduction	0.000650958	29
GO:0007140	Male meiotic nuclear division	0.000670873	17
GO:0010718	Positive regulation of epithelial to mesenchymal transition	0.000678471	28
GO:0046777	Protein autophosphorylation	0.000675278	83
GO:0005975	Carbohydrate metabolic process	0.000745776	67
GO:0007626	Locomotory behavior	0.000732213	35
GO:0019228	Neuronal action potential	0.000751623	19
GO:0035855	Megakaryocyte development	0.000752715	12
GO:0042073	Intraciliary transport	0.000751623	19
GO:0003222	Ventricular trabecula myocardium morphogenesis	0.000778887	9
GO:0060294	Cilium movement involved in cell motility	0.000778887	9
GO:0006936	Muscle contraction	0.000827288	39
GO:0097062	Dendritic spine maintenance	0.000869609	7
GO:0005576	Extracellular region	3.79602E-18	704
GO:0031012	Extracellular matrix	6.81415E-16	148
GO:0009986	Cell surface	3.45828E-15	276
GO:0031514	Motile cilium	2.67878E-14	80
GO:0005930	Axoneme	4.26751E-14	84
GO:0001669	Acrosomal vesicle	1.80123E-13	70
GO:0062023	Collagen-containing extracellular matrix	2.45118E-13	135
GO:0005737	Cytoplasm	3.28725E-11	2289
GO:0036126	Sperm flagellum	5.50691E-11	57
GO:0005929	Cilium	5.21728E-10	129
GO:0016324	Apical plasma membrane	2.15207E-09	189
GO:0005856	Cytoskeleton	6.31881E-09	230
GO:0016323	Basolateral plasma membrane	8.34977E-09	121
GO:0005814	Centriole	1.34331E-08	94
GO:0005788	Endoplasmic reticulum lumen	2.24558E-08	106
GO:0045121	Membrane raft	9.51612E-08	137
GO:0036064	Ciliary basal body	1.10433E-07	93
GO:0005912	Adherens junction	1.49897E-07	88
GO:0005874	Microtubule	2.14419E-07	171
GO:0030425	Dendrite	2.04588E-07	220
GO:0005858	Axonemal dynein complex	2.75138E-07	15
GO:0070062	Extracellular exosome	3.23383E-07	433

GO:0005901	Caveola	6.37581E-07	44
GO:0005925	Focal adhesion	7.77686E-07	158
GO:0043025	Neuronal cell body	7.71519E-07	170
GO:0045211	Postsynaptic membrane	7.54519E-07	81
GO:0032809	Neuronal cell body membrane	1.48981E-06	21
GO:0009897	External side of plasma membrane	2.50973E-06	161
GO:0030424	Axon	2.53986E-06	158
GO:0015629	Actin cytoskeleton	3.70398E-06	116
GO:0030175	Filopodium	3.92303E-06	44
GO:0097228	Sperm principal piece	4.99601E-06	18
GO:0005615	Extracellular space	5.92325E-06	697
GO:0001725	Stress fiber	7.03335E-06	41
GO:0043235	Receptor complex	1.0272E-05	96
GO:0036157	Outer dynein arm	1.54769E-05	11
GO:0042383	Sarcolemma	1.71865E-05	56
GO:0000795	Synaptonemal complex	1.80191E-05	18
GO:0005813	Centrosome	2.19994E-05	242
GO:0031410	Cytoplasmic vesicle	6.04039E-05	150
GO:0005911	Cell-cell junction	6.54841E-05	87
GO:0048786	Presynaptic active zone	7.27671E-05	32
GO:0098978	Glutamatergic synapse	7.2672E-05	155
GO:0008076	Voltage-gated potassium channel complex	8.30612E-05	43
GO:0005604	Basement membrane	0.000101024	53
GO:0031528	Microvillus membrane	0.00011767	17
GO:0005794	Golgi apparatus	0.00013188	413
GO:0030018	Z disc	0.000157331	69
GO:0042734	Presynaptic membrane	0.00018412	36
GO:0098688	Parallel fiber to Purkinje cell synapse	0.000200888	15
GO:0097225	Sperm midpiece	0.000237765	26
GO:0000775	Chromosome, centromeric region	0.00042752	48
GO:0043197	Dendritic spine	0.000423096	77
GO:0034707	Chloride channel complex	0.000622515	30
GO:0031234	Extrinsic component of cytoplasmic side of plasma membrane	0.000643757	37
GO:0005940	Septin ring	0.000752715	12
GO:0032153	Cell division site	0.000752715	12
GO:0098686	Hippocampal mossy fiber to CA3 synapse	0.000751623	19
GO:0042824	MHC class I peptide loading complex	0.000869609	7
GO:0005509	Calcium ion binding	2.43156E-28	413
GO:0050839	Cell adhesion molecule binding	3.71827E-08	44
GO:0004222	Metalloendopeptidase activity	4.26752E-07	77
GO:0005524	ATP binding	4.74879E-07	736
GO:0008201	Heparin binding	1.84217E-06	94
GO:0008569	Minus-end-directed microtubule motor activity	2.69446E-06	17
GO:0005516	Calmodulin binding	3.26192E-06	121

GO:0005201	Extracellular matrix structural constituent	6.05503E-06	49
GO:0005251	Delayed rectifier potassium channel activity	6.86472E-06	22
GO:0005102	Signaling receptor binding	7.26756E-06	142
GO:0050840	Extracellular matrix binding	1.12725E-05	20
GO:0003777	Microtubule motor activity	1.2601E-05	38
GO:0003779	Actin binding	1.27417E-05	135
GO:0008017	Microtubule binding	1.57799E-05	138
GO:0004252	Serine-type endopeptidase activity	1.60921E-05	94
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	1.83167E-05	67
GO:1990837	Sequence-specific double-stranded DNA binding	4.53444E-05	201
GO:0003700	DNA-binding transcription factor activity	5.64791E-05	198
GO:0051015	Actin filament binding	5.93339E-05	113
GO:0008237	Metallopeptidase activity	7.47062E-05	45
GO:0015293	Symporter activity	0.00010058	39
GO:0048407	Platelet-derived growth factor binding	0.000116029	9
GO:0005543	Phospholipid binding	0.00014166	48
GO:0005178	Integrin binding	0.000164238	79
GO:0005249	Voltage-gated potassium channel activity	0.000192235	35
GO:0005518	Collagen binding	0.000214925	32
GO:0005080	Protein kinase C binding	0.000237519	27
GO:0043394	Proteoglycan binding	0.000317659	8
GO:0005262	Calcium channel activity	0.000322632	34
GO:0004713	Protein tyrosine kinase activity	0.000377301	39
GO:0002020	Protease binding	0.000401406	38
GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	0.000423337	185
GO:0004364	Glutathione transferase activity	0.000429232	23
GO:0004435	Phosphatidylinositol phospholipase C activity	0.00043871	14
GO:0030165	PDZ domain binding	0.000601457	48
GO:0051959	Dynein light intermediate chain binding	0.000778673	23
GO:0005021	Vascular endothelial growth factor receptor activity	0.000869609	7

Table S2. Gene primer sequences.

Genes	Primers sequence (5'- >3')	Accession number	Product length (bp)
SYCP2	F: ACCACCACTGCAAATGACGA R: CTGCTGCCTGTGAATCGAGA	XM_027976988	242
SYCP3	F: TCCGGGAAGTTGGCAAAACC R: GGTCTTCTCTTCAATGGCATCC	XM_019961171.1	117
CDK1	F: ATGGCTTGGATCTGCTCTCG R: TGCTCTTGACACAACACAGGA	NM_001142508.1	154
DDX4	F: AGTGCCCTGTTCTTGTTGCT R: ACGACCAGTACGCCCAATTC	XM_054352837	131
TNP1	F: GGCATGA GGAGGGGCAAGAAC R: TCACAAGTGGGAGCGCAAATTG	NM_003284	138
β-Actin	F: CTCTTCCAGCCTTCCTTCCT R: GGGCAGTGATCTCTTTCTGC	NM_001101	178

Table S3A. Differential gene expression in W-SI vs W-CTR group

Genes	BaseMean	Log2FoldChange	padj	Up/Down	Significant
SYCP2	373.2611	-3.31832	0.000771554	down	yes
SYCP3	1181.179	-3.1935	1.42E-16	down	yes
CDK1	93.93165	-3.202823606	1.50E-15	down	yes
DDX4	2582.497	-3.26615	3.68E-23	down	yes
TNP1	1697.903	-3.78439	2.55E-05	down	yes

Table S3B. Differential gene expression in H-SI vs H-CTR group

Genes	BaseMean	Log2FoldChange	padj	Up/Down	Significant
SYCP2	2068.168	0.079455535	0.84680444	up	no
SYCP3	267.0271	-0.393669119	0.374167887	down	no
CDK1	160.484196	0.139946713	0.787100424	up	no
DDX4	5105.318	0.212797637	0.533122991	up	no
TNP1	8805.094	0.884308519	0.122281352	up	no

Table S4 Significantly enriched pathways of DEGs associated with spermatogenesis.

Map	Name	pval	Up_Gene	Down_Gene
map04114	Oocyte meiosis	2E-04	ADCY4|K08044;ADCY5|K08045;ADCY8|K08048;CALML4|K02183;CAMK2A|K04515;CAMK2G|K04515;IGF1|K05459;IGF1R|K05087;ITPR2|K04959;LOC114117278|K06631;LOC114118477|K05868;LOC121816477|K08047;MAP2K1|K04368;MAPK11|K04441;MAPK12|K04441;MAPK14|K04441;PPP2CB|K04382;PPP2R1A|K03456;PPP2R5B|K11584;PPP3CA|K04348;PPP3R1|K06268;PRKACB|K04345;RPS6KA3|K04373;YWHAB|K16197;YWHAG|K16198;YWHAH|K16198;YWHAZ|K16197	ADCY1|K08041;ANAPC10|K03357;ANAPC11|K03358;ANAPC2|K03349;ANAPC5|K03352;AURKA|K11481;BUB1|K02178;CALM3|K02183;CCNB1|K05868;CCNB2|K21770;CCNE1|K06626;CCNE2|K06626;CDC20|K03363;CDC25C|K05867;CDK1|K02087;CDK2|K02206;CPEB1|K02602;CPEB2|K02602;CPEB3|K02602;CUL1|K03347;ESPL1|K02365;FBXO43|K10318;FBXO5|K10292;ITPR3|K04960;LOC101107408|K08694;LOC101114750|K03359;LOC101117953|K06269;LOC101121823|K16198;LOC105609549|K03363;LOC105614854|K02183;LOC114112929|K02183;LOC114112940|K03094;LOC114117819|K05868;LOC121817856|K13055;MAD2L1|K02537;PGR|K08556;PKMYT1|K06633;PLCZ1|K05861;PLK1|K06631;PPP1CC|K06269;PPP2R5C|K11584;PPP3R2|K06268;PTTG1|K06635;REC8|K13054;SGO1|K11580;SMC1B|K06636;SPDYA|K08694;STAG3|K13055
map04110	Cell cycle	0.015	CCND3|K10152;CDKN1A|K06625;CDKN1B|K06624;CHEK2|K06641;E2F2|K09389;GADD45A|K04402;GADD45B|K04402;GADD45G|K04402;LOC105609509|K02603;LOC114117278|K06631;LOC114118477|K05868;MYC|K04377;RAD21|K06670;SMAD3|K23605;STAG2|K06671;TGFB2|K13376;TP53|K04451;YWHAB|K16197;YWHAG|K16198;YWHAH|K16198;YWHAZ|K16197	ANAPC10|K03357;ANAPC11|K03358;ANAPC2|K03349;ANAPC5|K03352;BUB1|K02178;BUB1B|K06637;BUB3|K02180;CCNA1|K06627;CCNA2|K06627;CCNB1|K05868;CCNB2|K21770;CCND1|K04503;CCNE1|K06626;CCNE2|K06626;CDC14A|K06639;CDC20|K03363;CDC25A|K06645;CDC25C|K05867;CDC45|K06628;CDC6|K02213;CDK1|K02087;CDK2|K02206;CDKN2C|K06622;CDKN2D|K06623;CHEK1|K02216;CUL1|K03347;DBF4|K06629;E2F1|K17454;ESPL1|K02365;LOC101114750|K03359;LOC101121823|K16198;LOC105609549|K03363;LOC114112940|K03094;LOC114113021|K06621;LOC114117819|K05868;MAD2L1|K02537;MCM2|K02540;MCM4|K02212;MCM5|K02209;MCM6|K02542;PCNA|K04802;PKMYT1|K06633;PLK1|K06631;PTTG1|K06635;RBL1|K04681;SFN|K06644;SMC1B|K06636;TFDP1|K04683;TGFB3|K13377;TTK|K08866;WEE2|K06632

Table S5 Effect of Scrotal Insulation on Semen Quality, PMN, and Sperm Concentration in Wugu-Hu and Hu Sheep

Day	Concentration (Spz/ml)	Progressive motility (%)	PMN (%)	Concentration (Spz/ml)	Progressive Motility	PMN (%)
		Wugu-Hu sheep			Hu sheep	
CTR	4.03±0.10^{a**}	82.00±0.90^{a}	97.35±0.29^{a}	4.77±0.09^{a}	82.15±2.67^{a}	97.54±0.07^{a}
Day 11	4.59±0.42^{a*}	39.83±6.96^{c}	94.14±0.29^{c*}	5.77±0.15^{c}	37.45±5.86^{c}	95.93±0.51^{b}
Day 16	4.14±0.26^{a}	56.45±2.04^{c}	95.18±0.66^{c}	4.68±0.37^{a}	63.58±4.63^{b}	95.95±0.50^{b}
Day 21	3.11±0.22^{c}	77.87±3.92^{a}	94.75±0.63^{c}	3.61±0.44^{b}	83.06±3.22^{a}	94.98±0.42^{c}
Day 26	4.63±0.41^{a}	73.40±5.94^{a}	95.42±0.51^{c}	5.02±0.34^{a}	80.92±3.37^{a}	96.30±0.62^{a}
Day 31	3.26±0.09^{c}	84.08±2.69^{a}	94.21±0.64^{c}	3.49±0.08^{c}	89.91±1.19^{b}	95.81±0.64^{b}
Day 36	2.78±0.22^{c}	85.95±2.68^{a}	93.29±0.56^{c}	3.63±0.24^{c}	87.20±2.10^{a}	94.22±0.52^{c}
Day 41	3.13±0.39^{b}	75.76±3.12^{a*}	93.80±0.36^{c}	4.18±0.18^{b}	88.33±2.03^{a}	94.19±0.52^{c}
Day 46	4.28±0.53^{a}	84.58±2.57^{a*}	94.50±0.61^{c}	5.06±0.19^{a}	90.32±0.76^{b}	94.82±0.54^{c}
Day 51	3.83±0.18^{a**}	83.70±1.55^{a}	96.62±0.60^{a}	4.68±0.11^{a}	84.84±0.59^{a}	96.16±0.66^{a}
Day 56	4.10±0.07^{a**}	82.56±0.86^{a}	97.26±0.45^{a}	4.78±0.09^{a}	83.63±1.31^{a}	97.22±0.20^{a}

*a, b, c: Differing subscripts denote a significant difference within the group compared to CTR, with a: no significant difference (P>0.05); b: significant difference (P<0.05); and c: highly significant difference (P<0.01). * Significant difference between breeds on the same day (P<0.05); ** highly significant difference (P<0.01). The lack of differing superscripts indicates p>0.05.*