**Supplementary Tables**

**Table S1**. Concentrate components and nutrient levels of the basal diet (dry matter basis, %)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ingredients | Formula of concentrate | | Nutrients | TMR nutrient levels | |
| EG | LG | EG | LG |
| Corn | 53.00 | 50.00 | Dry matter | 92.35 | 90.96 |
| Soybean meal | 9.70 | 22.50 | Crude protein | 9.60 | 10.00 |
| Rapeseed meal | 12.00 | 7.00 | Ether extract | 3.51 | 4.52 |
| Wheat bran | 15.70 | 11.50 | Ash | 11.19 | 8.85 |
| Limestone | 1.00 | 1.00 | Neutral detergent fiber | 49.19 | 34.15 |
| CaHPO4 | 0.60 | 0.60 | Acid detergent fiber | 29.98 | 24.86 |
| NaHCO3 | 1.30 | 1.30 | Metabolizable energy 2) | 1.93 | 2.15 |
| NaCl | 0.80 | 0.40 | Calcium | 0.54 | 0.56 |
| Vitamin E | 0.40 | 0.10 | Phosphorus | 0.37 | 0.26 |
| Soybean oil | 0.30 | 0.40 |  | | |
| Premix1) | 5.00 | 5.00 |
| De-mold agent | 0.20 | 0.20 |
| Total | 100.00 | 100.00 |  |  |  |

TMR, total mixed ration; EG, early gestation; LG, late gestation.

1)Premix provide for per kilogram TMR: VA 30000 IU, VD 10000 IU, VE 100mg, Fe 90 mg, Cu 12.5 mg, Mn 50 mg, Zn 80 mg, Se 0.3 mg, I 0.8 mg, Co 0.5 mg.

2) Metabolizable energy value was calculated according to the NRC, unit: Mcal / kg

**Table S2**. Primers sequence for qRT-PCR verify test

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Genes name | Primers sequence (Forward, 5' to 3') | Primers sequence (Reverse, 5' to 3') |
| TW | *IGF1* | GTCACATCCTCCTCGCATCTCTTC | CCTGTCTCCGCACACGAACTG |
| *PRKAG2* | TCCGCTTCTTCTCTCGCTCCAG | GTCGCTCAGGCTCTTGCTTGTAG |
| *PRKAG3* | GCCTCTACTCCCGCTTTGATGTG | TCCTCCGCCTCAGTGCTTCTC |
| *PCK1* | GAGGGTGTCCGTGGTTGTCTTTG | TGAGTAGTTGAGGCCGTCTGAGAG |
| *SDS* | GCCTCTTGTGCGGAGTGGTTC | GCCTTGGTGGAAGCGTGGAAG |
| *SDSL* | CCTGGATGATGAGCGAACACTGG | GCACACGATGACCACGACTGAG |
| TR | *CCNE2* | TGATGGTGCTTGCAGTGAAGAGG | TAGCCAGGAGATGACCGTTACAGG |
| *CCNA2* | ACCACAGCACGCACAACAGTC | AGTGTCTCTGGTGGGTTGAGGAG |
| *CDC20* | GCAGACCTTCACCCAGCATCAAG | CCGCCAGTTGCCAGGACATTG |
| *BUB1* | TGGAGATGTCGCAGGGGAACC | TGCTGAGGGACGGCTGGAAG |
| *POLD1* | GTGAAGACATCGGTGCGGAAGG | GTCCAACACTTGTCGCCGTAGG |
| *POLD2* | TGCTGCTGGTGTCTGGTCTGG | CACTGCACTGCTCTCCTTCATCC |
|  | *GAPDH* | CGGCACAGTCAAGGCAGAGAAC | CACGTACTCAGCACCAGCATCAC |

TW, Twin lambs; TR, Triplet lambs; *IGF1*, Insulin Growth Factor 1; *PRKAG2,* Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 2; *PRKAG3,* Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 3; *PCK1,* Phosphoenolpyruvate Carboxykinase 1; *SDS,* Serine Dehydratase; *SDSL,* Serine Dehydratase Like; *CCNE2,* Cyclin E2; *CCNA2,* Cyclin A2; *CDC20,* Cell Division Cycle 20; *BUB1,* BUB1 Mitotic Checkpoint Serine/Threonine Kinase; *POLD1,* DNA Polymerase Delta 1, Catalytic Subunit; *POLD2,* DNA Polymerase Delta 2, Catalytic Subunit; *GAPDH,* Glyceraldehyde-3-Phosphate Dehydrogenase.

**Table S3.** Quality contronl of the raw and clean data

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Raw Reads | Raw Bases | Clean Reads | Clean Bases | Error Rate | Q20 | Q30 | GC Content |
| TW\_C\_1\_1 | 20487438 | 3.07G | 20130449 | 3.02G | 0.03% | 97.58% | 93.44% | 50.68% |
| TW\_C\_1\_2 | 20487438 | 3.07G | 20130449 | 3.02G | 0.03% | 97.41% | 92.96% | 50.67% |
| TW\_C\_2\_1 | 21408492 | 3.21G | 20976019 | 3.15G | 0.03% | 97.60% | 93.46% | 51.00% |
| TW\_C\_2\_2 | 21408492 | 3.21G | 20976019 | 3.15G | 0.03% | 97.17% | 92.43% | 50.98% |
| TW\_C\_3\_1 | 21260504 | 3.18G | 20812826 | 3.12G | 0.03% | 97.63% | 93.53% | 50.67% |
| TW\_C\_3\_2 | 21260504 | 3.18G | 20812826 | 3.12G | 0.03% | 97.29% | 92.66% | 50.65% |
| TW\_C\_4\_1 | 20913821 | 3.13G | 20347837 | 3.05G | 0.03% | 97.09% | 92.31% | 47.64% |
| TW\_C\_4\_2 | 20913821 | 3.2G | 20943947 | 3.14G | 0.03% | 97.60% | 93.45% | 50.30% |
| TW\_C\_5\_1 | 21336140 | 3.2G | 20943947 | 3.14G | 0.03% | 97.60% | 93.45% | 50.30% |
| TW\_C\_5\_2 | 21336140 | 3.2G | 20943947 | 3.14G | 0.03% | 96.57% | 91.10% | 50.28% |
| TW\_C\_6\_1 | 25775156 | 3.86G | 25253560 | 3.79G | 0.03% | 97.60% | 93.49% | 50.90% |
| TW\_C\_6\_2 | 25775156 | 3.86G | 25253560 | 3.79G | 0.03% | 97.33% | 92.73% | 50.89% |
| TR\_C\_1\_1 | 20478627 | 3.07G | 20048546 | 3.01G | 0.03% | 97.63% | 93.53% | 50.81% |
| TR\_C\_1\_2 | 20478627 | 3.07G | 20048546 | 3.01G | 0.03% | 97.33% | 92.75% | 50.79% |
| TR\_C\_2\_1 | 23606122 | 3.54G | 23127806 | 3.47G | 0.03% | 97.66% | 93.53% | 50.80% |
| TR\_C\_2\_2 | 23606122 | 3.54G | 23127806 | 3.47G | 0.03% | 97.57% | 93.23% | 50.80% |
| TR\_C\_3\_1 | 19731762 | 2.95G | 19456890 | 2.92G | 0.03% | 97.67% | 93.56% | 50.51% |
| TR\_C\_3\_2 | 19731762 | 2.95G | 19456890 | 2.92G | 0.03% | 97.03% | 92.01% | 50.50% |
| TR\_C\_4\_1 | 21645839 | 3.24G | 21249971 | 3.19G | 0.03% | 97.70% | 93.59% | 50.98% |
| TR\_C\_4\_2 | 21645839 | 3.24G | 21249971 | 3.19G | 0.03% | 97.54% | 93.11% | 50.97% |
| TR\_C\_5\_1 | 27596270 | 4.13G | 27200817 | 4.08G | 0.03% | 97.55% | 93.40% | 51.17% |
| TR\_C\_5\_2 | 27596270 | 4.13G | 27200817 | 4.08G | 0.03% | 97.06% | 92.24% | 51.16% |
| TR\_C\_6\_1 | 21906865 | 3.28G | 21479593 | 3.22G | 0.03% | 97.59% | 93.45% | 51.29% |
| TR\_C\_6\_2 | 21906865 | 3.28G | 21479593 | 3.22G | 0.03% | 97.39% | 92.89% | 51.28% |
| TW\_F16\_1\_1 | 21761460 | 3.26G | 21389835 | 3.21G | 0.03% | 97.72% | 93.60% | 50.53% |
| TW\_F16\_1\_2 | 21761460 | 3.26G | 21389835 | 3.21G | 0.03% | 97.25% | 92.46% | 50.52% |
| TW\_F16\_2\_1 | 20995832 | 3.14G | 20641977 | 3.1G | 0.03% | 97.58% | 93.38% | 50.55% |
| TW\_F16\_2\_2 | 20995832 | 3.14G | 20641977 | 3.1G | 0.03% | 97.46% | 93.00% | 50.55% |
| TW\_F16\_3\_1 | 21335097 | 3.2G | 20792643 | 3.12G | 0.03% | 97.75% | 93.69% | 50.69% |
| TW\_F16\_3\_2 | 21335097 | 3.2G | 20792643 | 3.12G | 0.03% | 97.86% | 93.83% | 50.70% |
| TW\_F16\_4\_1 | 20841157 | 3.12G | 20401608 | 3.06G | 0.03% | 97.65% | 93.47% | 50.99% |
| TW\_F16\_4\_2 | 20841157 | 3.12G | 20401608 | 3.06G | 0.03% | 97.59% | 93.23% | 50.97% |
| TW\_F16\_5\_1 | 22114610 | 3.31G | 21590822 | 3.24G | 0.03% | 97.66% | 93.57% | 50.66% |
| TW\_F16\_5\_2 | 22114610 | 3.31G | 21590822 | 3.24G | 0.03% | 97.58% | 93.24% | 50.65% |
| TW\_F16\_6\_1 | 21381500 | 3.2G | 20849209 | 3.13G | 0.03% | 97.64% | 93.56% | 51.31% |
| TW\_F16\_6\_2 | 21381500 | 3.2G | 20849209 | 3.13G | 0.03% | 97.30% | 92.72% | 51.30% |
| TW\_F32\_1\_1 | 22834346 | 3.42G | 22350783 | 3.35G | 0.03% | 97.69% | 93.57% | 51.12% |
| TW\_F32\_1\_2 | 22834346 | 3.42G | 22350783 | 3.35G | 0.03% | 97.66% | 93.39% | 51.11% |
| TW\_F32\_2\_1 | 21297730 | 3.19G | 20883809 | 3.13G | 0.03% | 97.69% | 93.59% | 51.19% |
| TW\_F32\_2\_2 | 21297730 | 3.19G | 20883809 | 3.13G | 0.03% | 97.49% | 93.06% | 51.18% |
| TW\_F32\_3\_1 | 22259102 | 3.33G | 21836089 | 3.28G | 0.03% | 97.70% | 93.64% | 51.22% |
| TW\_F32\_3\_2 | 22259102 | 3.33G | 21836089 | 3.28G | 0.03% | 97.54% | 93.14% | 51.21% |
| TW\_F32\_4\_1 | 20052612 | 3G | 19646476 | 2.95G | 0.03% | 97.66% | 93.51% | 50.78% |
| TW\_F32\_4\_2 | 20052612 | 3G | 19646476 | 2.95G | 0.03% | 97.41% | 92.86% | 50.77% |
| TW\_F32\_5\_1 | 21629413 | 3.24G | 21147784 | 3.17G | 0.03% | 97.63% | 93.47% | 50.67% |
| TW\_F32\_5\_2 | 21629413 | 3.24G | 21147784 | 3.17G | 0.03% | 97.41% | 92.86% | 50.65% |
| TW\_F32\_6\_1 | 24754210 | 3.71G | 24244607 | 3.64G | 0.03% | 97.58% | 93.38% | 50.40% |
| TW\_F32\_6\_2 | 24754210 | 3.71G | 24244607 | 3.64G | 0.03% | 97.38% | 92.83% | 50.38% |
| TR\_F16\_1\_1 | 25852753 | 3.87G | 25395813 | 3.81G | 0.03% | 97.54% | 93.34% | 51.51% |
| TR\_F16\_1\_2 | 25852753 | 3.87G | 25395813 | 3.81G | 0.03% | 97.54% | 93.22% | 51.52% |
| TR\_F16\_2\_1 | 21094517 | 3.16G | 20671782 | 3.1G | 0.03% | 97.63% | 93.55% | 51.18% |
| TR\_F16\_2\_2 | 21094517 | 3.16G | 20671782 | 3.1G | 0.03% | 97.23% | 92.58% | 51.17% |
| TR\_F16\_3\_1 | 24065134 | 3.6G | 23636959 | 3.55G | 0.03% | 97.60% | 93.52% | 51.46% |
| TR\_F16\_3\_2 | 24065134 | 3.6G | 23636959 | 3.55G | 0.03% | 97.13% | 92.41% | 51.44% |
| TR\_F16\_4\_1 | 22834416 | 3.42G | 22430132 | 3.36G | 0.03% | 97.66% | 93.57% | 50.78% |
| TR\_F16\_4\_2 | 22834416 | 3.42G | 22430132 | 3.36G | 0.03% | 97.40% | 92.92% | 50.78% |
| TR\_F16\_5\_1 | 21142551 | 3.17G | 20767025 | 3.12G | 0.03% | 97.63% | 93.55% | 50.85% |
| TR\_F16\_5\_2 | 21142551 | 3.17G | 20767025 | 3.12G | 0.03% | 97.35% | 92.80% | 50.84% |
| TR\_F16\_6\_1 | 23483148 | 3.52G | 23084691 | 3.46G | 0.03% | 97.61% | 93.50% | 50.85% |
| TR\_F16\_6\_2 | 23483148 | 3.52G | 23084691 | 3.46G | 0.03% | 97.06% | 92.21% | 50.84% |
| TR\_F32\_1\_1 | 21286242 | 3.19G | 20959733 | 3.14G | 0.03% | 97.69% | 93.67% | 50.73% |
| TR\_F32\_1\_2 | 21286242 | 3.19G | 20959733 | 3.14G | 0.03% | 97.50% | 93.13% | 50.72% |
| TR\_F32\_2\_1 | 25682961 | 3.85G | 25322381 | 3.8G | 0.03% | 97.60% | 93.50% | 50.50% |
| TR\_F32\_2\_2 | 25682961 | 3.85G | 25322381 | 3.8G | 0.03% | 97.28% | 92.67% | 50.50% |
| TR\_F32\_3\_1 | 23980401 | 3.59G | 23641549 | 3.55G | 0.03% | 97.58% | 93.48% | 51.66% |
| TR\_F32\_3\_2 | 23980401 | 3.59G | 23641549 | 3.55G | 0.03% | 97.37% | 92.91% | 51.64% |
| TR\_F32\_4\_1 | 23145904 | 3.47G | 22689295 | 3.4G | 0.03% | 97.70% | 93.63% | 50.65% |
| TR\_F32\_4\_2 | 23145904 | 3.47G | 22689295 | 3.4G | 0.03% | 97.69% | 93.49% | 50.65% |
| TR\_F32\_5\_1 | 23594742 | 3.53G | 23247237 | 3.49G | 0.03% | 97.62% | 93.51% | 50.69% |
| TR\_F32\_5\_2 | 23594742 | 3.53G | 23247237 | 3.49G | 0.03% | 97.27% | 92.63% | 50.66% |
| TR\_F32\_6\_1 | 21227110 | 3.18G | 20931509 | 3.14G | 0.03% | 97.54% | 93.38% | 51.05% |
| TR\_F32\_6\_2 | 21227110 | 3.18G | 20931509 | 3.14G | 0.03% | 97.26% | 92.61% | 51.08% |

**Table S4**. the results of clean data mapped to reference genome

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample name | Total reads | Total mapped | Multiple mapped | Uniquely mapped |
| TW\_C\_1 | 40260898 | 35250770 (87.56%) | 2535496 (6.3%) | 32715274 (81.26%) |
| TW\_C\_2 | 41952038 | 36675470 (87.42%) | 2622190 (6.25%) | 34053280 (81.17%) |
| TW\_C\_3 | 41625652 | 36583276 (87.89%) | 2801740 (6.73%) | 33781536 (81.16%) |
| TW\_C\_4\* | 40695674 | 35566344 (87.4%) | 12043828 (29.59%) | 23522516 (57.8%) |
| TW\_C\_5 | 41887894 | 36692762 (87.6%) | 3416054 (8.16%) | 33276708 (79.44%) |
| TW\_C\_6 | 50507120 | 42683988 (84.51%) | 3156022 (6.25%) | 39527966 (78.26%) |
| TR\_C\_1 | 40097092 | 35379746 (88.24%) | 2631644 (6.56%) | 32748102 (81.67%) |
| TR\_C\_2 | 46255612 | 40561044 (87.69%) | 2972828 (6.43%) | 37588216 (81.26%) |
| TR\_C\_3 | 38913780 | 33898546 (87.11%) | 2501224 (6.43%) | 31397322 (80.68%) |
| TR\_C\_4 | 42499942 | 37358176 (87.9%) | 2794344 (6.57%) | 34563832 (81.33%) |
| TR\_C\_5 | 54401634 | 47041566 (86.47%) | 3537882 (6.5%) | 43503684 (79.97%) |
| TR\_C\_6 | 42959186 | 37218086 (86.64%) | 2606258 (6.07%) | 34611828 (80.57%) |
| TW\_F16\_1 | 42779670 | 37011026 (86.52%) | 2655400 (6.21%) | 34355626 (80.31%) |
| TW\_F16\_2 | 41283954 | 35328970 (85.58%) | 3008984 (7.29%) | 32319986 (78.29%) |
| TW\_F16\_3 | 41585286 | 35916678 (86.37%) | 2836276 (6.82%) | 33080402 (79.55%) |
| TW\_F16\_4 | 40803216 | 35316342 (86.55%) | 2704336 (6.63%) | 32612006 (79.93%) |
| TW\_F16\_5 | 43181644 | 36888354 (85.43%) | 2820150 (6.53%) | 34068204 (78.9%) |
| TW\_F16\_6 | 41698418 | 35936014 (86.18%) | 2514736 (6.03%) | 33421278 (80.15%) |
| TW\_F32\_1 | 44701566 | 38583226 (86.31%) | 2772164 (6.2%) | 35811062 (80.11%) |
| TW\_F32\_2 | 41767618 | 36354372 (87.04%) | 2656962 (6.36%) | 33697410 (80.68%) |
| TW\_F32\_3 | 43672178 | 37985828 (86.98%) | 2720128 (6.23%) | 35265700 (80.75%) |
| TW\_F32\_4 | 39292952 | 34556956 (87.95%) | 2670932 (6.8%) | 31886024 (81.15%) |
| TW\_F32\_5 | 42295568 | 36417536 (86.1%) | 2779332 (6.57%) | 33638204 (79.53%) |
| TW\_F32\_6 | 48489214 | 42472708 (87.59%) | 3572320 (7.37%) | 38900388 (80.22%) |
| TR\_F16\_1 | 50791626 | 43487994 (85.62%) | 2678992 (5.27%) | 40809002 (80.35%) |
| TR\_F16\_2 | 41343564 | 35888146 (86.8%) | 2729222 (6.6%) | 33158924 (80.2%) |
| TR\_F16\_3 | 47273918 | 40637496 (85.96%) | 2707184 (5.73%) | 37930312 (80.24%) |
| TR\_F16\_4 | 44860264 | 39292564 (87.59%) | 3079932 (6.87%) | 36212632 (80.72%) |
| TR\_F16\_5 | 41534050 | 35481160 (85.43%) | 2688518 (6.47%) | 32792642 (78.95%) |
| TR\_F16\_6 | 46169382 | 39302190 (85.13%) | 2741336 (5.94%) | 36560854 (79.19%) |
| TR\_F32\_1 | 41919466 | 36875108 (87.97%) | 2626062 (6.26%) | 34249046 (81.7%) |
| TR\_F32\_2 | 50644762 | 43627098 (86.14%) | 3234712 (6.39%) | 40392386 (79.76%) |
| TR\_F32\_3 | 47283098 | 41556902 (87.89%) | 3016206 (6.38%) | 38540696 (81.51%) |
| TR\_F32\_4 | 45378590 | 39212498 (86.41%) | 3068082 (6.76%) | 36144416 (79.65%) |
| TR\_F32\_5 | 46494474 | 40332508 (86.75%) | 3240076 (6.97%) | 37092432 (79.78%) |
| TR\_F32\_6 | 41863018 | 35814540 (85.55%) | 2823428 (6.74%) | 32991112 (78.81%) |

\*TW\_C\_4 was removed in the following analysis because of it low quality (the significant higher multiple mapped percentage and lower uniquely mapped percentage than other samples).

**Table S5**. Candidate DEGs affected by litter size

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Log2(Fold change) | *P* value | Comparison groups |
| *CCNB2* (cyclin B2) | 1.09 | 0.0437 | TR-C vs TW-C |
| *CCNE2* (cyclin E2) | 1.25 | 0.0215 | TR-C vs TW-C |
| *CDC6* (cell division cycle 6) | 2.53 | 0.0465 | TR-C vs TW-C |
| *CDC20* (cell division cycle 20) | 3.60 | 0.0232 | TR-C vs TW-C |
| *CCNA2* (cyclin A2) | 2.73 | 0.0077 | TR-C vs TW-C |
| *E2F2* (E2F transcription factor 2) | 1.64 | 0.0082 | TR-C vs TW-C |
| *MCM4* (minichromosome maintenance complex component 4) | 1.65 | 0.0296 | TR-C vs TW-C |
| *BUB1* (BUB1 mitotic checkpoint serine/threonine kinase) | 2.62 | 0.0169 | TR-C vs TW-C |
| *TTK* (TTK protein kinase) | 2.31 | 0.0037 | TR-C vs TW-C |
| *POLE* (DNA polymerase epsilon, catalytic subunit) | 1.46 | 0.0214 | TR-C vs TW-C |
| *TK1* (thymidine kinase 1) | 1.86 | 0.0293 | TR-C vs TW-C |
| *TXNRD3* (thioredoxin reductase 3) | 0.71 | 0.0482 | TR-C vs TW-C |
| *RRM2* (ribonucleotide reductase regulatory subunit M2) | 3.10 | 0.0078 | TR-C vs TW-C |
| *DCK* (deoxycytidine kinase) | 1.16 | 0.0322 | TR-C vs TW-C |
| *DNA2* (DNA replication helicase/nuclease 2) | -0.86 | 0.0340 | TR-F32 vs TW-F32 |
| *MCM3* (minichromosome maintenance complex component 3) | -0.75 | 0.0290 | TR-F32 vs TW-F32 |
| *MCM6* (minichromosome maintenance complex component 6) | -0.77 | 0.0257 | TR-F32 vs TW-F32 |
| *POLD2* (DNA polymerase delta 2, accessory subunit) | -0.80 | 0.0260 | TR-F32 vs TW-F32 |
| *SMAD3* (SMAD family member 3) | -0.73 | 0.0457 | TR-F32 vs TW-F32 |
| *CHEK1* (checkpoint kinase 1) | -0.74 | 0.0436 | TR-F32 vs TW-F32 |

**Table S6**. Candidate DEGs affected by dietary FA supplementation in twin born lambs

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Log2(Fold change) | *P* value | Comparison groups |
| *IGF1* (insulin like growth factor 1) | 0.86 | 0.0058 | TW-F16 vs TW-C |
| *GADD45B* (growth arrest and DNA damage inducible beta) | 1.34 | 0.0495 | TW-F16 vs TW-C |
| *THBS1* (thrombospondin 1) | 2.25 | 0.0095 | TW-F16 vs TW-C |
| *IGFBP3* (insulin like growth factor binding protein 3) | -0.75 | 0.0091 | TW-F16 vs TW-C |
| *CCNG1* (cyclin G1) | -0.66 | 0.0234 | TW-F16 vs TW-C |
| *SLC7A9* (solute carrier family 7 member 9) | -0.97 | 0.0083 | TW-F16 vs TW-C |
| *SLC16A10* (solute carrier family 16 member 10) | -0.71 | 0.0238 | TW-F16 vs TW-C |
| *LMNB1* (lamin B1) | 0.74 | 0.0132 | TW-F32 vs TW-C |
| *TUBA3E* (tubulin alpha 3e) | 1.43 | 0.0097 | TW-F32 vs TW-C |
| *TUBA1C* (tubulin alpha 1c) | 0.85 | 0.0226 | TW-F32 vs TW-C |
| *TUBA8* (tubulin alpha 8) | 0.64 | 0.0350 | TW-F32 vs TW-C |
| *PRKAG2* (protein kinase AMP-activated non-catalytic subunit gamma 2) | -0.61 | 0.0172 | TW-F32 vs TW-C |
| *PRKAG3* (protein kinase AMP-activated non-catalytic subunit gamma 3) | -1.52 | 0.0344 | TW-F32 vs TW-C |
| *INSR* (insulin receptor) | -0.56 | 0.0221 | TW-F32 vs TW-C |
| *G6PC* (glucose-6-phosphatase catalytic subunit) | -1.46 | 0.0018 | TW-F32 vs TW-C |
| *PCK1* (phosphoenolpyruvate carboxykinase 1) | -0.85 | 0.0219 | TW-F16 vs TW-C |
| -0.83 | 0.0389 | TW-F32 vs TW-C |
| *CTH* (cystathionine gamma-lyase) | -1.35 | 0.0083 | TW-F32 vs TW-F16 |
| *SARDH* (sarcosine dehydrogenase) | -0.52 | 0.0444 | TW-F32 vs TW-F16 |
| *GLDC* (glycine decarboxylase) | -0.81 | 0.0318 | TW-F32 vs TW-F16 |
| *GNMT* (glycine N-methyltransferase) | -1.37 | 0.0011 | TW-F32 vs TW-F16 |
| *SDS* (serine dehydratase) | 1.30 | 0.0354 | TW-F16 vs TW-C |
| -2.19 | 0.0006 | TW-F32 vs TW-F16 |
| *SDSL* (serine dehydratase like) | -1.43 | 0.0105 | TW-F32 vs TW-C |
| -1.13 | 0.0081 | TW-F32 vs TW-F16 |

**Table S7**. Candidate DEGs affected by dietary FA supplementation in triplet born lambs

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Log2(Fold change) | P value | Comparison groups |
| *HSD17B2* (hydroxysteroid 17-beta dehydrogenase 2) | -1.79 | 0.0171 | TR-F16 vs TR-C |
| *HSD17B6* (hydroxysteroid 17-beta dehydrogenase 6) | -1.90 | 0.0197 | TR-F16 vs TR-C |
| *AKR1D1* (aldo-keto reductase family 1 member D1) | -0.77 | 0.0123 | TR-F16 vs TR-C |
| *CYP1A2* (cytochrome P450 family 1 subfamily A member 2) | -4.91 | 0.0008 | TR-F16 vs TR-C |
| *CYP1A1* (cytochrome P4501A1) | -4.94 | 0.0002 | TR-F16 vs TR-C |
| *UGT2A3* (UDP glucuronosyltransferase family 2 member A3) | -1.19 | 0.0002 | TR-F16 vs TR-C |
| *CCNB2* (cyclin B2) | -1.07 | 0.0279 | TR-F16 vs TR-C |
| *CDK1* (cyclin dependent kinase 1) | -2.18 | 0.0190 | TR-F16 vs TR-C |
| *CDKN2C* (cyclin dependent kinase inhibitor 2C) | -1.85 | 0.0402 | TR-F16 vs TR-C |
| *CDC20* (cell division cycle 20) | -2.75 | 0.0492 | TR-F16 vs TR-C |
| *CDC6* (cell division cycle 6) | -2.17 | 0.0493 | TR-F32vs TR-C |
| *MCM4* (minichromosome maintenance complex component 4) | -1.60 | 0.0195 | TR-F32vs TR-C |
| *MCM5* (minichromosome maintenance complex component 5) | -1.60 | 0.0411 | TR-F32vs TR-C |
| *POLD1* (DNA polymerase delta 1, catalytic subunit) | -0.87 | 0.0402 | TR-F32vs TR-C |
| *POLD2* (DNA polymerase delta 2, accessory subunit) | -1.02 | 0.0140 | TR-F32vs TR-C |
| *TYMS* (thymidylate synthetase) | -1.56 | 0.0197 | TR-F32vs TR-C |
| *TK1* (thymidine kinase 1) | -2.02 | 0.0118 | TR-F32vs TR-C |
| *DCK* (deoxycytidine kinase) | -1.20 | 0.0164 | TR-F32vs TR-C |
| *CCNA2* (cyclin A2) | -2.73 | 0.0052 | TR-F16 vs TR-C |
| -1.64 | 0.0491 | TR-F32vs TR-C |
| *CCNE2* (cyclin E2) | -1.24 | 0.0159 | TR-F16 vs TR-C |
| -1.11 | 0.0282 | TR-F32vs TR-C |
| *TTK* (TTK protein kinase) | -2.84 | 0.0004 | TR-F16 vs TR-C |
| -1.44 | 0.0406 | TR-F32vs TR-C |
| *BUB1* (BUB1 mitotic checkpoint serine/threonine kinase) | -3.01 | 0.0051 | TR-F16 vs TR-C |
| -1.85 | 0.0426 | TR-F32vs TR-C |
| *PKMYT1* (protein kinase, membrane associated tyrosine/threonine 1) | -1.17 | 0.0366 | TR-F16 vs TR-C |
| -1.19 | 0.0468 | TR-F32vs TR-C |

**Table S8**. Person correlation between the hepatic gene expression and biochemical indexes in twin lambs

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Items | *IGF1* | *PRKAG2* | *PRKAG3* | *PCK1* | *SDS* | *SDSL* | Folate | Met | Hcy | SAH | SAM | DNMT | SAM/SAH |
| *IGF1* | 1 | -.538\* | .298 | -.239 | .450 | .073 | .706\*\* | .628\*\* | -.258 | -.472 | .462 | .386 | .464 |
| *PRKAG2* |  | 1 | -.420 | .487\* | -.445 | .538\* | -.717\*\* | -.746\*\* | .398 | .424 | -.700\*\* | -.639\*\* | -.659\*\* |
| *PRKAG3* |  |  | 1 | -.338 | .665\*\* | -.254 | .709\*\* | .164 | -.295 | -.275 | .288 | .680\*\* | .428 |
| *PCK1* |  |  |  | 1 | -.415 | .114 | -.423 | -.404 | -.033 | -.078 | -.569\* | -.539\* | -.398 |
| *SDS* |  |  |  |  | 1 | -.139 | .603\* | .501\* | -.336 | -.240 | .335 | .459 | .415 |
| *SDSL* |  |  |  |  |  | 1 | -.323 | -.469 | .578\* | .408 | -.438 | -.481 | -.483\* |
| Folate |  |  |  |  |  |  | 1 | .653\*\* | -.486\* | -.582\* | .693\*\* | .859\*\* | .728\*\* |
| Met |  |  |  |  |  |  |  | 1 | -.358 | -.597\* | .758\*\* | .496\* | .799\*\* |
| Hcy |  |  |  |  |  |  |  |  | 1 | .492\* | -.335 | -.617\*\* | -.357 |
| SAH |  |  |  |  |  |  |  |  |  | 1 | -.533\* | -.489\* | -.796\*\* |
| SAM |  |  |  |  |  |  |  |  |  |  | 1 | .715\*\* | .805\*\* |
| DNMT |  |  |  |  |  |  |  |  |  |  |  | 1 | .695\*\* |
| SAM/SAH |  |  |  |  |  |  |  |  |  |  |  | . | 1 |

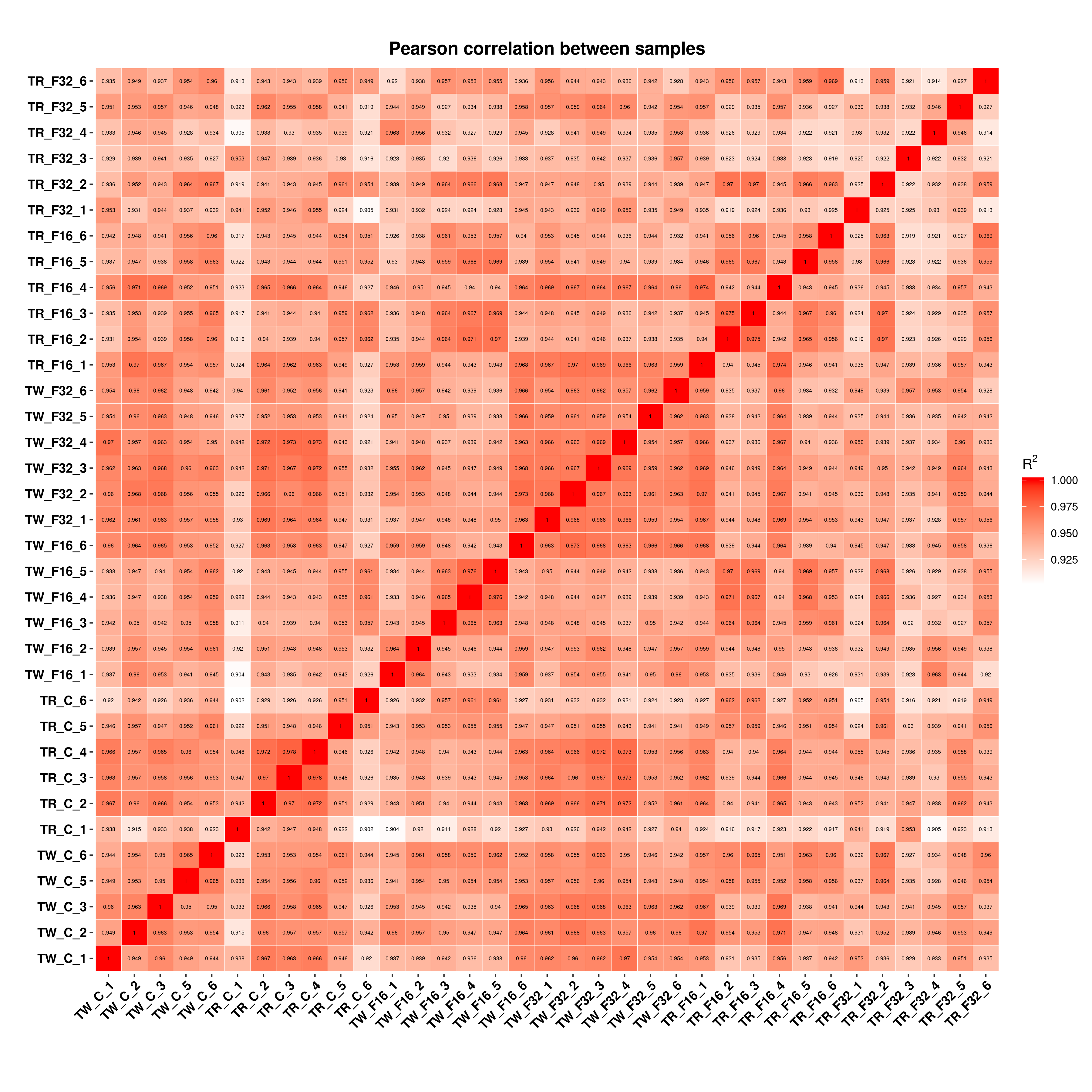
“\*” means *P* < 0.05. “\*\*” means *P* <0.01.

**Table S9**. Person correlation between the hepatic gene expression and biochemical indexes in triplet lambs

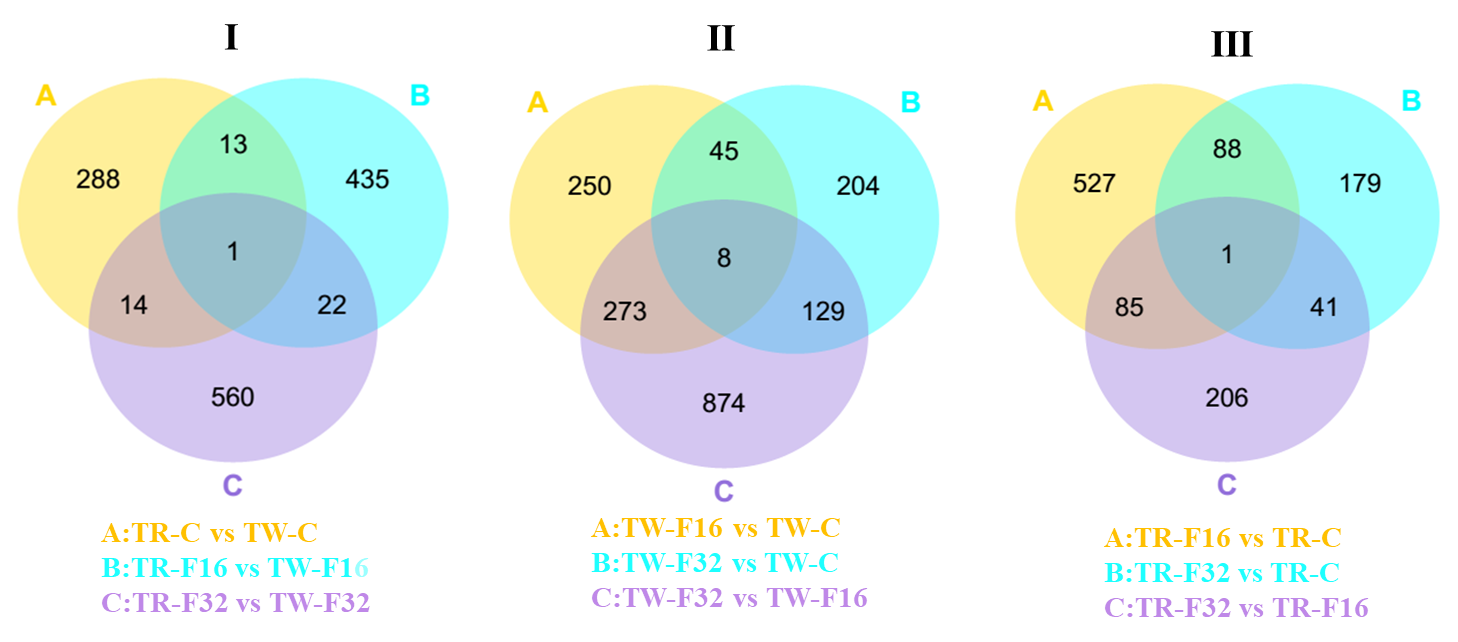
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Items | *CCNE2* | *CCNA2* | *CDC20* | *BUB1* | *POLD1* | *POLD2* | Folate | Met | Hcy | SAH | SAM | DNMT | SAM/SAH |
| *CCNE2* | 1 | .722\*\* | .387 | .422 | .773\*\* | .632\*\* | -.556\* | -.472\* | .653\*\* | .520\* | -.743\*\* | -.719\*\* | -.713\*\* |
| *CCNA2* |  | 1 | .663\*\* | .583\* | .740\*\* | .754\*\* | -.748\*\* | -.585\* | .639\*\* | .538\* | -.576\* | -.712\*\* | -.669\*\* |
| *CDC20* |  |  | 1 | .398 | .331 | .538\* | -.576\* | -.169 | .324 | .284 | -.308 | -.366 | -.383 |
| *BUB1* |  |  |  | 1 | .548\* | .774\*\* | -.342 | -.579\* | .371 | .223 | -.694\*\* | -.471\* | -.486\* |
| *POLD1* |  |  |  |  | 1 | .722\*\* | -.637\*\* | -.538\* | .712\*\* | .455 | -.601\*\* | -.774\*\* | -.619\*\* |
| *POLD2* |  |  |  |  |  | 1 | -.608\*\* | -.698\*\* | .597\*\* | .443 | -.833\*\* | -.767\*\* | -.706\*\* |
| Folate |  |  |  |  |  |  | 1 | .549\* | -.541\* | -.691\*\* | .385 | .544\* | .671\*\* |
| Met |  |  |  |  |  |  |  | 1 | -.288 | -.531\* | .614\*\* | .391 | .609\*\* |
| Hcy |  |  |  |  |  |  |  |  | 1 | .506\* | -.443 | -.552\* | -.646\*\* |
| SAH |  |  |  |  |  |  |  |  |  | 1 | -.465 | -.439 | -.893\*\* |
| SAM |  |  |  |  |  |  |  |  |  |  | 1 | .779\*\* | .773\*\* |
| DNMT |  |  |  |  |  |  |  |  |  |  |  | 1 | .693\*\* |
| SAM/SAH |  |  |  |  |  |  |  |  |  |  |  |  | 1 |

“\*” means *P* < 0.05. “\*\*” means *P* <0.01.

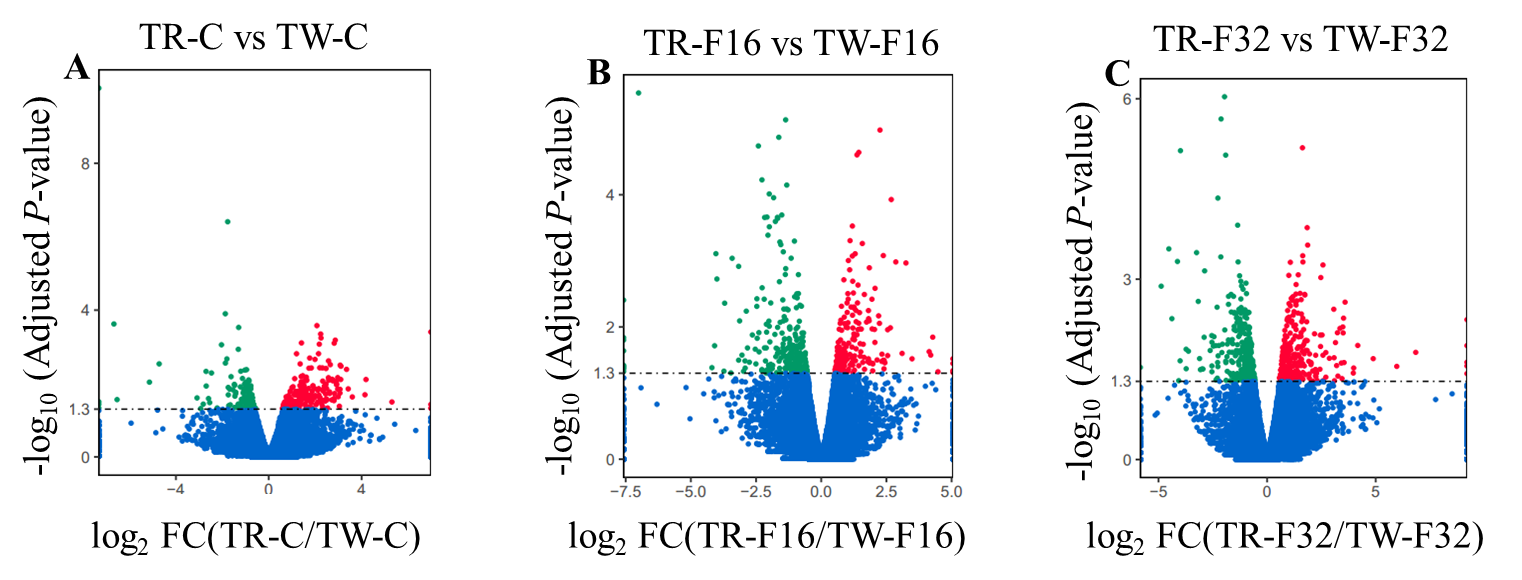
**Supplementary Figures**



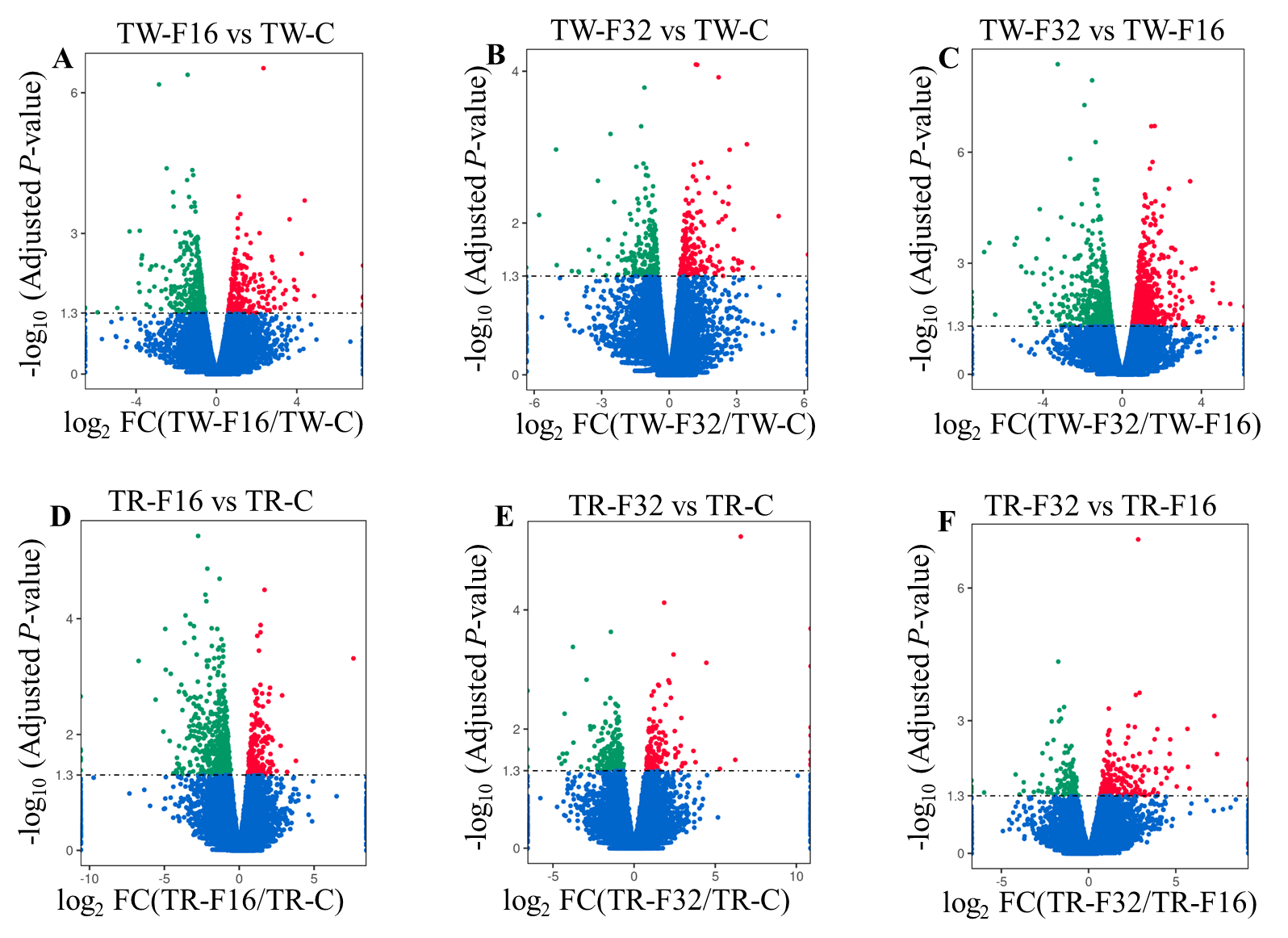
**Fig. S1.** Person correlation of transcript expression between samples. TW\_C and TR\_C indicate twin born and triplet born fed control diet, respectively. TW\_F16 and TR\_F16 mean twin- and triplet-born lambs from ewes fed control diet with 16 mg/(kg·DM) FA supplementation, respectively. TW\_F32 and TR\_F32 mean twin- and triplet-born lambs from ewes fed control diet with 32 mg/(kg·DM) FA supplementation, respectively.



**Fig. S2**. Venn diagram analysis of DEGs among different comparisons. TW-C, TW-F16 and TW-F32 indicate newborn twin lambs from ewes fed control diet supplemented with 0, 16 and 32 mg/(kg·DM) FA, respectively. TR-C, TR-F16 and TR-F32 indicate newborn triplet lambs from ewes fed with 0, 16 and 32 mg/(kg·DM) FA supplemented in control diet, respectively.



**Fig. S3**. Liver genes expression profile affected by litter sizes. The green, red and blue points indicate significant down-regulated, up-regulated and no significant difference genes, respectively. TW-C, TW-F16 and TW-F32 mean newborn twin lambs from ewes fed control diet supplemented with 0, 16 and 32 mg/(kg·DM) FA, respectively. TR-C, TR-F16 and TR-F32 mean newborn triplet lambs from ewes fed with 0, 16 and 32 mg/(kg·DM) FA supplemented in control diet, respectively. FC, fold change.



**Fig. S4**. Liver genes expression profile affected by dietary FA supplementation in twin- and triplet-born lambs. The green points indicated significant down-regulated genes and the red points indicated significant up-regulated genes, the blue points mean no significant difference between the two groups. TW-C, TW-F16 and TW-F32 represent newborn twin lambs from ewes fed control diet supplemented with 0, 16 and 32 mg/(kg·DM) FA, respectively. TR-C, TR-F16 and TR-F32 represent newborn triplet lambs from ewes fed with 0, 16 and 32 mg/(kg·DM) FA supplemented in control diet, respectively. FC, fold change.