Inosine monophosphate improved liver health and flesh quality of gibel carp (*Carassius auratus gibelio*) via activating AMPK signalling pathway and enhancing the contents of muscle fat and flavour substance

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**Part 3. References**

**Table S1.** Formulation and compositions of experimental diets (% dry matter).

|  |  |  |  |
| --- | --- | --- | --- |
| **Ingredients(%)** | **0%IMP** | **0.2%IMP** | **0.4%IMP** |
| Caseina | 41.00 | 41.00 | 41.00 |
| Corn starchb | 28.30 | 28.30 | 28.30 |
| Fish oil | 5.50 | 5.50 | 5.50 |
| Soy oil | 5.50 | 5.50 | 5.50 |
| Mineral premixc | 5.00 | 5.00 | 5.00 |
| Vitamin premixc | 0.39 | 0.39 | 0.39 |
| Choline chlorided | 0.11 | 0.11 | 0.11 |
| Carboxymethyl cellulose | 3.00 | 3.00 | 3.00 |
| Monocalcium phosphate | 2.00 | 2.00 | 2.00 |
| L-argininee | 0.76 | 0.76 | 0.76 |
| L-alaninee | 0.41 | 0.28 | 0.16 |
| 5'-IMPe | 0.00 | 0.20 | 0.40 |
| Cellulose | 8.03 | 7.96 | 7.88 |
| **Proximate composition** |  |  |  |
| Moisture | 7.60 | 8.49 | 8.58 |
| Crude protein | 40.96 | 40.34 | 40.38 |
| Crude lipid | 10.54 | 10.49 | 10.50 |
| Crude ash | 5.97 | 5.71 | 6.04 |
| Gross energy (MJ/kg) | 19.14 | 18.54 | 19.00 |
| 5'-IMP (%) | 0.00 | 0.16 | 0.35 |

a Casein was purchased from Gansu Hualing Casein Co., Ltd., Gansu, China.

b Corn starch was purchased fromYufeng Industrial Group Co., Ltd., Hebei, China.

c Mineral premix and vitamin premix were based on (Wei rt al., 2019).

d Choline chloride was composed of 50% choline chloride and 50% silicon dioxide.

e L-arginine, L-alanine and 5'-IMP were purchased from Yuanye Bio-Technology Co., Ltd., Shanghai, China.

**Table S2.** Effect of IMP supplementation on growth performance and whole-body composition of gibel carp.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | 0%IMP | 0.2%IMP | 0.4%IMP |
| *Growth performance* |  |  |  |
| IBW g1 | 8.02 ± 0.04 | 8.02 ± 0.03 | 8.03 ± 0.02 |
| FBW g2 | 48.51 ± 0.50 | 47.24 ± 1.28 | 47.80 ± 0.48 |
| WGR %3 | 504.19 ± 6.95 | 488.97 ± 15.58 | 494.71± 3.81 |
| SGR %/d4 | 3.00± 0.02 | 2.95 ± 0.04 | 2.97 ± 0.01 |
| FR %BW/d5 | 3..30 ± 0.07 | 3.38 ± 0.10 | 3.12 ± 0.05 |
| *Whole-body composition* | | | |
| Moisture % 71.73 ± 0.44 71.29 ± 0.20 71.28 ± 0.54 | | | |
| Crude protein % 15.05 ± 0.12 a 15.60 ± 0.07 b 15.42 ± 0.13 b | | | |
| Crude lipid % 7.97 ± 0.18 7.80 ± 0.10 7.53 ± 0.20 | | | |
| Ash % 3.35 ± 0.05 3.28 ± 0.06 3.33 ± 0.02 | | | |

Data are presented as the Means ± SE (n = 3). Values within the same row with different letters are significantly different (*P* < 0.05).

**1IBW**: initial body weight.

**2FBW**: final body weight.

**3WGR**: weight gain = 100 × (final mean weight − initial mean weight) / initial mean weight.

**4SGR**: specific growth rate (% d −1) = 100 × [ln (final body weight) − ln (initial body weight)] / days.

**5FR**: feeding rate (% body weight day −1) = 100 × (feed intake in dry matter) / [days × (initial body weight+ final body weight)/2].

**Table S3.** HPLC eluent compositions and program for nucleotide analysis.

|  |  |  |
| --- | --- | --- |
|  | Mobile phase A | Mobile phase B |
| Composition |  |  |
| Methanol | 0 ml | 500 ml |
| Water | 1000 ml | 0 ml |
| Potassium (KH2PO4) | 0.01 mol L-1 | 0 mol L-1 |
| Dipotassium (K2HPO4) | 0.1 mol L-1 | 0 mol L-1 |
| Time/min |  |  |
| 20 min | 98% | 2% |

The flow rate was 1.0 ml min-1.

**Table S4.** Result from quantitative enrichment analysis.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total Cmpd | Hits | Statistic Q | Expected Q | Raw p | Holm p | FDR |
| Purine metabolism | 65 | 12 | 48.01 | 14.29 | 2.52E-03 | 1.21E-01 | 1.13E-01 |
| Thiamine metabolism | 7 | 1 | 74.86 | 14.29 | 5.52E-03 | 2.59E-01 | 1.13E-01 |
| Pentose and glucuronate inter conversions | 18 | 1 | 66.14 | 14.29 | 1.41E-02 | 6.48E-01 | 1.13E-01 |
| Galactose metabolism | 27 | 1 | 66.14 | 14.29 | 1.41E-02 | 6.48E-01 | 1.13E-01 |
| Ascorbate and aldarate metabolism | 8 | 1 | 66.14 | 14.29 | 1.41E-02 | 6.48E-01 | 1.13E-01 |
| Starch and sucrose metabolism | 18 | 1 | 66.14 | 14.29 | 1.41E-02 | 6.48E-01 | 1.13E-01 |
| Alanine, aspartate and glutamate metabolism | 28 | 7 | 30.72 | 14.29 | 2.21E-02 | 9.28E-01 | 1.47E-01 |
| Pyrimidine metabolism | 39 | 6 | 36.51 | 14.29 | 2.45E-02 | 1.00E+00 | 1.47E-01 |
| Sphingolipid etabolism | 21 | 1 | 55.44 | 14.29 | 3.41E-02 | 1.00E+00 | 1.60E-01 |
| Amino sugar and nucleotide sugar metabolism | 37 | 2 | 37.73 | 14.29 | 3.69E-02 | 1.00E+00 | 1.60E-01 |
| Glyoxylate and dicarboxylate metabolism | 32 | 5 | 34.54 | 14.29 | 3.89E-02 | 1.00E+00 | 1.60E-01 |
| Tryptophan metabolism | 41 | 3 | 44.95 | 14.29 | 4.10E-02 | 1.00E+00 | 1.60E-01 |
| beta-Alanine metabolism | 21 | 2 | 35.10 | 14.29 | 4.90E-02 | 1.00E+00 | 1.60E-01 |
| D-Glutamine and D glutamate metabolism | 6 | 4 | 34.57 | 14.29 | 5.74E-02 | 1.00E+00 | 1.60E-01 |
| Nitrogen metabolism | 6 | 2 | 34.57 | 14.29 | 5.74E-02 | 1.00E+00 | 1.60E-01 |
| Glycolysis / Gluconeogenesis | 26 | 2 | 33.67 | 14.29 | 6.15E-02 | 1.00E+00 | 1.60E-01 |
| Fructose and  mannose metabolism | 20 | 1 | 46.38 | 14.29 | 6.30E-02 | 1.00E+00 | 1.60E-01 |
| Inositol phosphate metabolism | 30 | 1 | 46.38 | 14.29 | 6.30E-02 | 1.00E+00 | 1.60E-01 |
| Cysteine and  methionine metabolism | 33 | 4 | 30.37 | 14.29 | 6.33E-02 | 1.00E+00 | 1.60E-01 |
| Pantothenate and CoA biosyn thesis | 19 | 4 | 27.48 | 14.29 | 7.40E-02 | 1.00E+00 | 1.78E-01 |
| Arginine biosynthesis | 14 | 7 | 29.95 | 14.29 | 8.46E-02 | 1.00E+00 | 1.93E-01 |
| Glycerolipid metabolism | 16 | 2 | 37.74 | 14.29 | 9.49E-02 | 1.00E+00 | 2.06E-01 |
| Aminoacyl-tRNA biosynthesis | 48 | 14 | 24.24 | 14.29 | 9.87E-02 | 1.00E+00 | 2.06E-01 |
| Primary bile acid biosynthesis | 46 | 2 | 28.11 | 14.29 | 1.35E-01 | 1.00E+00 | 2.59E-01 |
| Glycerophospholipid metabolism | 36 | 6 | 24.64 | 14.29 | 1.42E-01 | 1.00E+00 | 2.59E-01 |
| Vitamin B6 metabolism | 9 | 1 | 31.92 | 14.29 | 1.44E-01 | 1.00E+00 | 2.59E-01 |
| Arginine and proline metabolism | 38 | 8 | 19.75 | 14.29 | 1.45E-01 | 1.00E+00 | 2.59E-01 |
| Histidine metabolism | 16 | 4 | 22.14 | 14.29 | 1.93E-01 | 1.00E+00 | 3.19E-01 |
| Phosphonate and  Phosphinate metabolism | 6 | 1 | 26.23 | 14.29 | 1.94E-01 | 1.00E+00 | 3.19E-01 |
| Glycine, serine and threonine metabolism | 33 | 7 | 21.16 | 14.29 | 1.99E-01 | 1.00E+00 | 3.19E-01 |
| Nicotinate and  nicotinamide metabolism | 15 | 5 | 20.70 | 14.29 | 2.17E-01 | 1.00E+00 | 3.35E-01 |
| Taurine and hypotaurine metabolism | 8 | 1 | 22.82 | 14.29 | 2.31E-01 | 1.00E+00 | 3.37E-01 |
| Caffeine metabolism | 10 | 2 | 21.25 | 14.29 | 2.37E-01 | 1.00E+00 | 3.37E-01 |
| Glutathione metabolism | 28 | 3 | 20.96 | 14.29 | 2.42E-01 | 1.00E+00 | 3.37E-01 |
| Lysine degradation | 25 | 3 | 20.27 | 14.29 | 2.46E-01 | 1.00E+00 | 3.37E-01 |
| Butanoate metabolism | 15 | 2 | 20.88 | 14.29 | 2.59E-01 | 1.00E+00 | 3.45E-01 |
| Riboflavin metabolism | 4 | 1 | 19.64 | 14.29 | 2.71E-01 | 1.00E+00 | 3.52E-01 |
| Porphyrin and  chlorophyll metabolism | 30 | 3 | 17.06 | 14.29 | 3.30E-01 | 1.00E+00 | 4.17E-01 |
| Steroid hormone biosynthesis | 85 | 1 | 12.29 | 14.29 | 3.95E-01 | 1.00E+00 | 4.86E-01 |
| Valine, leucine and isoleucine degradation | 40 | 2 | 9.92 | 14.29 | 4.57E-01 | 1.00E+00 | 5.35E-01 |
| Pyruvate metabolism | 22 | 2 | 11.05 | 14.29 | 4.80E-01 | 1.00E+00 | 5.48E-01 |
| Ether lipid metabolism | 20 | 1 | 4.48 | 14.29 | 6.15E-01 | 1.00E+00 | 6.74E-01 |
| Citrate cycle (TCA cycle) | 20 | 2 | 7.92 | 14.29 | 6.18E-01 | 1.00E+00 | 6.74E-01 |
| Ubiquinone and other terpenoid quinone biosynthesis | 9 | 1 | 1.03 | 14.29 | 8.11E-01 | 1.00E+00 | 8.41E-01 |
| Phenylalanine metabolism | 10 | 2 | 2.38 | 14.29 | 8.24E-01 | 1.00E+00 | 8.41E-01 |
| Tyrosine metabolism | 42 | 2 | 1.09 | 14.29 | 9.14 E-01 | 1.00 E+00 | 9.14E-01 |
| Phenylalanine, tyrosine and  tryptophan biosynthesis | 4 | 2 | 2.38 | 14.29 | 8.24E-01 | 1.00E+00 | 8.41E-01 |

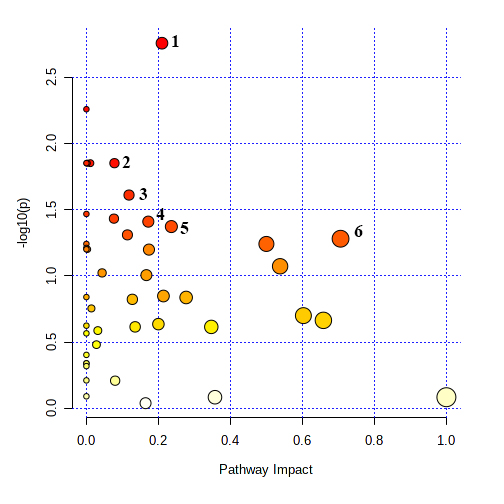
**Table S5.** Primers used for quantitative RT-PCR (qPCR).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Function classifications | Accession no. | Gene name | Primer sequence (5’-3’) | Product length(bp) |
| Lipid synthesis | KF499584 | *acc*1 | F: GAGCTGTCTATCAGAGGAGACTTCA  R: GACGCTCGGCCTGCATCTTCT | 139 |
|  | KX898507 | *srebp1*2 | F: GGCCCTCTACTGCGTGGCACA  R: ACCACCATTTGGAGTGAGGGTCAC | 194 |
|  | KF511494 | *fas*3 | F: CCACACCATGGACCCACAGCT  R: CTGGGTCTTTACTGAAGGCCTCT | 158 |
|  | XM026220745.1 | *pparγ4* | F: AATGGAAGCCAGAGGCCTTGT  R: AAACTTGAACCCGAGTCTCCTC | 133 |
| Lipolysis | FJ204474.1 | *lpl5* | F: GTAGTGGACTGGTTGTCCCG  R: CATCGGGGGAAAGAGTGCTT | 277 |
|  | MH536187 | *hsl*6 | F: GAAGAGTGTTTCTATGCCTACT  R: CCGTGAGACATTGCCCTCAT | 140 |
| Fatty acids β- oxidation | KX898509 | *cpt1α*7 | F: GAAGCTCATCAGGCTGTGGCCTT  R: TTCCAGGAGTGAAGTCCGGAGAG | 113 |
|  | KX898510 | *aco3*8 | F: TGTGGAGGACACGGTTACCTTGC  R: AGTTGCTGGTCTGCTGCAGAAGG | 115 |
| Gluconeogenesis | KX898505 | *g6pase*9 | F: CCTTACTGGTGGGTCCATGAGACT  R: TGGGCCGGTCTCACAGGTCAT | 90 |
|  | KX898506 | *pepck*10 | F: AGACAAACCCTCATGCCATGGCAAC  R: GGGTCTATGATGGGGCACTGG | 226 |
| Glycolysis | KX898498 | *gk11* | F: GAGGAGATGCGTAAGGTGGAGCT  R: TTCTCATACAGCTGATGTCCAGGGTT | 167 |
|  | KX898500 | *6pfk12* | F: ACACCGGATGCCGCAGAAGCA  R: TCGATCTCTCCGGTCACATACTCG | 105 |
|  | KX898502 | *pk13* | F: GCATCTGTGTCTGCTGGACATCGA  R: TGAGAGCCGTGAGAGAAGTTCAGTC | 144 |
| Glucose transport | KY328742 | *glut414* | F: CACTCTCTGTGGCCATCTTCTCCAT  R: TCACCAACATACATGGGCACCAATCCT | 241 |
| Inflammatory cytokines | XM\_026196241.1 | *TGF-β*15 | F: ATTGGACGGAACCCCAAACA  R: GTGACATCAAACGACAGCCA | 237 |
|  | XM\_026289280.1 | *IL-6*16 | F: TGTTCTCAGGGCATTCGCTT  R: GGAGTTGTAGTGCCCTTGGT | 161 |
|  | XM\_026282152.1 | *TNF-α*17 | F: TTGAGCAGGAGATGGGAACCG  R: AGAGCCTCAGGGCAACGGAAA | 115 |
|  | XM\_026220359.1 | *IL-1β*18 | F: GTGGTGAACATCATCATTGCTGT  R: TTCTCCAAGAAGAAGCTGAGCA | 101 |
| Inernal reference | AB039726.2 | *β-actin* | F: TTGAGCAGGAGATGGGAACCG  R: AGAGCCTCAGGGCAACGGAAA | 115 |
|  | AB056104 | *ef1α*19 | F: GTTGGAGTCAACAAGATGGACTCCAC  R: CTTCCATCCCTTGAACCAGCCCAT | 198 |

1*acc*: Acetyl-CoA carboxylase; 2*srebp1*: Sterol regulatory element binding protein 1; 3*fas*: Fatty acid synthase; *4pparγ:* Peroxisome proliferator-activated receptor γ; *5lpl*: Lipoprotein lipase; 6*hsl*: Hormone-sensitive lipase; 7*cpt1α*: Carnitine palmitoyl transferase 1 isoform a; 8*aco3*: Acyl-CoA oxidase 3; 9*g6pase*: Glucose-6-phosphatase; 10*pepck*: Phosphoenolpyruvate carboxykinase; 11*gk*: Glucokinase; *126pfk*: 6-phosphofructokinase; *13pk*: Pyruvate kinase; *14glut4*: Glucose transporter type 4; 15*TGF-β*: Transforming growth factor β; 16*IL-6*: Interleukin-6; *TNF-α*17: Tumor necrosis factor α; 18*IL-1β*: Interleukin-1β; 19*ef1α*: Translation elongation factor 1-alpha.

**Table S6.** Linear regression analysis.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | Regression line | | | R2 | | F value | | *P* value | |
| Growth performance | | | | | | | | | |
| FBW g2 | | y = -1.78x + 48.21 | | 0.051 | | 0.374 | 0.560 | | |
| WGR %3 | | y = -23.70x + 500.70 | | 0.062 | | 0.460 | 0.519 | | |
| SGR %/d4 | | y = -0.07x + 2.99 | | 0.059 | | 0.439 | 0.529 | | |
| FR %BW/d5 | | y = -0.28x + 2.35 | | 0.151 | | 1.242 | 0.302 | | |
| Whole-body composition | | | | | | | | | |
| Moisture % | y = -1.14x + 71.66 | | | 0.089 | | 0.681 | 0.436 | | |
| Crude protein % | y = 1.06x + 15.16 | | | 0.268 | | 5.847 | 0.028 | | |
| Crude lipid % | y = -1.1x + 7.99 | | | 0.222 | | 3.713 | 0.076 | | |
| Ash % | y = -0.02x + 3.32 | | | 0.003 | | 0.021 | 0.889 | | |
| Haematology indicators | | | | | | | | | |
| TG mmol/L | y = -3.98x + 6.64 | | 0.174 | | 2.952 | | | | 0.108 |
| HDL-C mmol/L | y = -0.3x + 2.48 | | 0.062 | | 1.053 | | | | 0.320 |
| LDL-C mmol/L | y = -0.69x + 1.85 | | 0.147 | | 2.768 | | | | 0.116 |
| ALT U/L | y = -10x + 12.32 | | 0.520 | | 15.166 | | | | 0.002 |
| AST U/L | y = -912.4x + 615.8 | | 0.379 | | 9.173 | | | | 0.008 |
| Expression of Inflammatory cytokines genes | | | | | | | | | |
| *TGF-β* | y = -0.913x +1.25 | | 0.049 | | 0.816 | | | | 0.380 |
| *IL-6* | y = -1.015x + 1.04 | | 0.482 | | 14.890 | | | | 0.001 |
| *TNF-α* | y = -0.137x + 1.07 | | 0.004 | | 0.065 | | | | 0.802 |
| *IL-1β* | y = -2.171x + 1.00 | | 0.757 | | 49.854 | | | | 0.000 |
| Hepatic glycolipid metabolism | | | | | | | | | |
| Hepatic fat content (%) | y =-38.695x + 28.93 | | 0.815 | | 57.447 | | | | 0.00 |
| Hepatic glycogen content  (mg/g) | y = -33.537x + 90.67 | | 0.298 | | 5.101 | | | | 0.043 |
| Expression of hepatic glycolipid metabolism genes | | | | | | | | | |
| *acc* | y = -0.779x + 0.99 | | 0.316 | | 7.396 | | | | 0.015 |
| *srebp1* | y = -0.009x + 1.01 | | 0.000 | | 0.001 | | | | 0.978 |
| *fas* | y = -0.732x + 1.06 | | 0.324 | | 7.677 | | | | 0.014 |
| *cpt1α* | y = 1.455x + 1.06 | | 0.569 | | 21.153 | | | | 0.000 |
| *hsl* | y = 0.620x + 0.99 | | 0.090 | | 1.580 | | | | 0.227 |
| *aco3* | y = -0.067x + 0.96 | | 0.004 | | 0.067 | | | | 0.799 |
| *pepck* | y = -1.642x + 1.00 | | 0.670 | | 32.480 | | | | 0.000 |
| *g6pase* | y = -0.889x + 1.08 | | 0.389 | | 10.190 | | | | 0.006 |
| *gk* | y = 0.809x + 1.10 | | 0.035 | | 0.585 | | | | 0.455 |
| *glut4* | y = -1.52x + 0.99 | | 0.746 | | 47.032 | | | | 0.000 |
| *pfk* | y = 0.186x + 0.95 | | 0.032 | | 0.531 | | | | 0.477 |
| *pk* | y = -0.156x + 1.00 | | 0.008 | | 0.129 | | | | 0.724 |
| Hepatic IMP content (μmol/g) | y = 0.12x + 0.19 | | 0.416 | | 9.991 | | | | 0.007 |
| Hepatic ATP content (μmol/g) | y = 0.036x + 0.56 | | 0.008 | | 0.127 | | | | 0.726 |
| Hepatic AMP content (μmol/g) | y = 0.555x + 1.09 | | 0.703 | | 30.830 | | | | 0.000 |
| Hepatic AMP/ATP | y = -0.206x + 2.01 | | 0.006 | | 0.062 | | | | 0.808 |
| Muscle fat content (%) | y =-0.541x + 10.565 | | 0.004 | | 0.059 | | | | 0.811 |
| Muscle protein content (%) | y = -1.126x + 82.45 | | 0.014 | | 0.226 | | | | 0.641 |
| Muscle ATP content (μmol/g) | y = 0.013x + 0.21 | | 0.000 | | 0.006 | | | | 0.940 |
| Muscle AMP content (μmol/g) | y = 0.008x + 0.41 | | 0.000 | | 0.002 | | | | 0.962 |
| Muscle IMP content (μmol/g) | y = 5.522x + 2.53 | | 0.780 | | 35.546 | | | | 0.000 |
| Muscle AMP/ATP | y = -0.7638x + 2.47 | | 0.017 | | 0.273 | | | | 0.608 |
| Expression of muscle lipid metabolism genes | | | | | | | | | |
| *acc* | y = 0.673x + 0.935 | | 0.179 | | 3.482 | | | | 0.080 |
| *srebp1* | y = 1.218x + 0.959 | | 0.529 | | 17.966 | | | | 0.001 |
| *pparγ* | y = 0.259x + 0.920 | | 0.016 | | 0.257 | | | | 0.619 |
| *cpt1α* | y = -0.413x + 1.07 | | 0.088 | | 1.538 | | | | 0.233 |
| *hsl* | y = -0.543x + 1.073 | | 0.101 | | 1.807 | | | | 0.198 |
| *lpl* | y = -1.236x + 1.047 | | 0.367 | | 9.261 | | | | 0.008 |



**Fig. S1.** Summary of pathway analyses with MetaboAnalyst 5.0, as visualized by bubble plots. 1. Purine metabolism; 2. Pentose and glucuronate interconversions; 3. Pyrimidine metabolism; 4. Glyoxylate and dicarboxylate metabolism; 5. Tryptophan metabolism; 6. Alanine, aspartate and glutamate metabolism.



Fig. S2. A statistical diagram of KEGG pathway enrichment of metabolite sets. Each point in the figure represents a KEGG pathway. The name of the pathway is shown on the left coordinate axis, and the abscissa shows the enrichment factor.

 Fig. S3. Plot of purine metabolism pathway from the KEGG database. the blue indicates that metabolites were not in our data but used as background for enrichment analysis, the red means the metabolites with significant differences in the data, the yellow means the metabolites with differences in the data. From the KEGG IDs to the metabolite name: C00064, L-Glutamine; C00330, Deoxyguanosine; C00130, IMP; C00387, Guanosine; C00360, adenyl-deoxyribonucleotide (dAMP); C00020, AMP; C00212, Adenosine; C00294, Inosine; C00147, Adenine; C05512, Deoxyinosine; C00262, Hypoxanthine.

**References**

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