Use of antimicrobial peptides as a feed additive for juvenile goats

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Although antimicrobial peptides (AMPs) have been used as feed additives, only a few studies have examined their use in ruminants. In this study, we evaluated the use of AMPs (recombinant swine defensin and a fly antibacterial peptide were mixed by 1:1) as a medicated feed additive for juvenile goats. Dietary treatments included control groups (group I: 300 g concentrate; group III: 600 g concentrate), and AMP-supplemented groups (group II: 300 g concentrate + 3.0 g AMPs; group IV: 600 g concentrate + 3.0 g AMPs). AMP-treated groups exhibited an increase in bacterial genera, including Fibrobacter, Anaerovibrio, and Succiniclasticum, and the ciliate genus Ophryoscolex; as well a reduction in bacterial genera, such as Selenomonas, Succinivibrio, and Treponema, and the ciliate genera Polyplastron, Entodinium, and Isotricha. The changes in Fibrobacter, Anaerovibrio, Ophryoscolex, Polyplastron, Entodinium, and Isotricha were related to the concentrate. AMP treatment led to increased body weight, average daily weight gain, enzymatic activity (pectinase, xylanase, and lipase), especially in the normal concentrate group, and influence on ruminal fermentation function. In addition, goats treated with AMPs had higher rumen microorganism diversity indices than the control groups. Our results demonstrate that AMPs can be utilized as feed additives for juvenile goats.

The microbial environment in the rumen is quite complex and dynamic; this is due to several factors including type of diet. The microbial community consists of bacteria (10^10–10^11 cells/mL), methanogenic archaea (10^7–10^9 cells/mL), ciliate protozoa (10^4–10^6 cells/mL), anaerobic fungi (10^3–10^6 cells/mL), and bacteriophages (10^9–10^10 particles/mL) present. A major function of the microbiome is to ferment plant materials that can be ingested by ruminant animals. Rumen regulation is one of the most important methods for improving feed efficiency, rumen health, and rumen livestock production performance. Several antibiotic compounds, such as monensin, hainanmycin, and virginiamycin, have been used to improve ruminal fermentation and the efficiency of nutrient utilization. However, the overuse of antibiotics has raised concerns regarding product safety and environmental health, therefore, the use of antibiotics as animal feed additives has been banned in the European Union (European Union, 2003).

Antimicrobial peptides (AMPs) are widespread in bacteria, animals, and plants and provide opportunities for novel research. In addition to antimicrobial properties, previous studies have demonstrated antifungal, antiviral, anti-parasitic, and antitumor activities. AMP-induced immunoregulatory and antioxidant activities have been shown to be mediated by cationic charge, amphipathicity, amino acid composition, and structure. AMPs have also been demonstrated to improve performance, nutrient retention, and intestinal morphology, and to reduce the incidence of diarrhoea in livestock animals. Peng et al. demonstrated that dietary supplementation with crude rpBD2 (recombinant porcine β-defensin 2) has beneficial effects on growth and intestinal morphology of weaned piglets, reducing the incidence of post-weaning diarrhoea and the numbers of potential pathogens in the caecum. AMPs could therefore serve as potential alternatives to antibiotics in livestock production. However, there is insufficient information on the effects of AMPs on rumen digestion, as only a limited number of inconclusive studies have examined the use of AMPs as alternatives to feed antibiotics and growth promoters in ruminant nutrition. Previous studies in our laboratory have shown that adding AMPs (composed of recombinant swine defensin and a fly antibacterial peptide at a blending ratio of 50:50) in feed can improve growth and immunity of weaned piglets. Based on our previous findings and the reported bactericidal effects of AMPs, we hypothesized that dietary AMP supplementation could affect rumen microbiology, and therefore...
cellulase (CMCase), and protease activity could be detected between AMP-treated and control animals (P< 0.05, group III compared to group I). The activity of xylanase, lipase, and amylase was associated with concentrate. Xylanase activity was higher in AMP-supplemented goats than in control groups (I, III; P< 0.05). The activity of lipase was higher in AMP-supplemented groups than in control groups (I, III; P< 0.05) but did not change with double concentrate; lipase increased with normal concentrate (significant difference on day 20). The activity of protease was significantly lower in AMP-treated groups than in control groups (I, III; P< 0.05); whereas pectinase (except on day 60) and lipase (except on day 20) activity increased in AMP-treated groups.

Rumen microorganisms. Bacterial community structure. Following the removal of low-quality reads from sequencing data, we obtained 1,786,781 total reads for bacteria, with an average of 49,632 reads per sample. The identified bacterial phyla and genera are detailed in Tables 4 and 5 and their respective community compositions.
### Table 2. Changes in ruminal fermentation parameters in goat rumen fluid. *Mean significant difference (P < 0.05).*

| Parameter              | Groups | Parameter | Groups |
|------------------------|--------|-----------|--------|
|                        | I      | II        | III     | IV     | P-Value |
|                        | 0d     | 20d       | 60d     | 0d     | 20d     |
| pH                     |        |           |         |        |         |
|                        | 6.89 ± 0.03 | 6.88 ± 0.03 | 6.88 ± 0.03 | 6.87 ± 0.04 | 0.215 | 0.893 | 0.504 | 0.714 |
| Ammonia (mg/100 mL)    |        |           |         |        |         |
|                        | 11.19 ± 0.21 | 11.19 ± 0.36 | 11.01 ± 0.24 | 11.06 ± 0.22 | 0.99 | 0.067 | 0.256 | 0.334 |
| Acetate (mmol/l)       |        |           |         |        |         |
|                        | 51.70 ± 1.60 | 51.61 ± 0.91 | 51.46 ± 1.59 | 51.31 ± 1.52 | 0.849 | 0.76 | 0.73 | 0.719 |
| Propionate (mmol/l)    |        |           |         |        |         |
|                        | 15.30 ± 0.40 | 15.27 ± 0.20 | 15.22 ± 0.34 | 15.41 ± 0.41 | 0.909 | 0.354 | 0.671 | 0.573 |
| Butyrate (mmol/l)      |        |           |         |        |         |
|                        | 8.60 ± 0.15 | 8.59 ± 0.14 | 8.60 ± 0.19 | 8.61 ± 0.23 | 0.945 | 0.782 | 0.885 | 0.926 |
| Urea nitrogen (mg/100 mL) | 75.59 ± 1.25 | 88.83 ± 2.13 | 86.97 ± 2.27 | 72.67 ± 2.32 | 0.001 | 0.001 | 0.001 | 0.002 |
| MCP (mg/mL)            |        |           |         |        |         |
|                        | 41.94 ± 1.36 | 45.51 ± 2.07 | 46.91 ± 1.78 | 40.14 ± 1.54 | 0.096 | 0.002 | 0.003 | 0.064 |
| Butyrate to Propionate ratio | 3.95 ± 0.20 | 3.94 ± 0.09 | 3.95 ± 0.17 | 3.89 ± 0.19 | 0.906 | 0.195 | 0.973 | 0.634 |
| 0.15 ± 0.27 | 0.12 ± 0.08 | 0.17 ± 0.06 | 0.17 ± 0.04 | 0.229 | 0.432 | 0.924 | 0.409 |
| 1.96 ± 0.17 | 1.77 ± 0.22 | 3.07 ± 0.61 | 2.09 ± 0.38 | 0.215 | 0.037 | 0.05 | 0.304 |
| 2.59 ± 0.28 | 2.55 ± 0.27 | 3.83 ± 0.66 | 2.63 ± 0.58 | 0.898 | 0.116 | 0.081 | 0.766 |
| 1.30 ± 0.07 | 1.51 ± 0.04 | 1.33 ± 0.05 | 1.33 ± 0.04 | 0.88 | 0.486 | 0.607 | 0.221 |
| 1.35 ± 0.03 | 1.37 ± 0.09 | 2.31 ± 0.48 | 2.39 ± 0.09 | 0.613 | 0.794 | 0.025 | 0.001 |
| 1.33 ± 0.02 | 1.35 ± 0.04 | 3.04 ± 0.33 | 3.60 ± 0.26 | 0.67 | 0.003 | 0.002 | 0.001 |
| CMCase (U/mL)          |        |           |         |        |         |
|                        | 74.39 ± 1.87 | 73.50 ± 1.50 | 73.13 ± 1.39 | 73.20 ± 1.38 | 0.54 | 0.79 | 0.453 | 0.824 |
| Xylanase (U/mL)        |        |           |         |        |         |
|                        | 10.03 ± 0.33 | 10.02 ± 0.41 | 10.24 ± 0.47 | 10.20 ± 0.35 | 0.975 | 0.701 | 0.424 | 0.216 |
| Pectinase (U/mL)       |        |           |         |        |         |
|                        | 45.51 ± 3.81 | 45.15 ± 2.14 | 45.14 ± 1.65 | 45.16 ± 1.71 | 0.728 | 0.684 | 0.829 | 0.992 |
| β-glucosidase (U/ mL)  |        |           |         |        |         |
|                        | 68.90 ± 4.03 | 62.40 ± 2.67 | 60.79 ± 2.69 | 66.18 ± 3.98 | 0.168 | 0.177 | 0.005 | 0.006 |
| Protease (mg /min mL−1) | 50.89 ± 0.49 | 55.97 ± 2.79 | 50.82 ± 3.54 | 59.33 ± 3.61 | 0.13 | 0.051 | 0.016 | 0.004 |
| Amylase (U/DL)         |        |           |         |        |         |
|                        | 20.92 ± 0.78 | 20.89 ± 0.37 | 20.76 ± 1.17 | 20.78 ± 0.68 | 0.962 | 0.967 | 0.854 | 0.778 |
| Lipase (U/L)           |        |           |         |        |         |
|                        | 24.88 ± 0.33 | 21.17 ± 1.50 | 25.59 ± 0.83 | 25.92 ± 0.61 | 0.029 | 0.244 | 0.25 | 0.024 |
| 27.62 ± 0.59 | 25.02 ± 0.58 | 26.26 ± 1.14 | 27.71 ± 1.04 | 0.006 | 0.163 | 0.145 | 0.033 |
| 19.24 ± 1.69 | 18.99 ± 1.09 | 19.92 ± 1.40 | 19.85 ± 1.38 | 0.862 | 0.861 | 0.316 | 0.529 |
| 18.81 ± 1.12 | 23.05 ± 1.36 | 18.25 ± 2.48 | 18.76 ± 0.69 | 0.037 | 0.634 | 0.742 | 0.007 |
| 21.13 ± 2.32 | 30.50 ± 3.37 | 32.42 ± 4.18 | 33.38 ± 3.82 | 0.041 | 0.713 | 0.014 | 0.468 |

**Table 3. Changes in activity of enzymes in rumen fluid of goats. *Mean significant difference (P < 0.05).***
Table 4. Influence of AMPs on proportion of different bacterial phyla. *mean significant difference (P < 0.05).

| Bacterial phylum       | Groups |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                        | I      | II    | III   | IV    | I VS II | III VS IV | I VS III | II VS IV |
| Bacteroidetes          | 0d     | 36.00 ± 2.07 | 36.15 ± 5.15 | 36.20 ± 4.35 | 35.93 ± 3.71 | 0.964 | 0.638 | 0.939 | 0.828 |
|                        | 20d    | 40.87 ± 2.19 | 43.68 ± 3.53 | 34.30 ± 3.67 | 38.52 ± 2.95 | 0.483 | 0.287 | 0.026 | 0.105 |
|                        | 60d    | 47.12 ± 1.10 | 52.77 ± 4.33 | 33.57 ± 2.66 | 35.94 ± 3.72 | 0.213 | 0.585 | 0.023 | 0.065 |
| Firmicutes             | 0d     | 27.02 ± 5.86 | 28.08 ± 2.58 | 27.79 ± 4.10 | 27.57 ± 3.68 | 0.645 | 0.735 | 0.732 | 0.537 |
|                        | 20d    | 27.19 ± 1.77 | 29.65 ± 3.32 | 35.29 ± 1.53 | 31.91 ± 1.98 | 0.387 | 0.058 | 0.016 | 0.474 |
|                        | 60d    | 18.05 ± 1.07 | 22.70 ± 1.70 | 33.72 ± 3.06 | 26.76 ± 3.56 | 0.051 | 0.199 | 0.022 | 0.296 |
| Proteobacteria         | 0d     | 19.92 ± 6.46 | 20.69 ± 4.14 | 18.99 ± 1.55 | 18.65 ± 3.53 | 0.763 | 0.805 | 0.859 | 0.213 |
|                        | 20d    | 19.23 ± 2.88 | 7.73 ± 2.46 | 12.54 ± 2.55 | 7.20 ± 1.31 | 0.032 | 0.042 | 0.042 | 0.800 |
|                        | 60d    | 19.99 ± 1.07 | 3.29 ± 0.46 | 14.00 ± 0.33 | 10.64 ± 0.51 | 0.001 | 0.001 | 0.001 | 0.006 |
| Verrucomicrobia        | 0d     | 4.60 ± 1.73 | 5.06 ± 0.38 | 5.57 ± 1.23 | 4.58 ± 2.66 | 0.613 | 0.557 | 0.563 | 0.811 |
|                        | 20d    | 4.34 ± 0.34 | 4.45 ± 0.40 | 6.66 ± 2.20 | 7.89 ± 0.53 | 0.760 | 0.393 | 0.215 | 0.005 |
|                        | 60d    | 2.69 ± 0.35 | 7.81 ± 2.43 | 4.82 ± 0.87 | 8.23 ± 2.02 | 0.086 | 0.043 | 0.034 | 0.863 |
| Tenericutes            | 0d     | 1.75 ± 0.40 | 2.45 ± 1.23 | 2.51 ± 0.42 | 2.60 ± 1.60 | 0.524 | 0.936 | 0.194 | 0.562 |
|                        | 20d    | 1.83 ± 0.58 | 3.72 ± 0.92 | 1.94 ± 0.50 | 3.67 ± 1.33 | 0.094 | 0.203 | 0.825 | 0.970 |
|                        | 60d    | 2.43 ± 0.44 | 4.56 ± 0.96 | 3.12 ± 0.52 | 5.39 ± 0.71 | 0.026 | 0.020 | 0.317 | 0.479 |
| Spirochaetes           | 0d     | 0.95 ± 0.24 | 0.69 ± 0.32 | 0.90 ± 0.25 | 0.88 ± 0.31 | 0.499 | 0.900 | 0.696 | 0.428 |
|                        | 20d    | 1.25 ± 0.17 | 0.41 ± 0.08 | 3.02 ± 0.43 | 1.43 ± 0.40 | 0.007 | 0.001 | 0.023 | 0.058 |
|                        | 60d    | 3.00 ± 0.71 | 1.35 ± 0.21 | 4.01 ± 0.32 | 2.73 ± 0.64 | 0.077 | 0.101 | 0.216 | 0.031 |
| Cyanobacteria          | 0d     | 1.67 ± 0.72 | 1.24 ± 0.44 | 1.49 ± 0.41 | 1.48 ± 0.79 | 0.550 | 0.999 | 0.786 | 0.352 |
|                        | 20d    | 1.13 ± 0.19 | 2.48 ± 0.20 | 1.30 ± 0.07 | 1.60 ± 0.15 | 0.003 | 0.143 | 0.355 | 0.004 |
|                        | 60d    | 0.60 ± 0.11 | 1.45 ± 0.35 | 0.77 ± 0.03 | 1.61 ± 0.55 | 0.056 | 0.130 | 0.076 | 0.769 |
| Fibrobacteres          | 0d     | 5.24 ± 1.00 | 5.14 ± 0.97 | 4.68 ± 1.21 | 5.49 ± 1.10 | 0.938 | 0.611 | 0.696 | 0.778 |
|                        | 20d    | 3.93 ± 0.26 | 5.37 ± 0.18 | 3.01 ± 0.26 | 4.25 ± 0.18 | 0.002 | 0.002 | 0.025 | 0.006 |
|                        | 60d    | 2.63 ± 0.40 | 4.36 ± 0.31 | 2.74 ± 0.21 | 4.39 ± 0.36 | 0.008 | 0.017 | 0.761 | 0.463 |

Ciliate community structure. A total of 631,179 quality protozoa sequences were obtained from the 36 samples, with an average of 17,532 reads per rumen sample. Although all animal groups were fed the same diet, there was a high level of variation between individuals in terms of ciliate community composition at the genus level and their respective community compositions are detailed in Fig. S2. The only characteristic in common was the dominant role of *Polyploastron* and *Ophryoscolex* (Table 8).

Compared with the control groups (I and III), *Ophryoscolex* appeared to increase in the AMP-supplemented groups (Table 8), although a significant increase was only apparent with normal concentrate. *Polyploastron*, *Entodinium*, and *Isotrachia* appeared to decrease in the AMP-supplemented groups, although a significant
Table 6. Diversity estimation based on sequence analysis of 16S rRNA gene libraries of the goat rumen. *The operational taxonomic units (OTUs) were defined with 3% dissimilarity. The diversity indices (Chao1, ACE, Shannon and Simpson) were calculated. **Values with different superscripts in the same row differ significantly (P < 0.05).
Table 7. Diversity estimation based on sequence analysis of 18S rRNA gene libraries of the goat rumen. The operational taxonomic units (OTUs) were defined with 3% dissimilarity. The diversity indices (Chao1, ACE, Shannon and Simpson) were calculated.

| Parameter | Ciliate | I | II | III | IV |
|-----------|---------|---|----|-----|----|
| OUT       | 0d      | 116 ± 19 | 121 ± 8 | 119 ± 21 | 124 ± 8 |
|           | 20d     | 123 ± 23 | 130 ± 18 | 103 ± 15 | 110 ± 15 |
|           | 60d     | 118 ± 19 | 141 ± 22 | 108 ± 14 | 122 ± 7 |
| Chao1     | 0d      | 91 ± 13 | 95 ± 18 | 89 ± 10 | 96 ± 12 |
|           | 20d     | 98 ± 23 | 95 ± 18 | 76 ± 14 | 85 ± 19 |
|           | 60d     | 98 ± 24 | 116 ± 14 | 87 ± 14 | 95 ± 3 |
| ACE       | 0d      | 103 ± 24 | 104 ± 17 | 105 ± 10 | 101 ± 21 |
|           | 20d     | 106.42 ± 27.99 | 104.06 ± 20.98 | 83.82 ± 12.15 | 99.17 ± 106.42 |
|           | 60d     | 107.07 ± 24.21 | 128.27 ± 21.05 | 95.50 ± 17.63 | 108.40 ± 1.72 |
| Simpson   | 0d      | 0.764 ± 0.073 | 0.747 ± 0.046 | 0.758 ± 0.028 | 0.765 ± 0.012 |
|           | 20d     | 0.766 ± 0.142 | 0.720 ± 0.128 | 0.728 ± 0.091 | 0.769 ± 0.082 |
|           | 60d     | 0.784 ± 0.055 | 0.769 ± 0.071 | 0.741 ± 0.070 | 0.811 ± 0.050 |
| Shannon   | 0d      | 2.987 ± 0.133 | 3.019 ± 0.233 | 3.029 ± 0.058 | 2.991 ± 0.126 |
|           | 20d     | 3.014 ± 0.666 | 2.819 ± 0.664 | 2.707 ± 0.593 | 2.918 ± 0.572 |
|           | 60d     | 3.081 ± 0.563 | 3.074 ± 0.431 | 2.780 ± 0.311 | 3.146 ± 0.230 |

Table 8. Influence of diet and AMPs on proportion of ciliates genera. *mean significant difference (P < 0.05).

| Protozoal genus | Groups | Parameter | I | II | III | IV |
|-----------------|--------|-----------|---|----|-----|----|
| Polyplastron    | 0d     | 40.07 ± 4.64 | 41.23 ± 4.37 | 40.21 ± 4.06 | 42.57 ± 2.07 | 0.785 |
|                 | 20d    | 45.37 ± 0.64 | 53.17 ± 4.71 | 51.44 ± 7.60 | 49.09 ± 7.80 | 0.031* |
|                 | 60d    | 56.78 ± 4.55 | 41.28 ± 1.70 | 65.59 ± 2.93 | 63.67 ± 2.74 | 0.031* |
| Diplodactron    | 0d     | 7.39 ± 1.41 | 6.80 ± 1.45 | 7.46 ± 1.27 | 6.98 ± 0.30 | 0.719 |
|                 | 20d    | 6.17 ± 1.04 | 6.41 ± 0.32 | 2.60 ± 0.91 | 2.83 ± 1.71 | 0.642 |
|                 | 60d    | 3.31 ± 0.54 | 3.36 ± 0.37 | 1.51 ± 0.27 | 1.81 ± 0.75 | 0.881 |
| Entodinium      | 0d     | 4.43 ± 1.05 | 4.12 ± 0.78 | 3.67 ± 0.66 | 4.07 ± 0.21 | 0.776 |
|                 | 20d    | 2.65 ± 0.50 | 0.46 ± 0.16 | 0.94 ± 0.18 | 1.01 ± 0.49 | 0.022* |
|                 | 60d    | 1.38 ± 0.12 | 0.60 ± 0.13 | 1.50 ± 0.21 | 1.04 ± 0.29 | 0.002* |
| Ophryoscolex    | 0d     | 10.86 ± 1.43 | 11.30 ± 2.98 | 9.90 ± 4.22 | 10.31 ± 1.72 | 0.866 |
|                 | 20d    | 14.99 ± 7.23 | 45.07 ± 4.14 | 24.64 ± 2.60 | 33.19 ± 4.77 | 0.006* |
|                 | 60d    | 27.98 ± 3.44 | 52.09 ± 2.13 | 29.09 ± 2.56 | 31.52 ± 2.07 | 0.001* |
| Enoplilastrium  | 0d     | 6.79 ± 0.84 | 0.16 ± 0.14 | 0.0 ± 0.0 | 0.023* |
|                 | 60d    | 5.79 ± 1.40 | 0.16 ± 0.14 | 0.0 ± 0.0 | 0.023* |
| Dasytricha      | 0d     | 0.99 ± 0.25 | 0.79 ± 0.47 | 1.02 ± 0.29 | 0.81 ± 0.19 | 0.626 |
|                 | 20d    | 0.32 ± 0.40 | 0.74 ± 0.32 | 0.99 ± 0.42 | 0.78 ± 0.54 | 0.397 |
|                 | 60d    | 0.0 ± 0.42 | 0.89 ± 0.20 | 0.93 ± 0.13 | 0.126 |
| Isotricha       | 0d     | 36.09 ± 3.74 | 37.20 ± 3.16 | 38.02 ± 4.44 | 36.70 ± 2.92 | 0.538 |
|                 | 20d    | 29.87 ± 5.49 | 13.95 ± 1.36 | 18.80 ± 5.11 | 12.89 ± 4.01 | 0.042* |
|                 | 60d    | 4.21 ± 0.90 | 2.01 ± 0.46 | 1.42 ± 0.31 | 1.04 ± 0.40 | 0.038* |

Discussion

Microbial community composition in ruminants has previously been linked with animal production traits. In the present study, we found that Bacteroidetes was the dominant phylum in all samples (except group III), followed by Firmicutes, Proteobacteria, and Verrucomicrobia. This structure is similar to the rumen bacterial community of sheep inferred from multiplex 454 Titanium pyrosequencing. At the genus level, Prevotella, known as an abundant member of the rumen microbiome, was the most abundant genus detected, followed by Butyrivibrio, [Paraprevotellaceae]CF231, Fibrobacter, Succinivibrio, and Anaerovibrio. Many of these genera...
include organisms that are important cellulose and hemicellulose-degraders; this indicates a rumen bacterial community highly oriented towards fibre degradation. *Polylastraon* and *Ophryoscolex* were the most abundant ciliate genera in this study; the protozoal community composition is similar to that of the A type (dominated by *Polylastraon*, *Ostracodinium*, *Dasytrichia*, and *Entodinium*)[25,32]. However, many studies have identified *Entodinium* as the predominant protozoal group in ruminants[28–31]. This discrepancy may be due to diet. In this study, forage grass was the main fodder and xylanase and glucanase activities of *Polylastraon* and *Ophryoscolex* are much higher than those of *Entodinium*[27]. In addition, high-throughput sequencing technology could also affect the true composition of rumen ciliates. Kittelmann et al. reported that smaller-celled genera, such as *Entodinium*, *Charonina*, and *Diploidium*, tended to be underrepresented, while larger-celled genera, such as *Metadinium*, *Epistrium*, *Eudytoplodinium*, *Ostracodinium*, and *Polylastraon*, tended to be overrepresented using the pyrosequencing approach.

Antimicrobial peptides possess broad-spectrum antimicrobial activity and have been used as a new type of feed additive in animal husbandry. A number of recent studies have suggested that dietary supplementation containing an antimicrobial peptide, such as lactoferrin and the lactoferrampin fusion peptide, potato protein, cecropin AD, or antimicrobial peptide PS, reduced the total numbers of aerobes while simultaneously enhancing the total amount of anaerobes and beneficial lactobacilli, thus improving growth performance in weanling pigs[33–35]. In this study, we have shown that dietary supplementation with AMPs improved growth of juvenile goats under two types of concentrate conditions. These results suggest that AMPs can be used to promote growth performance in goats. This is consistent with the finding of Yoon et al., who observed an improvement in the average daily gain and feed efficiency of weanling pigs fed diets supplemented with antimicrobial peptide-A3. Similarly, Jin et al. observed an improvement in the average daily gain (ADG) of weanling pigs fed diets supplemented with antimicrobial peptides from *Solanum tuberosum*, improving their intestinal balance and creating gut microecological conditions[38–40]. In this study, we found that *Proteobacteria* were significantly decreased in the AMP-supplemented groups, while *Fibrobacteres* were significantly increased. This may be due to the fact that *Fibrobacteres* are anaerobic bacteria[41], whereas *Proteobacteria* consist of aerobic bacteria that are mostly pathogenic[42]; the antibacterial peptide could have inhibited the pathogenic bacteria while enhancing the total amount of anaerobes. Dietary supplementation with AMPs has the potential to increase bacterial genera, such as *Fibrobacter*, *Anaerovibrio*, *Succiniclasticum*, and the ciliate genus *Ophryoscolex*, while reducing bacterial genera, including *Selenomonas*, *Succinivibrio*, and *Treponema*, and ciliate genera such as *Polylastraon*, *Entodinium*, and *Isotricha*. However, changes in *Fibrobacter*, *Anaerovibrio*, *Ophryoscolex*, *Polylastraon*, *Entodinium*, and *Isotricha* were related to the amount of concentrate that no significant different in the double concentrate group. Of these, *Fibrobacter* [43,44], *Treponema* [45], *Ophryoscolex* [26], and *Polylastraon* are cellulose-degrading microbes and *Succiniclasticum*, *Entodinium*, and *Isotricha* are starch-degrading microbes. *Selenomonas* and *Succinivibrio* degrade both starch and cellulose, and *Anaerovibrio* are fat-degrading bacteria. Therefore, we hypothesize that the increase in the relative abundance of *Fibrobacter* and *Ophryoscolex* in the normal concentrate group was due to an increase in xylanase and pectinase activities. Similarly, the decrease in the relative abundance of *Isotricha* and *Entodinium* was caused by a decrease in amylase activity in the normal concentrate group; whereas the increase in the relative abundance of *Anaerovibrio* was due to an increase in lipase activity in the same group.

Moreover, the fermentation products of *Fibrobacter*, *Anaerovibrio*, *Treponema*, *Selenomonas*, *Ophryoscolex*, *Polylastraon*, and *Isotricha* are acetate, propionate, and succinate; the fermentation product of *Succinivibrio* is succinate; and the fermentation products of *Butyrivibrio* are acetate and butyrate. Therefore, an increase in the relative abundance of *Fibrobacter*, *Anaerovibrio*, *Ophryoscolex* in the normal concentrate group may have caused an increase in acetate; whereas a decrease in the relative abundance of *Treponema*, *Selenomonas*, *Polylastraon*, and *Isotricha* in the double concentrate group may have led to the decrease in acetate. Lack of any variation to the relative abundance of *Butyrivibrio* prevented a change in butyrate. Acetate, propionate, and butyrate are the main components in VFAs, accounting for 95% of the total volatile matter content[30]. A change of the acetate + butyrate-to-propionate ratio is related to rumen fermentation mode. Thus, changes in acetate can cause alterations to T-VFA content (increase with normal concentrate and decrease with double concentrate) and the acetate + butyrate-to-propionate ratio (significant decrease with double concentrate). These results indicate that the effects of AMPs on rumen fermentation function and rumen microorganisms in goats were related to the amount of concentrate. It is possible that increased dosage causes similar changes in the double concentrate groups. However, additional studies will be needed to thoroughly elucidate these changes. The alpha diversity indices were elevated in the AMP-supplemented groups in this study, especially on day 60; indicating that AMP supplementation could increase microbial diversity in the rumen.

Previous studies have demonstrated the importance of concentrate supplementation in goat growth and productivity. In this study, we found that the ADG increased with increasing concentrate amount. This result is consistent with the findings of Salim et al., who reported that feeding grazing goats with concentrate supplement may optimize growth performance. The main reason for this may be changes in the rumen bacterial composition of ruminants driven by the amount of dietary concentrate. In the present study, Firmicutes replaced Bacteroidetes as the dominant phylum in group (III) and *Proteobacteria* were significantly fewer compared to group I. This is in good agreement with data reported by Liu et al. who reported that Firmicutes increased with a high concentrate diet. Similarly, Wetzel et al. observed that *Proteobacteria* decreased and Firmicutes increased with increasing concentrate doses because of the ability of many Firmicutes to easily degrade fermentable carbohydrates. In terms of bacterial and ciliate genera, *Prevotella* increased with increasing concentrate amount, as reported also by Khafipour et al. and Metzler-Zebeli et al. *Prevotella* is one of the most abundant genera in the rumen of goats because these bacteria possess highly diverse functions, in particular following a high-grain feeding regime. *Anaerovibrio* (on day 60) and *Treponema* increased significantly, whereas *Succinivibrio*, *Fibrobacter* (on day 20), *Selenomonas* (on day 60), and *Diplopylastron* (on day 60) decreased significantly with increasing
concentrate dosage. Therefore, the higher relative abundance of *Prevotella* and *Anaerovibrio* was due to augmented pectinase and lipase activities, which led to a further increase in T-VFA, ammonia, acetate, and MCP, and ultimately to enhanced goat growth performance.

Moreover, all indices were lower in group III compared to group I, indicating that bacterial diversity depended on dietary concentrate dosage. Similarly, Lillis et al.\(^{58}\) reported that bacterial diversity was affected to a greater degree by a 90:10 than a 50:50 concentrate:forage ratio.

In summary, this study demonstrates that dietary supplementation with AMPs has beneficial effects on the growth performance, ruminal fermentation function, enzymatic activity, and rumen morphology of juvenile goats; and that these effects are related to concentrate amount. Therefore, AMPs could potentially be used as feed additives for juvenile goats on commercial farms. The detailed mechanism(s) by which AMPs promote growth of juvenile goats and improve their rumen microbial community structure require further clarification.

### Materials and Methods

#### Ethics statement.
All experimental procedures and animal care performed in the present study were approved according to the recommendations of the Guide of the Sichuan Agricultural University Animal Care and Use Committee (Sichuan Agricultural University, Sichuan, China) under permit NO. DKYB20100805, and all efforts were made to minimize suffering. Field studies did not involve endangered or protected species. Chuanzhong black goats were housed at the experimental farm of the Animal Nutrition Institute of Sichuan Agricultural University.

#### Materials.
Antimicrobial peptides used were provided by Rota BioEngineering Co., Ltd. (Sichuan, China). AMPs were composed of recombinant swine defensin PBD-mI(DHYICAKKGGTCNFSPCPLFNRIEGTCYGAKCCIR) and a fly antibacterial peptide LUC-n(ATCDLLSGTVKHSACAAHCLLRGNRGGYCNGRAICVCRN) at a blending ratio of 1:115.

#### Animal handling.
Twenty-four, approximately four-month old, non-castrated Chuanzhong black goats, of average weight (16.17 ± 0.72 kg), were acclimated for 7 days prior to the experiment. All goats were caged and randomly allotted to four dietary treatment groups: I-normal concentrate group (300 g concentrate [per head per day]), II-normal concentrate and antimicrobial peptide group (300 g concentrate + 3.0 g AMPs), III-double concentrate group (600 g concentrate), and IV-double concentrate and antimicrobial peptide group (600 g concentrate + 3.0 g AMPs).

The diet included concentrate (Table 9) and forage (fresh grass). The groups were composed of three replicate pens with 2 goats each, animals were maintained in a house with free access to water, and fed twice daily (at 09:00 and 18:00); the animals maintained their normal herd behaviour.

#### Sampling and DNA extraction.
Rumen fluid samples were collected using a stomach tube on days 0, 20 and 60, prior to morning feeding; the first part of the rumen fluid was discarded to prevent saliva interference. Three goats were selected from each group for sampling (one goat per pen). Rumen pH was measured immediately after collection using a portable pH meter (Model PHB-4, Shanghai Leica Scientific Instrument Co., Ltd., Shanghai, China). Solid feed particles were removed from the rumen fluid by filtration through 4 layers of cheesecloth. Samples were stored at −80 °C for later analysis. Microbial genomic DNA was extracted from rumen samples using a stool DNA kit (OMEGA Bio-Tek, Norcross, GA, USA), according to the manufacturer’s instructions.

#### Ruminal fermentation function and enzyme activity analysis.
Samples were prepared for VFA analysis and chromatography according to Luo et al.\(^{46}\). The concentration of NH₃-N was analyzed using visible-light spectrophotometry (Scientific BioMate 3 s, Thermo). NH₄Cl standards were prepared according to Broderick and Kang\(^{40}\). Microbial protein (MCP) in the rumen was analyzed by trichloroacetic acid protein precipitation\(^{41}\). The activities of CMCase, xylanase, pectinase and β-glucosidase were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA). Protease activity was measured as follows: a reaction mixture containing 1 mL casein and 4 mL protease enzyme was incubated for 4 h at 38 °C; at this point, the reaction was stopped by adding 10% trichloroacetic acid. The sample was then centrifuged at 3500 × g for 15 min. Next, 1 mL of supernatant was removed and mixed with 5 mL 0.4 M Na₂CO₃ and 1 mL Folin-Ciocalteu’s phenol solution and incubated on the laboratory bench for 15 min. The hydrolysed protein was measured using visual-light

### Table 9. Composition and nutrient levels of the concentrate (DM basis). \(^{1)}\)Premix provides the following per kg of the diet: Fe (as ferrous sulfate) 30 mg, Cu (as copper sulfate) 10 mg, Zn (as zinc sulfate) 50 mg, Mn (as manganese sulfate) 60 mg, VA 2 937 IU, VD 343 IU, VE 30 IU.

| Ingredients          | Content(%) | Nutrient levels | Content(%) |
|----------------------|------------|-----------------|------------|
| Corn grain           | 51         | DE/(MJ/kg)      | 13.34      |
| Wheat bran           | 23         | DM              | 84.27      |
| Rapsease meal        | 10         | CP              | 16.66      |
| Rapsease cake        | 10         | CF              | 4.17       |
| Fish meal            | 3          | NDF             | 13.72      |
| NaCl                 | 1          | ADF             | 6.91       |
| Premix\(^{1)}\)      | 2          |                 |            |
| Total                | 100        |                 |            |

In summary, this study demonstrates that dietary supplementation with AMPs has beneficial effects on the growth performance, ruminal fermentation function, enzymatic activity, and rumen morphology of juvenile goats; and that these effects are related to concentrate amount. Therefore, AMPs could potentially be used as feed additives for juvenile goats on commercial farms. The detailed mechanism(s) by which AMPs promote growth of juvenile goats and improve their rumen microbial community structure require further clarification.
spectrophotometry at 680 nm. Concentration and activity of lipase and amylase were measured using commercially available kits (Nanjing JianCheng Bioengineering Institute, Nanjing, China).

**Rumen microbial community analysis.** The V4 regions of bacterial 16S rRNA genes and ciliate protozoal 18S rRNA genes were amplified. Bacterial sequences were amplified using primers 520 F 5′-GACCTAACTTGGYDIAAGNG-3′ and 802 R 5′-TACNVGGTTATCTAATCC-3′; ciliate sequences were amplified using primers V547F 5′-CCAGLSXACGCGGTATATTCC-3′ and VAR 5′-ACTTTCGTTCTGATYRA-3′. The bacterial amplification mixture consisted of 1 μL (10 μM) of each primer, 1 μL template DNA, 5 μL × reaction buffer, 5 μL × high GC buffer, 0.5 μL 10 mM dNTPs, 0.25 μL Q5 high-fidelity DNA polymerase and 11.25 μL ddH₂O. The ciliate PCR was carried out in triplicate using 25 μL mixtures containing 1 μL (10 μM) of each primer, 2 μL template DNA, 5 μL × 5 Q5 reaction buffer, 5 μL × 5 Q5 GC high enhancer, 2 μL 2.5 mM dNTPs, and 0.25 μL (5 U/μL) Q5 polymerase. Amplification was performed as follows: initial denaturation at 98 °C for 5 min; 27 cycles of denaturation at 98 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s; plus a final 5-min extension step at 72 °C. PCR products were excised from 2% agarose gels and purified with a Qiagen Gel extraction kit (Qiagen, Venlo, The Netherlands). The remaining DNA was stored at −20 °C until it was sequenced. High quality DNA, was sent to Shanghai Paisennuo Biological Technology Co. Ltd for sequencing using an Illumina MiSeqPE250 (Illumina, San Diego, CA, USA).

**Data analysis.** Sequence reads were processed and analysed using QIIME pipeline software (version 1.8.0). Chimeric sequences were removed to generate high quality sequences. High-quality sequences were divided and aligned into Operational Taxonomic Units (OTUs) with 97% sequence similarity using the QIIME pipeline software. The highest abundance sequences were compared with template regions in the Greengenes database (Release 13.8, http://greengenes.secondgenome.com/) (bacterial) and NCBI (http://www.ncbi.nlm.nih.gov) database (Ciliate protozoal), and were used to acquire taxonomic information for each OTU and species composition. Alpha diversity indices (including the Simpson index and Shannon index) were obtained using QIIME pipeline software. R software was used to analyze microfloral population structures. The results of these various analyses are expressed as means ± standard error of the mean (SEM). Statistical comparisons were made using paired sample t test via a commercially available statistical software package (SPSS 19.0, Business Machines Corporation, Armonk, NY, USA). Differences among treatments were regarded as significant at P < 0.05.

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Conceived and designed the experiments: Junliang Deng, Qi Liu, Zhihua Ren, Liuhong Shen. Performed the experiments: Qi Liu, Shuhua Yao, Yun Chen. Analyzed the data: Qi Liu, Shuhua Yao. Contributed reagents/materials/analysis tools: Qi Liu, Yanyi Yang, Shuang Gao, Hengmin Cui, Xiaoping Ma, Yanchun Hu, Shumin Yu. Wrote the paper: Qi Liu, Shuhua Yao.

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