Supplementary data

Materials and methods

Molecular assay

Molecular evaluation of the bacteria was performed by PCR method using specific primers (5'-TTGGAGAGTTTGATCCTGGCTC-3'/5'-AGGAGGTGATCCAACCGCA-3') (supplementary file). The required materials for PCR, except DNA, was provided from CinnaGen (Tehran, Iran). For PCR reactions, the main stock of primer was diluted to 5 mM by adding sterilized distilled water. The utilized primers for the identification of *Pediococcus Lolii* are shown in Table 1. The master mixed includes template DNA, reverse primer, PCR buffer, dNTP, $MgCl_2$, Taq DNA polymerase, forward primer, and distilled water. The required amounts from these components were poured into individual vials according to Table 2.

Table 1. Nucleotide sequences of *Pediococcus Lolii* and primers were used in the present study.

Nucleotide sequence	Primer name
TTGGAGAGTTTGATCCTGGCTC- 3'	16s- 27F:
AGGAGGTGATCCAACCGCA – 3'	16s-1492R: 5'-

Table 2. The master mixed components and their required amounts.

Matrix component	Required amount
Template DNA	100 mg
PCR 10X buffer	5 µl
$MgCl_2$	3 µl
dNTPs	1 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
Taq DNA polymerase	0.5 µl
Distilled water	up to 50 µl

Subsequently, 18 µL of the prepared master mixed together with 2 µL of extracted DNA were poured into a 0.2 mL vial, and the vial was put into the thermocycler and the PCR was conducted according to the following protocol:

a) Initial denaturalization at 94 °C for 5 min,
b) 35 cycles of the following steps,
    b-1) 94 °C for 60 s,
    b-2) 60 °C for 40 s,
    b-3) 72 °C for 90 s,
c) One cycle at 72 °C for 10min.

Results

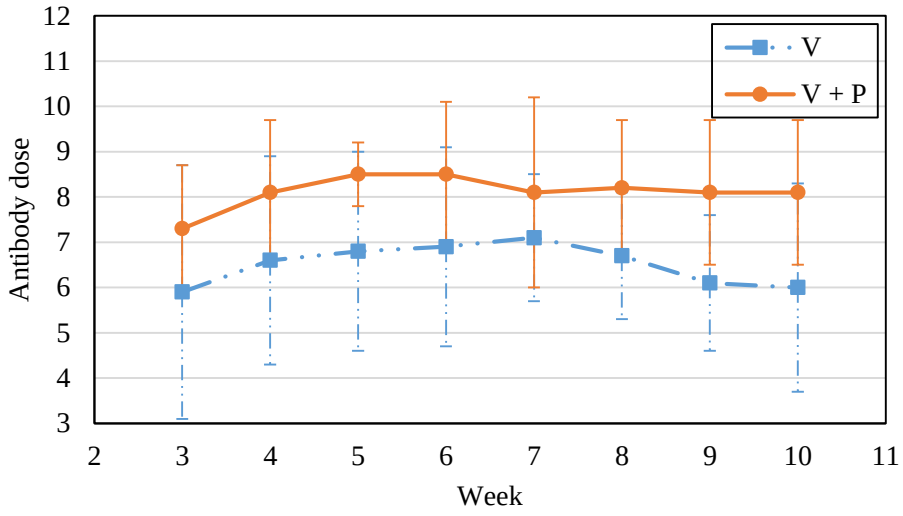

Fig. 1. Antibody changes after vaccination against influenza virus (■-..-) vaccine alone, (-●-) vaccine + probiotics

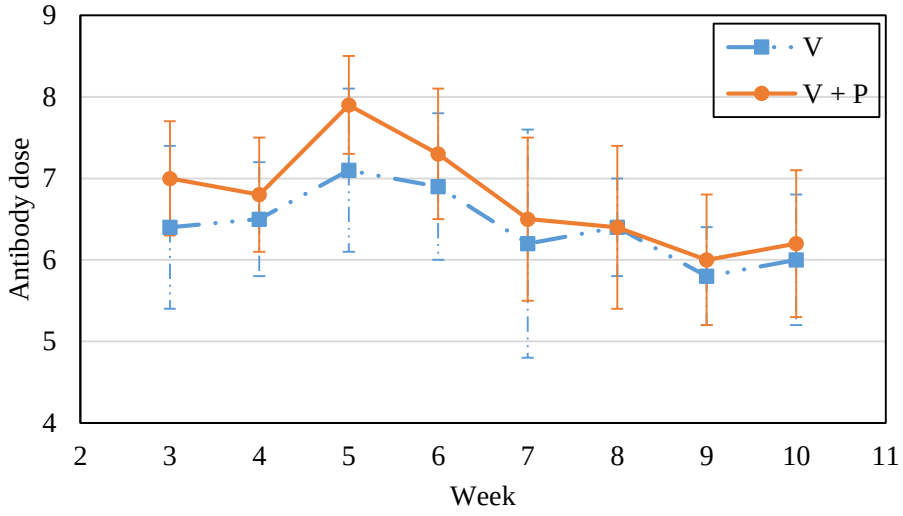

Fig. 2. Antibody changes after vaccination against Newcastle virus (■-..-) vaccine alone, (-●-) vaccine + probiotics