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IN VITRO AND IN VIVO ANALYSIS OF BIOACTIVE SUBSTANCES GROWTH AND ANTIOXIDANT ACTIVITIES

Modou Mangan (ESR4)

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

Abstract

IN VITRO AND IN VIVO ANALYSIS OF BIOACTIVE SUBSTANCES GROWTH AND ANTIOXIDANT ACTIVITIES

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Heat stress is a major problem in the poultry industry, causing severe economic loss due to its adverse effects on chickens' health and performance. Thus, the main aim of this study was to screen in vitro bioactive substances to find those most suitable for in-ovo modulation of chicken microbiota and heat stress mitigation in chickens. To achieve this, we first determined the kinetic growth curve of the selected probiotics (*Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, *Limosilactobacillus reuteri*, *Lacticaseibacillus rhamnosus*). Subsequently, these probiotics were combined with prebiotics (raffinose, galactooligosaccharide (GOS), long-chain inulin) and plant extracts (green tea, turmeric, garlic extract). The growth curve of the probiotics in the presence of prebiotics or plant extracts was determined by optical density (OD) at 600 nm. Finally, the antioxidant activities of these bioactive substances were assessed using the 2,2-diphenyl-1-picrylhydrazyl DPPH) assay.

From the results obtained, *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* had the highest growth curve (OD 2). From the DPPH results, *Lactiplantibacillus plantarum* exhibited antioxidant activity of 69% and thus was selected for in-ovo injection on day 12 of embryonic development. The second bioactive used for in-ovo injection and microbiota modulation was prebiotic GOS which was selected due to its positive effects in mitigating heat stress in poultry. These bioactive substances were delivered in-ovo to test their effects on hatchability and zootechnical parameters on day-old chicks.

Preliminary results show that the hatchability was higher in the negative control (not injected) and GOS treatment. The day-old chick body weight was higher ($P < 0.05$) when treated with *Lactiplantibacillus plantarum* compared to the other groups. The day-old chick length was lowest in the negative control. There was no difference between the treatments for the Pasgar score.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N°955374.

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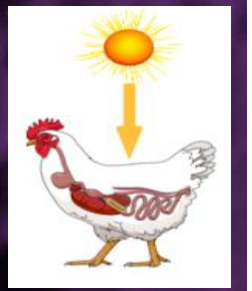
INTRODUCTION



HIGH TEMPERATURES – CHALLENGE FOR POULTRY PRODUCTION

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HIGH TEMPERATURES – CHALLENGE FOR POULTRY PRODUCTION

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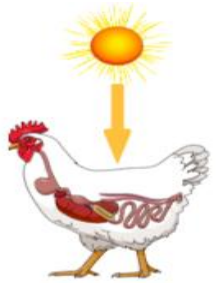
Physiological problems

- Acid-base imbalance
- Oxidative stress
- Respiratory alkalosis
- Shift in the microbial profile



Performance parameters

- Reduced feed intake
- Poor feed conversion ratio
- Reduce body weight
- Poor meat quality
- Increased mortality
- Economic losses

Possible intervention strategies

- Good house design
- Nutritional management
- Genetic selection

Bioactive substances increase antioxidant potential, reducing oxidative stress and heat stress thereby enhancing a balanced gut microbiota in poultry.

Solution: In *ovo* delivery of bioactive substances?

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Objectives

- To in vitro select bioactive substances for *in ovo* injection
- To determine the antioxidant potentials of the selected bioactive substances
- To determine the effects of the selected bioactive substances on hatchability and day-old chick quality

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METHODOLOGY – IN VITRO SELECTION

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- **Probiotics**
- *Lacticaseibacillus rhamnosus*
- *Lacticaseibacillus casei*
- *Limosilactobacillus reuteri*
- *Lactiplantibacillus plantarum*

- **Prebiotics**
- Inulin
- Raffinose

- **Plant extracts**
- Green Tea
- Garlic
- Turmeric

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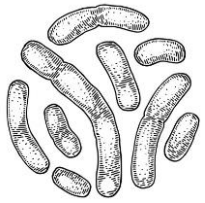
Why these bioactive substances?

Promote early gut colonization

Stimulate immune function



Antioxidant potentials

Pathogen exclusion

Stimulate gut microflora

Radical scavenging ability

Stimulate immune function

Why the DPPH assay?

- Simple and inexpensive
- Direct measurement of antioxidant activity
- High sensitivity

Oxidative stress can be cause by heat stress

Heat stress can cause gut dysbiosis leading to pathogen infection and diseases

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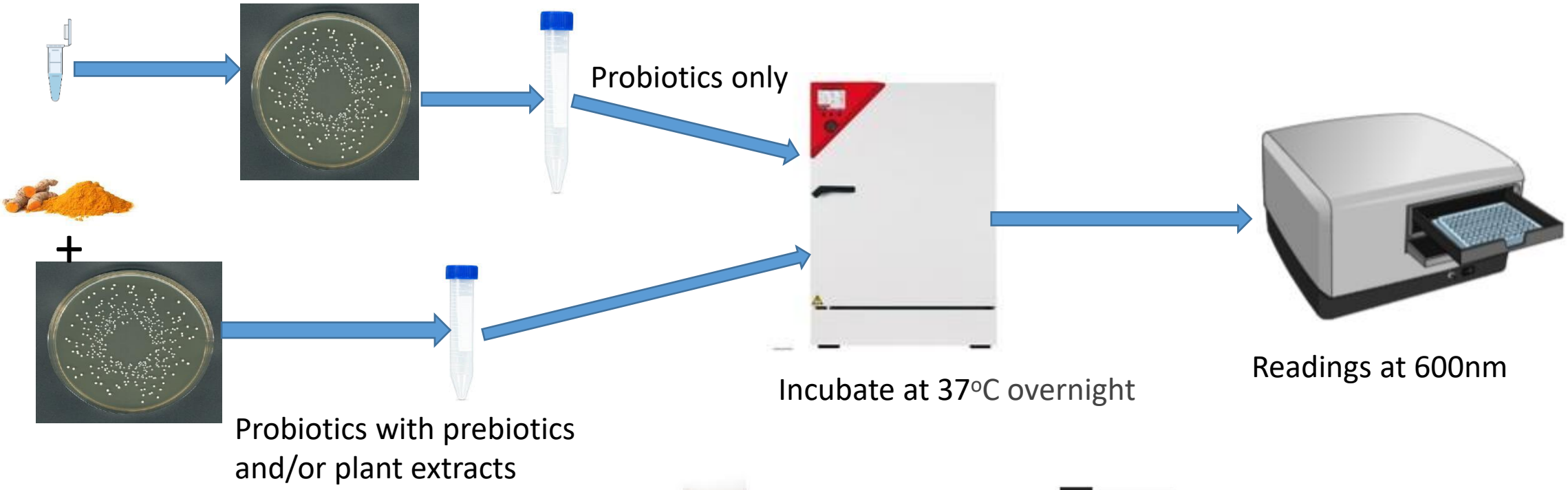
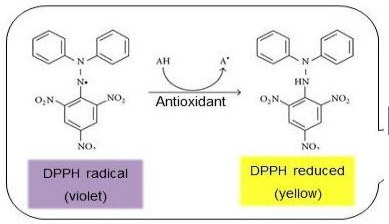


DPPH ASSAY

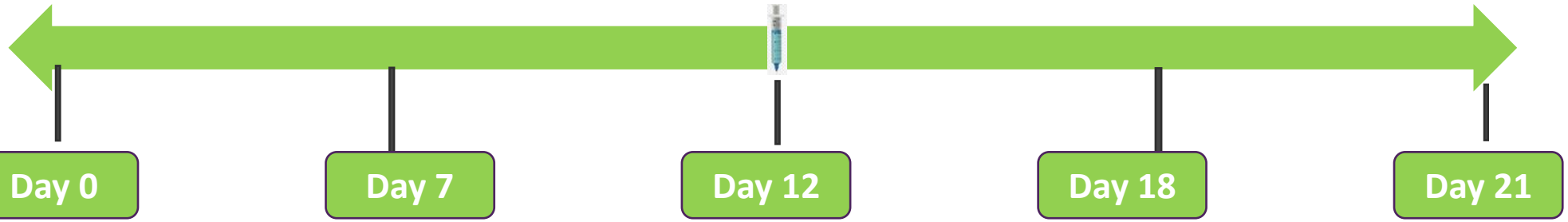


Antioxidant quantification at 520nm

Inhibition (%) : (A control −A sample)/ (A control) X 100

Data was analysed using STATISTICA version 14. One way ANOVA was used, Statistical differences was tested using Tukey test. Graphs were plotted using OringinPro

METHODOLOGY: IN *OVO* PROGRAMMING



Temperature 37.5°C and RH 60%

Treatment groups

- *Lactiplantibacillus plantarum*
- Positive control
- Negative control

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RESULTS- IN VITRO

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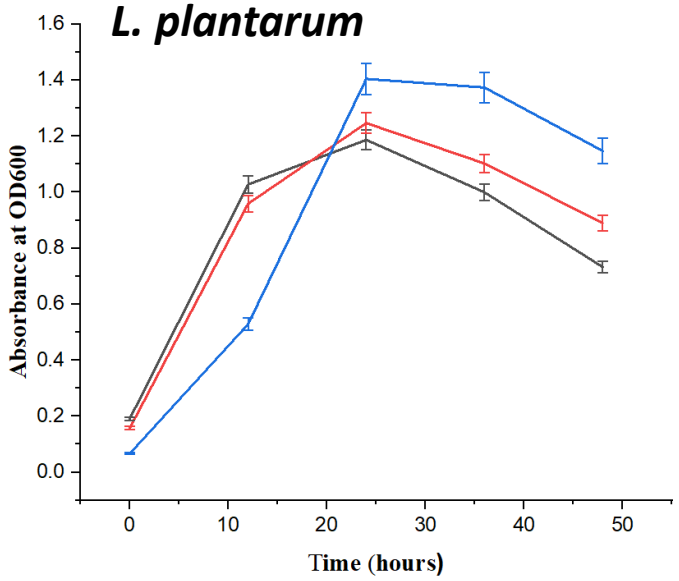
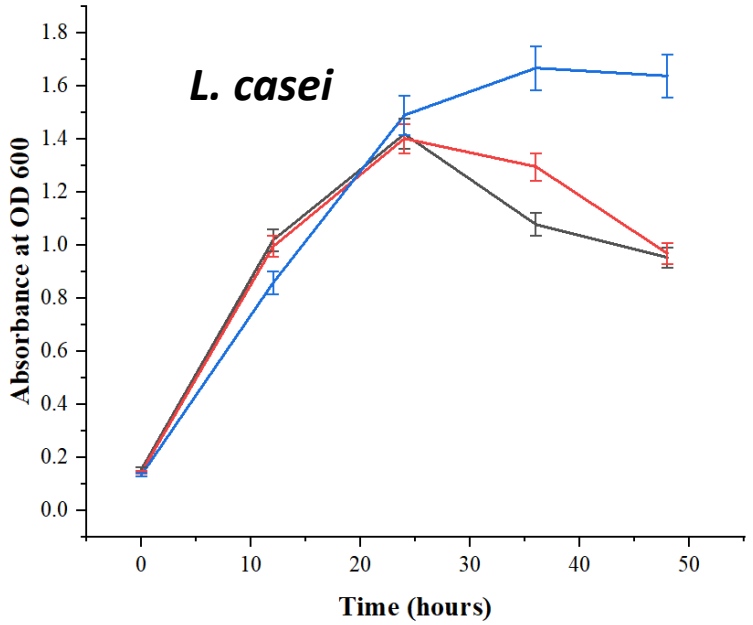
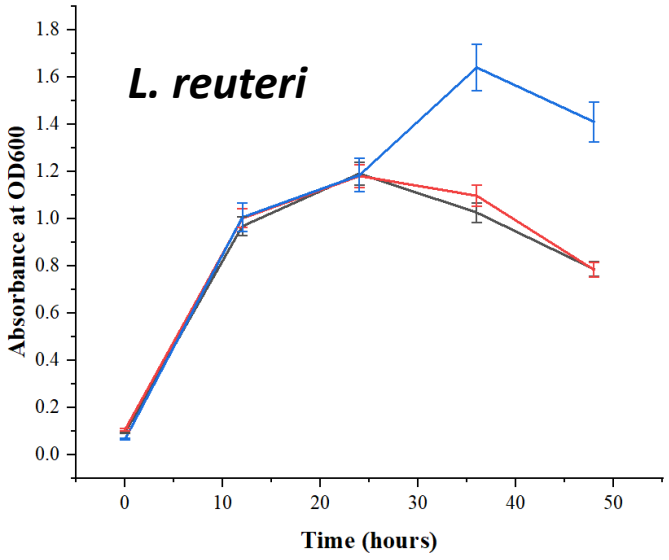
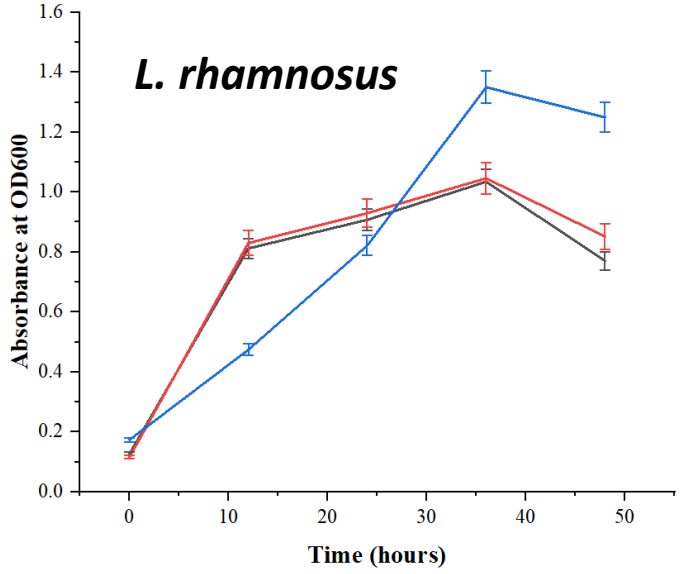
Growth curve of probiotics combined with prebiotics







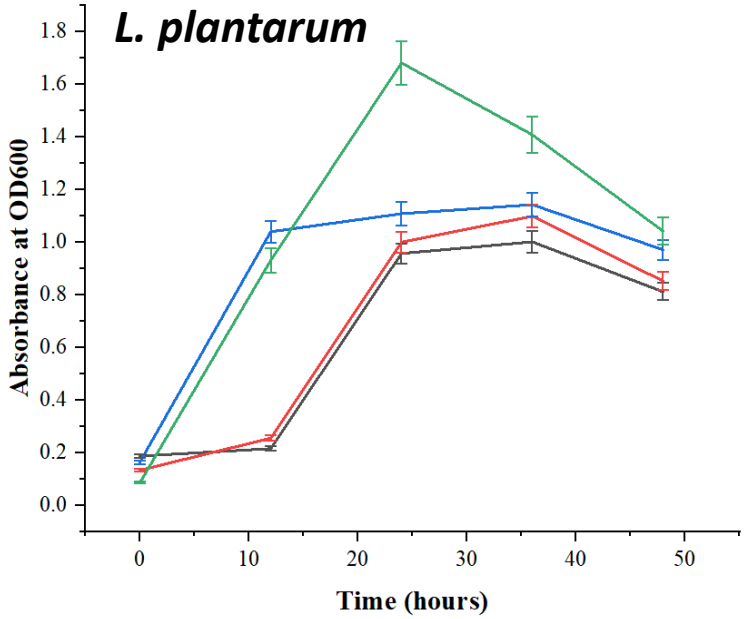


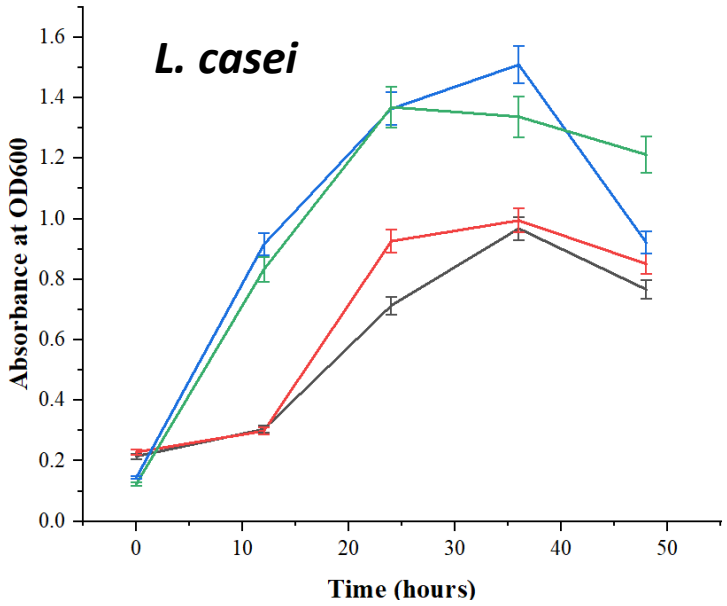
Probiotics with raffinose

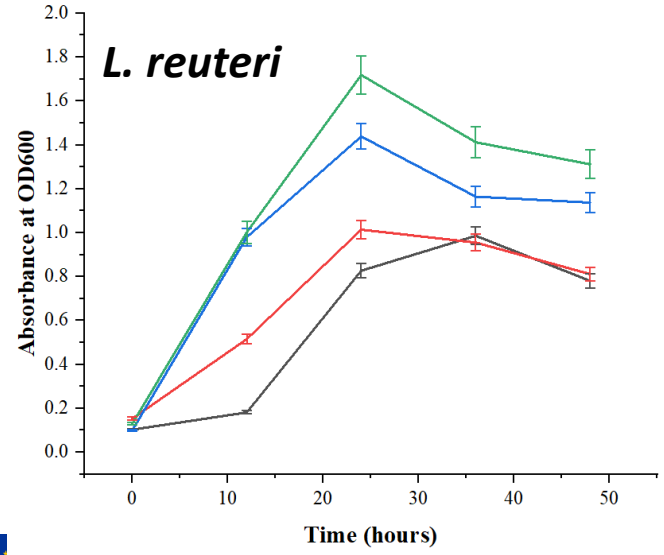
Probiotics with inulin

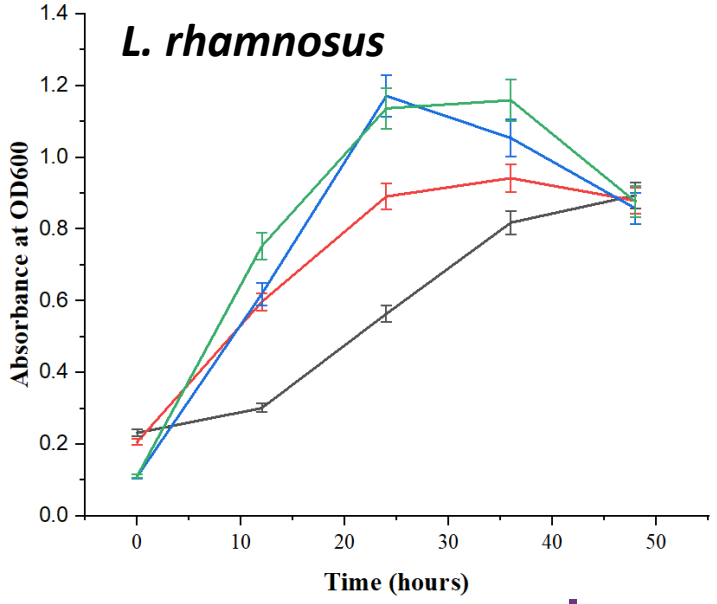
Probiotics alone

Growth curve of probiotics combined with plant extracts









- Probiotics with greentea
- Probiotics with turmeric
- Probiotics with garlic
- Probiotics alone

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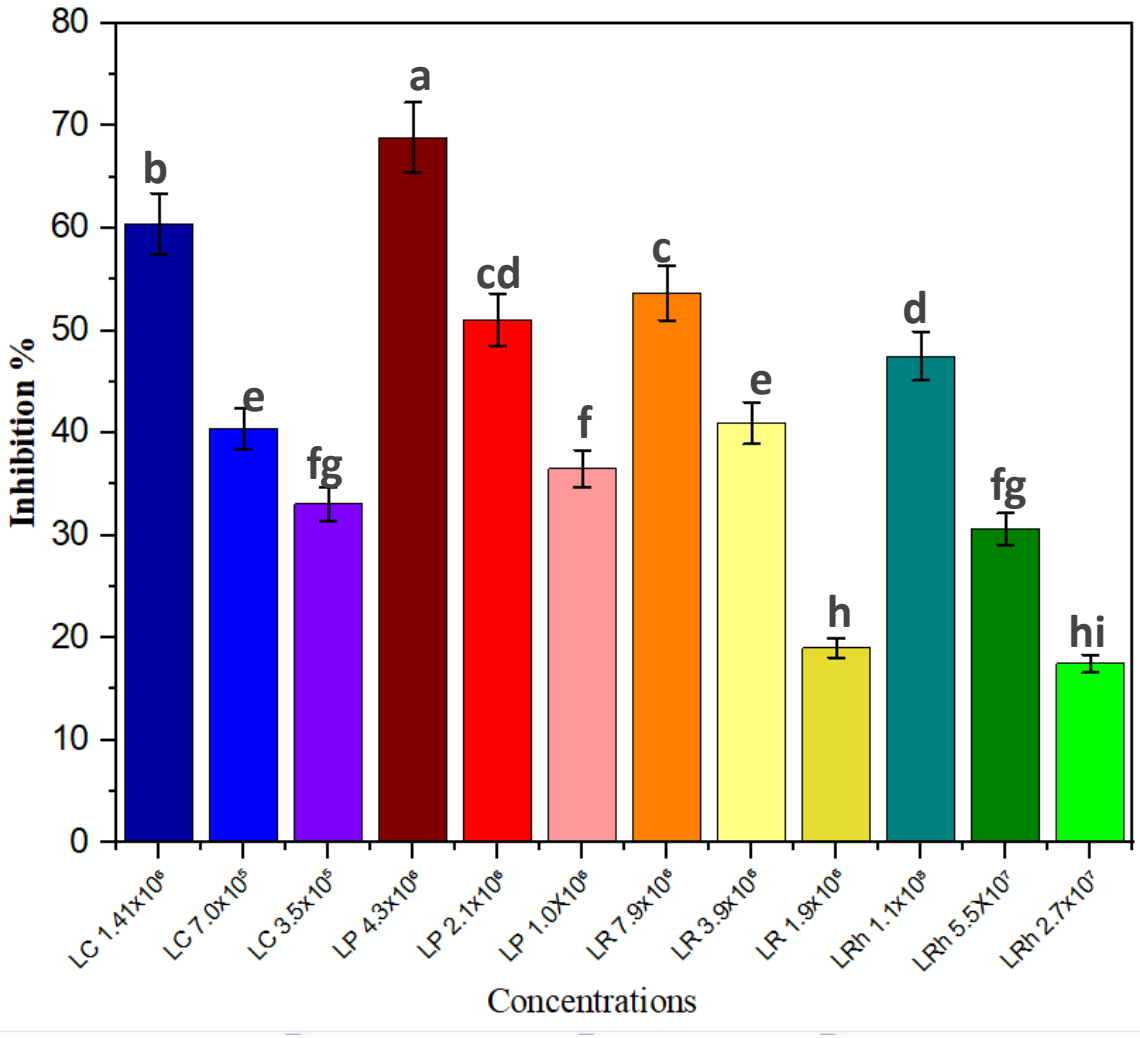
DPPH assay for probiotics only



L.C: *Lacticaseibacillus casei*
L.P: *Lactiplantibacillus plantarum*
L.R: *Limosilactobacillus reuteri*
L.Rh: *Lacticaseibacillus rhamnosus*

a,b letters having different superscripts differs significantly (P< 0.05)

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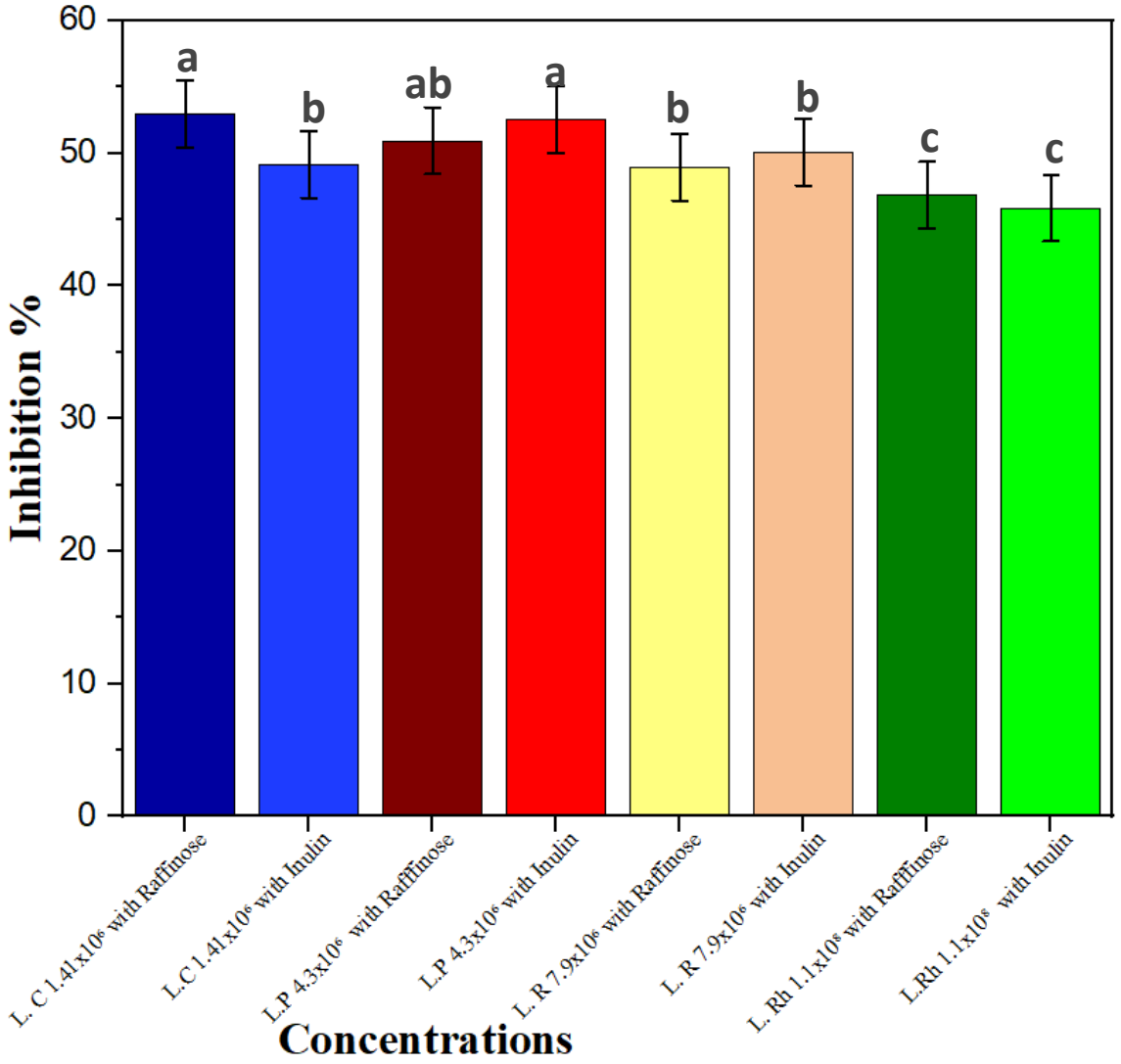
DPPH assay of probiotics combined with prebiotics



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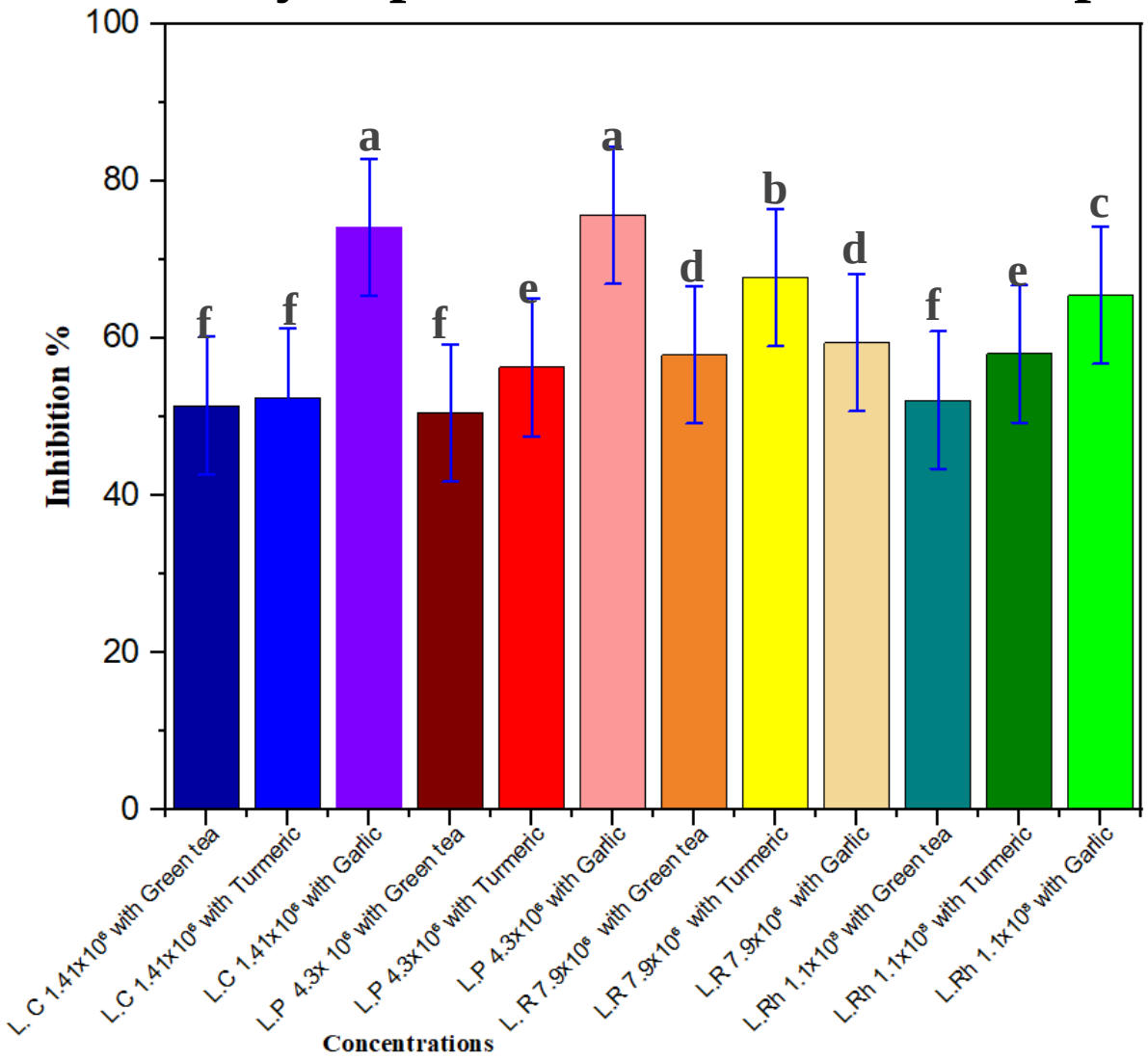
DPPH assay of probiotics combined with plant extracts



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RESULTS- IN VIVO

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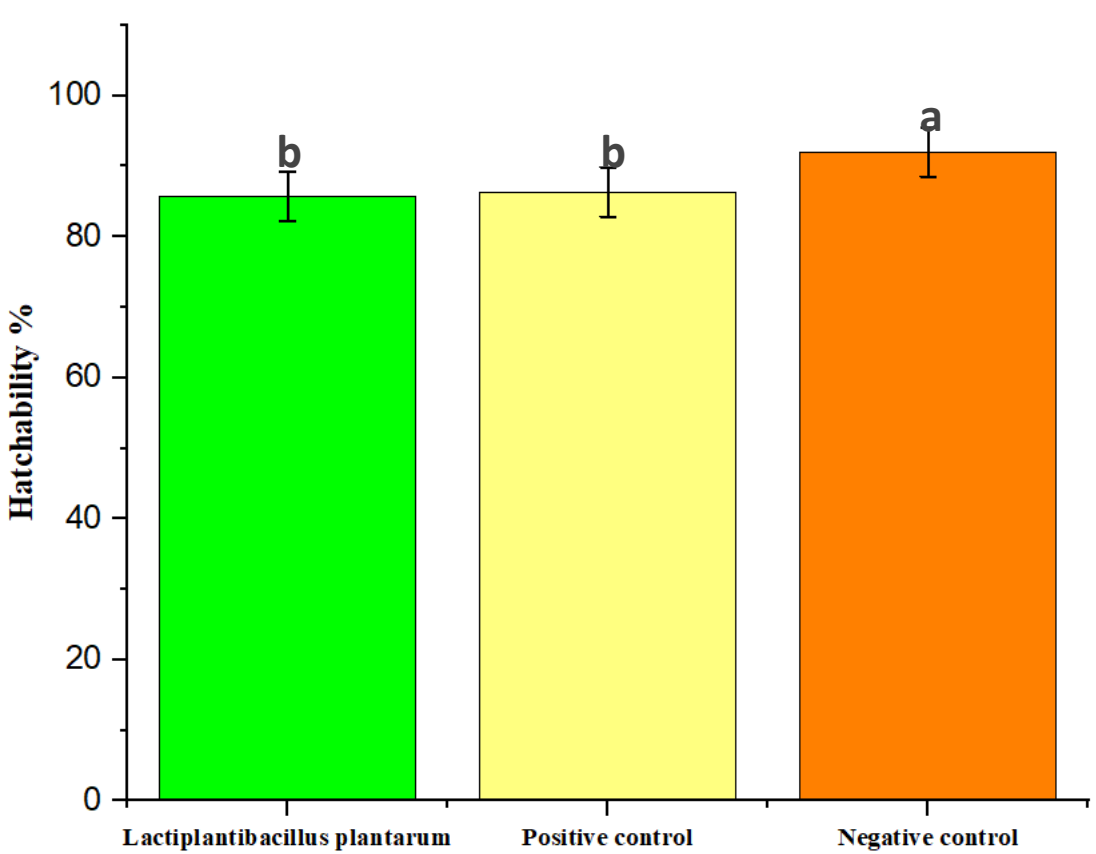
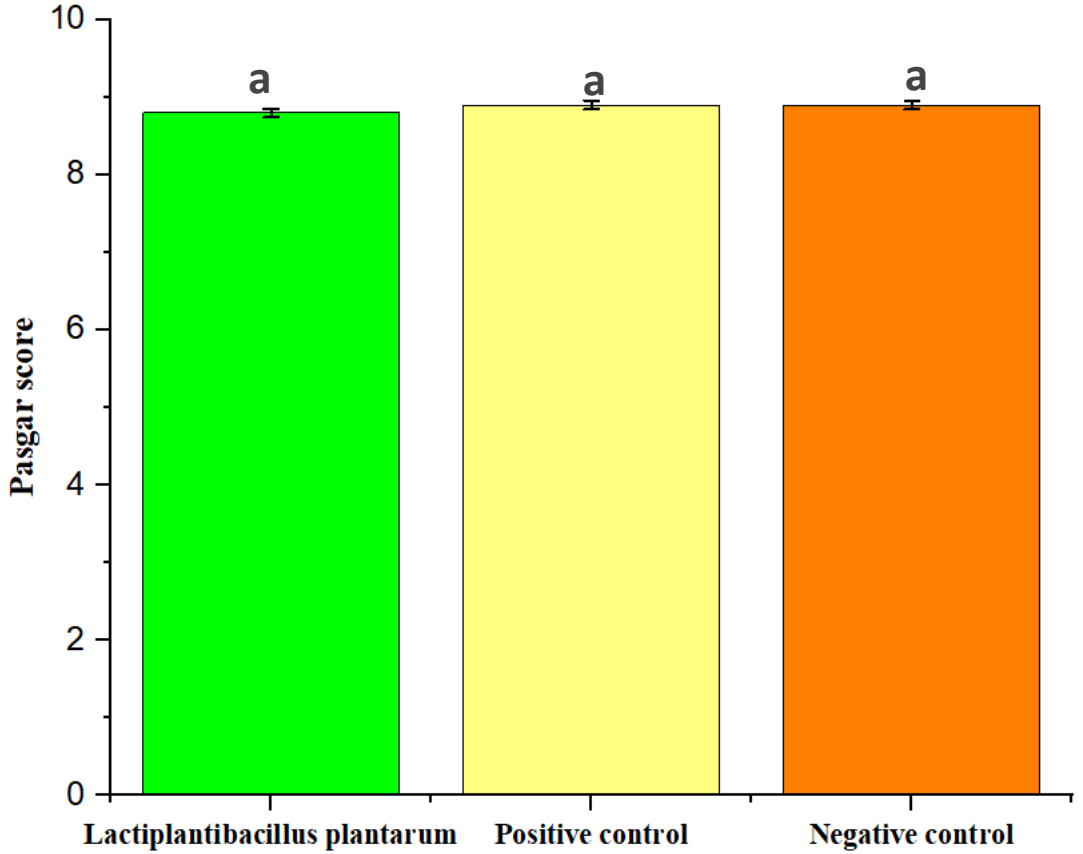
Hatchability rate and pasgar scores of one-day-old chicks





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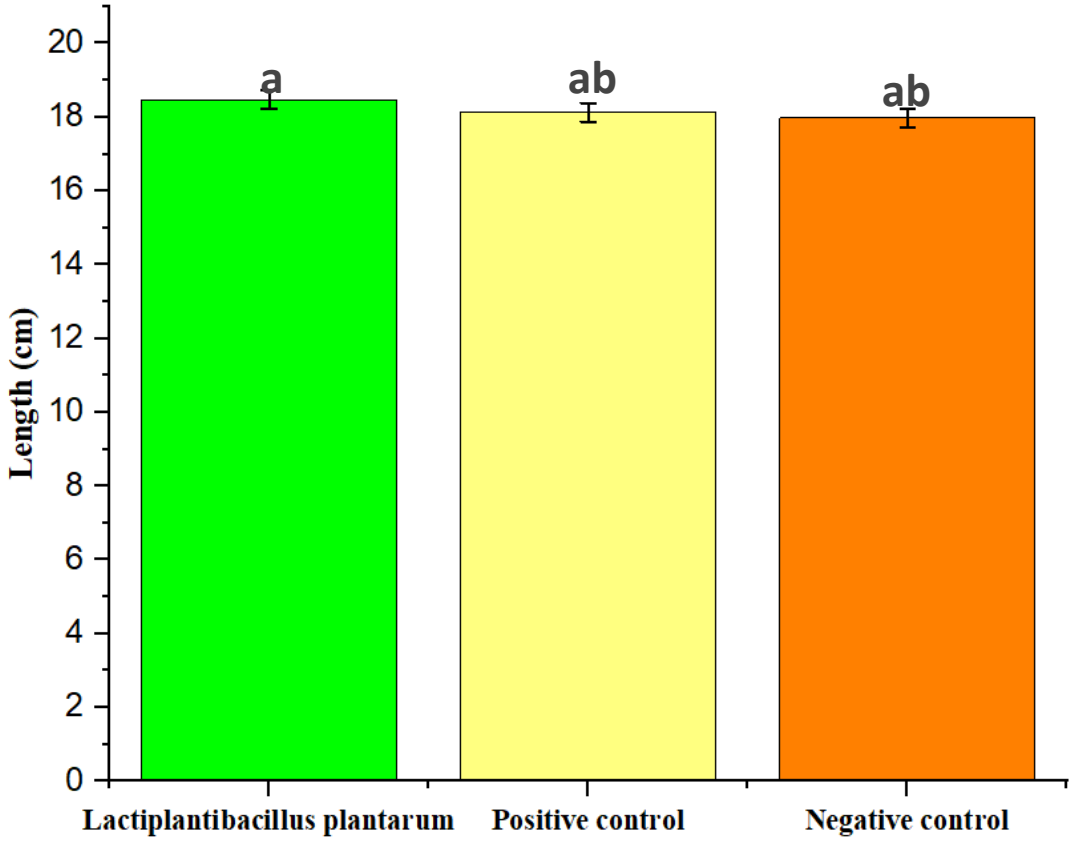
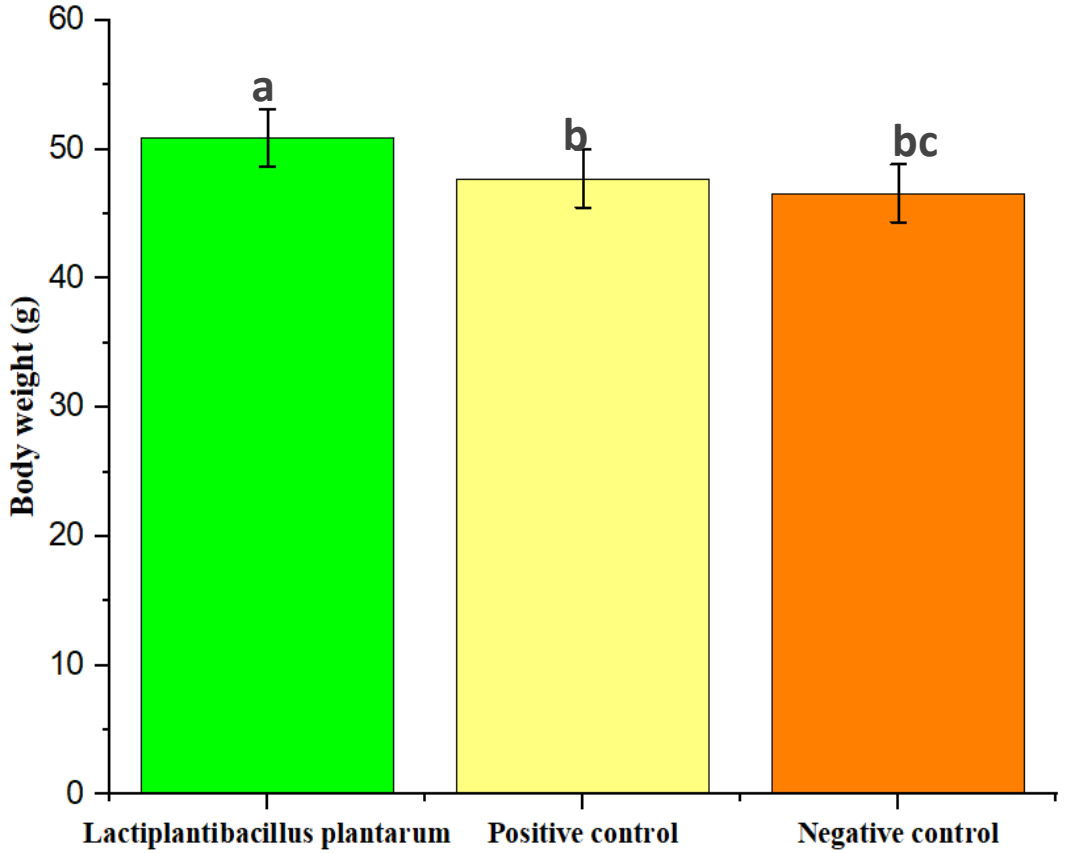
Lengths and body weights of one-day-old chicks





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TAKE- HOME MESSAGE

- The current in vitro data show that *Lactiplantibacillus plantarum* exhibited the highest antioxidant activity compared to all other probiotics tested.
- *Lactiplantibacillus plantaru*m increased the body weight of day-old chicks, length of day-old chicks, improved chick quality and does not affect hatchability.

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THANK YOU

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Supervisors:

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