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# THE EFFECT OF BLENDED PROJECT-BASED LEARNING INTEGRATED WITH 21ST-CENTURY SKILLS ON PRE-SERVICE BIOLOGY TEACHERS' HIGHER-ORDER THINKING SKILLS

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## ABSTRACT

Educational institutions are required to prepare competent and competitive pre-service teacher graduates who have skills according to the needs of the 21st-century. This study aims to find the effect of blended Project-Based Learning (PjBL) integrated with 21st-century skills on higher-order thinking skills of students as pre-service biology teachers on immunology. This research is an experimental study with a mixed method, in which the qualitative observation data used a questionnaire with self-assessment and peer assessment methods supported by experimental data using the HOTS instrument analyzed quantitatively. The application of blended PjBL integrated with 21st-century skills used a quasi-experimental research method with a quantitative descriptive approach. Pre-service teachers in this study are still studying at educational institutions, especially teacher faculty, and categorized as pre-service teachers. The research sample was students taking the immunology course in the Biology Education Study Program. They were 57 students from IAIN Palangka Raya, 60 students from Universitas Palangka Raya, and 83 students from Universitas Negeri Malang. Student grouping based on the initial ability of HOTS is carried out in all groups from which the student sample is referred to as a cluster. The total sample of 200 people was then grouped into three groups based on their basic abilities, namely low-level class, medium-level class, and high-level class. The study results proved that the blended PjBL integrated with 21st-century skills significantly affected students' high-level thinking skills based on the paired sample test (Sig. 2-tailed 0.000<0.05). The mean value in the experimental group (75.53) and the control group (56.35) strengthens the data significance of students' higher-order thinking skills based on the N-Gain value and the independent sample T-test. The findings of this study are that blended PjBL integrated with 21st-century skills can increase HOTS on indicators of evaluating (78.15) and creating (79.21). This blended learning integration can be used as an innovative learning model solution to increase pre-service biology teachers' higher-order thinking skills according to the demands of 21st-century skills.



Keywords: 21st-century skills; blended learning; immunology; project-based learning

## INTRODUCTION

The 21st-century skills are the primary targets in the curriculum of educational institutions around the world (Alismail & McGuire, 2015; Yusuf et al., 2017; Wang et al., 2018; Haviz et al., 2018) because a good skill in the 21st century has become one of the solutions to answer the challenges of the industrial revolution 4.0 era. The 21st-century skills equalize the ability to think, which is needed in the 21st century. One of the main skills in 21st-century skills is creative, critical, and problem-solving, or higher-order thinking skills (Van Laar et al., 2017; Rahman,

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2019). Critical thinking needs to be developed for students because it is a cognitive thinking process (Sardone & Devlin-Scherer, 2010). Creative thinking skills produce products through new ideas (Hanni et al., 2018), create ideas, change thinking flexibly, and develop ideas to find problem solutions (Katz et al., 2018). Problem-solving is a thought process that stimulates students to treat a problem and analyze it, aiming to solve the problem, training individuals to collaborate procedurally and systematically, developing creativity, expanding thinking processes, increasing intellectual abilities, increasing individual motivation, and increasing individual motivation activity in the learning process. The problem-solving ability needs to be developed for each individual (Martin et al., 2017; Villena et al., 2019). The development of HOTS can be done in the learning process, including biology learning.

Educators have prepared various strategies in training students and pre-service teachers with the demands of the 21st-century (Teo, 2019). Quality education is one factor that determines the progress of a nation. Therefore, educational institutions must prepare the nation's generation with special skills, otherwise known as 21st-century skills (Geisinger, 2016). 21st-century skills, known as 4C, include critical thinking and problem-solving, creativity and innovation, communication, and collaboration. Overall, these competencies are needed to survive in facing global problems (Greiff & Kyllonen, 2016; Jia et al., 2016). Thus, it is vital to research all fields, including 21st-century skills that involve students and pre-service biology teachers.

Haviz et al. (2018) explained that global competition and technological developments in the 21st-century are a fast and dynamic development of the century and require individuals who have 4C skills or soft skills implemented in everyday life. Larson and Miller (2011) also argued that soft skills that can be implemented directly in real life are more important than hard skills. Education is an academic forum expected to produce graduates who can follow scientific developments in science and technology. As a human resource, teachers have a vital role in the education system. Preparing qualified teachers is one of the responsibilities of educational institutions to produce competent and competitive teacher candidates. This competence is an absolute requirement for pre-service teachers according to the needs of the 21st century, so it becomes an important point in this research.

Aspiring teachers need 21st-century skills to compete in the 21st century. Research by Haviz et al. (2020) reported the importance of 21st-century integrative skills mastered by pre-service teachers and education administrators. Those skills can increase the ability to sell power (marketability), ability to work (employability), and readiness for citizenship (Sang et al., 2018; Zainuddin & Perera, 2019). Critical thinking and higher-order thinking skills are needed to perform various analyses, assessments, evaluations, reconstruction, decision-making that lead to rational and logical action (Hudha & Batlolona, 2017). Higher-order thinking skills in 21st-century skills are one aspect that can be achieved through the Project-Based Learning (PjBL) model.

Analysis of several previous studies reported that blended learning and PjBL were quite influential in improving students' creative thinking skills (Yustina et al., 2020), students' metacognitive behavior (Listiana et al., 2016), problem-solving abilities (Nawani et al., 2019), and generic science skills (Haviz et al., 2018). It also encourages creativity (Lucas, 2016) and positively correlates with teacher analysis skills (Aslan & Zhu, 2017). Maryuningsih et al. (2019) emphasized the advantages of PjBL integration in science learning, which aims to determine the level of thinking skills and assess the perspective of Biology teachers. The results of a quasi-experimental study of 37 biology teachers as respondents reported a significant increase in the thinking skills and perspectives of biology teachers in learning chromosome inheritance material through online discussion forums. This research illustrates the importance of integrating science learning with 21st-century skills. Integrated learning focuses more on competency content, so exploration of 21st-century skills and thinking skills in learning more broadly is essential (Zainuddin & Attaran, 2016).

Strengthening higher-order thinking skills (HOTS) is student-centered and influenced by strategies and innovative models of learning (Haviz et al., 2020). It means that students' thinking skills are also influenced by the role of the teacher in designing and using strategies or learning models that are appropriate to the characteristics of the material. Therefore, exploring learning models is essential to improve thinking skills (Fitriani et al., 2019; Maryuningsih et al., 2019). The learning model must be designed appropriately to accustom students to think at higher levels (Listiana et al., 2016). Strengthening HOTS can be achieved when students actively understand and integrate knowledge with their experiences (Anderson & Krathwohl, 2015). To develop HOTS, students must understand factual, conceptual,

and procedural knowledge to apply their practiced knowledge and then analyze the process to find solutions. Lecturers guide students through observing activities, forming concepts, giving responses, analyzing, comparing, and giving the necessary considerations (Yerdelen et al., 2015; Suwarma & Apriyani, 2022). In line with this, Wang et al. (2018) stated that project-based learning is the ideal model for meeting 21st-century educational goals because it involves the 4C principles.

The PjBL, as a learning model, uses projects as learning media. Students explore, assess, interpret, synthesize, and information to achieve learning goals. The learning model is problem-oriented as a first step in collecting and integrating new knowledge based on experience and is designed to analyze solutions to complex problems in investigating. The blended PjBL, which is integrated with 21st-century skills, is expected to influence better the HOTS of pre-service biology teachers (Haviz et al., 2020). This research hopes that it can explore innovative learning models that are appropriate in increasing the HOTS of pre-service biology teachers, one of which is in the immunology material.

The material of the mucosal immune system in immunology courses is abstract (Sumarno et al., 2012; Milliana et al., 2014; Sumarno et al., 2015), so it requires understanding and the ability to analyze higher basic concepts. A study of the students' learning outcomes at IAIN Palangka Raya indicated that 86.67% of the immunology material was inappropriate, 63.33% of students were less able to construct their understanding, and 60% of them were not able to develop sensitive attitudes towards technological developments related to infection and immunity. The characteristics of this material require better critical analysis skills, where analytical skills are part of thinking skills. Through immunology material, it is hoped to stimulate students' thinking and analysis skills. The target of learning outcomes in the immunology course is to understand the basic concepts of immunology, which include mechanisms at the cellular, tissue, organ, and organ system levels. Students can apply various immunology concepts in everyday life, analyze multiple problems that develop in the environment as an implementation of the concept in immunology, and communicate the results of using the basic concepts of immunology based on scientific written observations. As a protein secreted by plasma cells that binds to antigens and functions as an effector of the humoral immune system, Immunoglobulin A (IgA) is essential to understand more explicitly its role in the immune system against infection (Petersen et al., 2012).

Infections due to microorganisms and how to deal with them are basic knowledge that everyone must have in facing the current pandemic (Yustina et al., 2020). Transfer of knowledge concepts in learning requires a high level of understanding and critical analysis of students to be understood optimally. The blended PjBL integrated 21st-century skills are recommended as an innovative learning model that has characteristics in line with the basic competencies of immunology material, which aims to improve students' HOTS and critical thinking skills. The integration of 21st-century skills in the blended learning model is reported by Haviz et al. (2020) to improve the pre-service biology teachers' thinking skills significantly. The results of Haviz's study become the basis for the analysis of the importance of exploring the learning model for pre-service biology teachers in learning biology material in this study so that it is more innovative and able to stimulate higher-order thinking skills.

This study aims to explore innovative learning models to improve students' HOTS as pre-service biology teachers through the blended PjBL integrated with 21st-century skills in immunology material. The innovative learning targeted in this study is the renewal of the learning model by integrating one of the 21st-century skill components into the PjBL model, which is also a novelty of the previous learning model. The 21st-century skill component referred is critical thinking and problem solving, which was inserted in the 5th stage (testing process and learning outcomes) and the 6th (project evaluation) in PjBL (Choi et al., 2019). Student HOTS targeted through the blended PjBL model is the students' ability to analyze, evaluate, and create in connection with learning projects of the importance of probiotic supplementation in increasing the body's immune system. Blended PjBL integrated 21st-century skills implemented referring to in vivo probiotic supplementation practicum in group projects. One month, the probiotic lactobacillus reuteri supplementation in this project against Balb/c mice was given. The stimulated immunomucosa response was measured based on the level of s_IgA in the serum of mice. In this project, students are required to analyze the correlation of supplementation with the secretion of s-IgA as the body's defense system against infection.

According to several studies, the blended learning used in the PjBL model can overcome the problem of time constraints (Sumarni & Kadarwati, 2020) because it involves students in organized and meaningful activities in designed projects (Ummah et al., 2019). Therefore, the

blende model in this study used modified practicum-based learning tools, both in material and assessment aspects. Blended learning is expected to show significant results as one of the results of exploring an innovative learning model for immunology subjects that is appropriate in increasing students' HOTS. Implementing the four skills of the 21st-century requires multiple evaluations to apply to different environments. Therefore, the focus of this study is HOTS on several indicators of higher-order thinking criteria, as one of the exploratory findings of this study.

## METHODS

This research used mixed-method, in which experimental research is integrated into educational research through a project-based blended learning model (Creswell, 2016). The mix method in blended PjBL integrated with 21st-Century Skills" in this research is a combination or mixture of online and offline learning, which is a learning strategy that combines face-to-face learning and learning that uses online learning resources. The online learning resources are supported by various literature sources, which can be accessed via the internet (online). The information collected is discussed through an offline, face-to-face meeting and becomes a discussion material for the experimental project. This study's implementation stages of blended learning refer to the PjBL integrated with 21st-century skills stages. The experimental stage in this research is designed to be part of a project implemented in the PjBL model and be integrated into educational research through the mucosal immune system practicum activities. There were three research locations: The Laboratory of Microbiology of Institut Agama Islam Negeri Palangka Raya, the Laboratory of Analytical Chemistry of Universitas Palangka Raya, and the Laboratory of Biomolecular of Universitas Negeri Malang from August to September 2020.

The 21st-century skills to improve students' HOTS were analyzed from each indicator's difference tests and score analysis. The research subjects used as research samples were Biology Education undergraduate students taking Immunology courses. They were 57 students from Institut Agama Islam Negeri (IAIN) Palangka Raya, 60 students from Universitas Palangka Raya (UPR), and 83 students from Universitas Negeri Malang (UM). The research sample used in this study were students studying at the teacher training faculty, so the students were referred to as pre-service teachers. The whole research subjects were grouped into three groups based on the results of the preliminary test analysis of students' initial HOTS: low-level class, medium level class, and high-level class. The grouping is based on the results of the preliminary test analysis of students' HOTS initial abilities, where students whose score less than 56 are categorized in low-level group, a score between 56 and 71 is considered a medium level group, and a score greater than 71 is categorized as a high-level group (Table 1). Student grouping based on the initial HOTS is carried out in all groups from which the student sample is referred to as a cluster. The grouping aims to determine the effectiveness of the implemented blended learning model, whether it is more effective for groups of students with initial abilities of the low-level class, medium level class, or high-level class.

**Table 1.** The Classification of HOTS Score Groups

| Range of Score | Number | Category |
|---|---|---|
| 72 - 100 | 3.5 – 4.0 | High |
| 56 - 71 | 2.5 - 3.0 | Medium |
| ≤ 39 - 55 | 0 – 2.0 | Low |

The implementation stage of the blended PjBl integrated 21st-century skills of the research is summarized and presented in Figure 1.

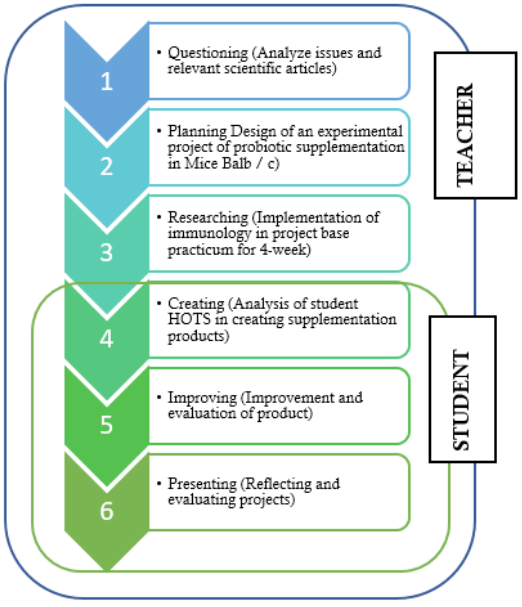



**Figure 1.** Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020)

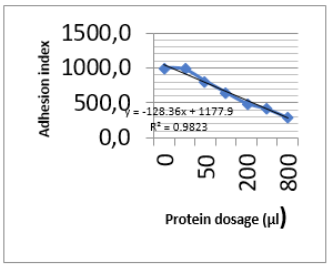
The research stages in Figure 1 begin with 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of preparing questions or project assignments that come from local issues, contextual in real life, and are adjusted to the basic competencies of the material. The presentation of the issue begins with a critical analysis of articles relevant to the research topic; 3) designing a collaborative project plan. The project was designed with only one design problem in a working group: the mucosal immune system—probiotic supplementation in producing s-IgA in serum Balb/c mice as an immune system. The work project is designed with laboratory experimentation; 4) arranging the schedule for project completion, which includes the timeline, final target, project deadlines, planning for problem-solving methods, and scientific reasons for choosing the particular method; 5) project monitoring and project evaluation assignments for students independently; 6) testing the results through project presentations to determine the achievement of student competencies and evaluate the project's achievement; 7) evaluation and reflection of activities, analysis of project result individually and in groups at the end of the project. The integration of 21st-century skills into PjBL at this stage is through skills in analyzing, evaluating, and creating, as these four skills are HOTS indicators. HOTS indicators raised in the experimental project include the ability to analyze the concept of immunology and the body's integrity system, evaluate the immunomodulatory mechanisms and immunoregulators, and create basic concepts for protein-based supplementation products.

Yustina et al. (2020) combine PjBL with PBL on pre-service creative thinking skills, so researchers must refer to the blended learning stage in the study to modify the PjBL model in its integration with 21st-century skills. Research design, determination of control groups, and online and offline methods in this study also refer to the research. Modifications were made after the questioning (issue analysis) and planning stages because the integrated 21st-century PjBL blended learning continued with the research stage.

The instrument used to measure HOTS is an assessment of multiple-choice questions that are compiled based on the three indicators of 21st-century skill achievement, namely skills to analyze, evaluate, and create. The observation process can be carried out by lecturers and students using self-assessment and peer assessment (Bahri et al., 2019). Students did self-assessment or individual assessment, while group members and lecturers carried out peer assessment. The test scores and observation sheets were reviewed descriptively and then presented.

The instrument in this study are:

1. Look at the 65kDa test result of protein adhesion subunit Yersinia enterocolitica in the following diagram.

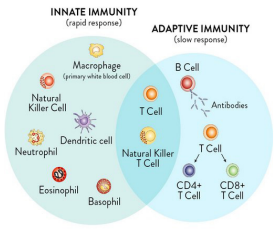



1. The higher the dose, the greater the adhesion index
2. The dose of protein adhesin 65kDa pili sub unit Yersinia enterocolitica is directly proportional to the adhesion index
3. There is no effect on the percentage of the adhesion index with the treatment of the dose of adhesin protein 65kDa pili sub unit Yersinia enterocolitica
4. Treatment of the dose of protein adhesin 65kDa pili sub unit Yersinia enterocolitica affects the adhesion index

From the four statements above, which is the correct statement based on the observed data:

a. 1 and 3
b. 2 and 3
c. 1 and 4
d. 3 only

2. Look at the following picture:




What conclusions can be concluded to reinforce the above regulatory facts?

a. Receptors link the interaction between non-specific and specific immune responses in recognizing pathogens

b. There is an activation of T cells and NK cells that migrate to the infection site. Cytokines produced during the non-specific immune response process as indicators of specific immune responses to the infection site

c. The non-specific immune system and the specific immune system interact and work together to produce a more effective combined immune response to destroy the antigen

d. The non-specific immune system acts as a specific immune system stimulant

This study used a quasi-experimental design with a non-equivalent control model (Campbell & Stanley, 2015). The design is presented in Table 2.

**Table 2.** Study Design

| Pretest | Implementation | Posttest |
|---|---|---|
| $O_{1\ (experimental)}$ | $X_1$ | $O_{2\ (experimental)}$ |
| $O_{3\ (Control)}$ | $X_2$ | $O_{4\ (Control}$ |

Note:
$X_1$: Project-Based Learning integrated with 21st-century skills
$X_2$: Learning Model based on practicum
$O_1$: Pretest experimental group
$O_2$: Posttest experimental group
$O_3$: Pretest control group
$O_4$: Posttest control group

This research used a quantitative descriptive analysis method. Previously, the data were tested with assumptions using the normality test and the homogeneity of variance, then continued with data analysis and hypothesis testing. Hypothesis testing used the N-gain, paired, and Independent T-test assisted by the SPSS-22 program. The N-gain score is the difference between the pretest value (before treatment) and posttest (after treatment). The difference in value between the pretest and posttest indicates the effectiveness of implementing innovative models in the experimental group and the control group (low-level class, middle-level class, and high-level class). The paired test determines the effect of the blended PjBL integrated with 21st-century skills on students' HOTS. Independent T-test aims to determine how much influence the implementation of the blended PjBL integrated with 21st-century skills on students' HOTS, both in the experimental and control groups.

Learning outcomes increase if the students' posttest ($X_2$) is higher than the pretest results ($X_1$) or ($X_2$>$X_1$). The N-gain value is the difference between the pretest and posttest data, where the score is categorized based on the acquisition range. The interpretation of HOTS N-gain value (g) refers to the classification of Hake, 1999. The score category index is presented in Table 3.

**Table 3.** Normalized Gain Index Score and its Classification/Effectiveness

| Quality | $N_{gain}$ | Category |
|---|---|---|
| Greatly increased | g≥0.7 | High |
| Increased | 0.3<g<0.7 | Medium |
| Quite increased | g≤0.3 | Low |

The N-gain value obtained is then interpreted in the form of a percentage to know the effectiveness category of the N-gain acquisition. The N-gain score (%) used as an indicator of the effectiveness of the treatment in the following formula:

$$N - Gain\ score\ (\%) = \frac{Posttest\ score - pretest\ score}{100 - pretest\ score}\ x\ 100$$

The difference between the pretest and posttest score in N-Gain score (%) indicates the effectiveness of the treatment so that the effectiveness level interpreted in the category of the effectiveness of the N-Gain score is based on certain intervals. The interpretation category of the N-gain effectiveness in percentage (%) is presented in Table 4.

**Table 4.** Interpretation Category of the N-gain Effectiveness

| Percentage (%) | Interpretation |
|---|---|
| <40 | Ineffective |
| 40-55 | Less Effective |
| 56-75 | Quite Effective |
| > 76 | Effective |

The research data were analyzed descriptively by determining the average value, then the result category was determined based on Table 5.

**Table 5.** The Category of HOTS Score

| Range of Score | Number | Category |
|---|---|---|
| 80-100 | 4.0 | Very Good |
| 72-79 | 3.5 | Good |
| 64- 31 | 3.0 | More than Enough |
| 56-63 | 2.5 | Enough |
| 48-55 | 2.0 | Poor |
| 40-47 | 1.0 | Very Poor |
| ≤39 | 0 | Failed |

The descriptively-analyzed HOTS assessment data will be interpreted according to the standards in the HOTS category (Table 4) based on each strengthening indicator of HOTS as measured in the study.

## RESULTS AND DISCUSSION

First, students were given a pretest to determine their basic ability to understand the immune system and its potential for body integrity against infection. Students' initial skills were obtained from the pretest given to all research samples using the online test. The blended learning stage begins with the questioning stage, where analyzing issues and relevant scientific articles also use the online method. The experimental stage is designed based on an integrated project in quasi-experimental research implemented in the PjBL model through practicum activities using the offline method. Posttest was carried out to determine the achievement of students' understanding of the material, which was carried out offline. The pretest and posttest results of students' HOTS are presented in Table 6.

**Table 6**. The Results of Posttest and Pretest

| | Cluster | Students' Skill Level | Pre test | Post test | Gain | NGain Score | NGain Score (%) | Min | Max |
|---|---|---|---|---|---|---|---|---|---|
| Experimental | A | Low | 24.33 | 67.67 | 43.33 | 0.57 | 57.16 | 47.37 | 69.57 |
| | | Medium | 33.59 | 72.31 | 38.72 | 0.58 | 57.60 | 42.19 | 69.57 |
| | | High | 34.07 | 81.33 | 47.26 | 0.71 | 71.37 | 57.89 | 80.01 |
| | | | | | 43.10 | | 62.04 | | |
| | B | Low | 26.19 | 68.52 | 42.33 | 0.57 | 57.04 | 47.37 | 69.57 |
| | | Medium | 35.88 | 73.14 | 37.26 | 0.59 | 58.57 | 42.10 | 73.91 |
| | | High | 29.44 | 81.67 | 52.22 | 0.80 | 79.42 | 53.68 | 76.55 |
| | | | | | 43.94 | | 65.01 | | |
| | C | Low | 28.89 | 68.89 | 40.00 | 0.56 | 56.13 | 42.10 | 65.01 |
| | | Medium | 38.03 | 75.61 | 37.58 | 0.61 | 60.54 | 42.10 | 70.00 |
| | | High | 35.78 | 86.13 | 50.36 | 0.78 | 78.29 | 57.61 | 82.61 |
| | | | | | 42.65 | | 64.99 | | |
| Control | A | Low | 22.67 | 41.67 | 19.00 | 0.25 | 24.47 | 6.67 | 33.34 |
| | | Medium | 32.22 | 54.20 | 21.98 | 0.32 | 32.23 | 19.7 | 47.37 |
| | | High | 31.00 | 61.53 | 30.53 | 0.44 | 44.22 | 35.97 | 52.39 |
| | | | | | 23.84 | | 33.64 | | |
| | B | Low | 23.75 | 49.79 | 38.33 | 0.34 | 34.11 | 18.18 | 47.83 |
| | | Medium | 32.90 | 55.11 | 22.21 | 0.33 | 32.92 | 22.72 | 47.37 |
| | | High | 31.33 | 59.72 | 28.38 | 0.41 | 41.28 | 25.00 | 50.01 |
| | | | | | 29.64 | | 36.27 | | |
| | C | Low | 29.17 | 52.21 | 23.04 | 0.33 | 32.62 | 10.50 | 47.37 |
| | | Medium | 35.84 | 61.18 | 25.34 | 0.39 | 38.88 | 19.27 | 48.18 |
| | | High | 32.75 | 61.42 | 28.67 | 0.42 | 42.17 | 19.27 | 68.18 |
| | | | | | 25.68 | | 37.89 | | |

Table 6 shows an increase in the average pretest and posttest scores in the experimental and control groups. The Gain value evidenced the increase in students' HOTS in all clusters, where the experimental group (A=43.10, B=43.94, C=42.65) was higher than the control group (A=23.84, B=29.64, C=37.89). Supported by an average minimum and maximum value in each cluster. The mean minimum value of the experimental group (A = 49.15, B = 47.72, C = 47.27) was higher than the control group (A = 20.78, B = 21.97, C = 16.35), while the mean maximum value for the experimental group (A = 73.05, B = 73.34, C = 72.54) is higher than the control group

(A = 44.37, B = 48.40, C = 54.58).The average increase in students' HOTS at all ability levels for all clusters in the experimental group (43.23) was more significant than the control group (26.39), which illustrated that implementing the innovative PjBL model integrated with 21st-century skills is effective in increasing students' HOTS. The learning model's effectiveness can be seen through the N-Gain score obtained (Mayub et al., 2020). The effectiveness of the learning model implementation is supported by the N-Gain score (%) in Figure 2.

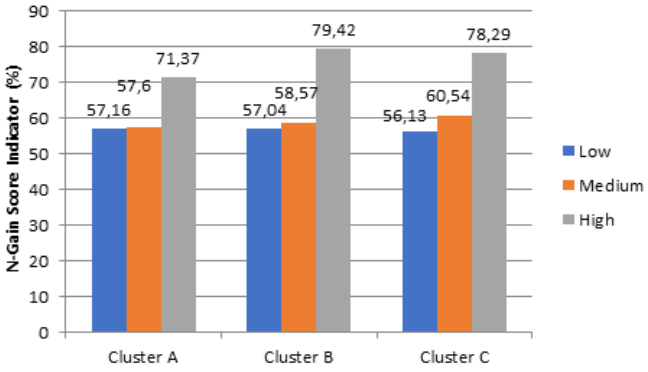



**Figure 2.** The Analysis Result of Average of the HOTS Increase based on the N-Gain Score (%)

Based on the N-gain score (%) in Figure 2, it appears that the implementation of the innovative PjBL model integrated with 21st-century skills is quite effective in learning in all clusters for students in the low-level class (mean=56.78), students in the medium level class (mean=58.90), and students in the high-level class (mean=76.36) that are interpreted in the effective category. This interpretation showed that the implementation of the learning model is more effective in increasing HOTS at high-level classes than low-level classes and medium-level classes.

Thinking skills apply thinking processes in complex situations, where higher-order thinking processes need encouragement and enthusiasm (Zulfiani et al., 2020). HOTS at high-level classes has better-thinking skills than low-level and medium-level classes. HOTS at the high-level classes has higher skills in solving the problems presented (Safarudin et al., 2020), is more active in processing and analyzing new information that is considered more relevant (Retnowati, 2020), then arranging it into interrelated units into further information (Darling et al., 2020). Activities in analyzing ideas and information to be more specific, differentiating, selecting, identifying, assessing, and developing them in a perfect direction require more critical thinking skills (Usmeldi et al., 2017). The project-based learning emphasizes students' critical analysis so that protein-based probiotic supplementation can be used as new information in stimulating the body's immune system against infection with microorganisms. The increase in students' HOTS was measured by referring to the HOTS indicator arranged on the instrument, including the ability to analyze, evaluate, and create. It is presented in Table 7.

The HOTS increase based on HOTS indicators in Table 6 showed that the experimental class is higher than the control class for the entire cluster. The average increase of HOTS in the experimental group was in the moderate category and was interpreted as quite effective on the indicator of analyzing (62.10), high categories and interpreted as effective on the indicator of evaluating (78.15) and creating (79.21). The increase in the student's HOTS N-gain score in Table 7 shows the increase in the students' HOTS N-gain score. It indicates the improvement of students' analysis skills, such as discussing, identifying problems, formulating problems, conducting studies of relevant literature, designing project frameworks, presenting hypotheses, determining research parameters and instruments, and using appropriate data collection and data analysis methods. All components in the analytical ability indicator provide a solid basis for pre-service biology teachers in responding to actual problems and then communicating/implementing the benefits of project achievements obtained in real life. The increase in students' HOTS on the indicators of evaluating and creating shows the development of students' thinking skills. The increase in HOTS N-gain in the ability to evaluate becomes a barometer of pre-service biology teachers' ability to utilize technology.

**Table 7**. The N-Gain Index for Each Indicator of HOTS and its Classification

| Group | Cluster | HOTS | Analyze | Evaluate | Creating |
|---|---|---|---|---|---|
| | | | **Immunology Concepts and Body Integrity System** | **Mechanism of Immunomodulator, Immunostimulator, and Immunoregulator** | **Protein-based Supplementation** |
| Experiment | A | N-Gain Index | 0.65 | 0.78 | 0.80 |
| | | Classification | Medium | High | High |
| | | N-Gain (%) | 65.01 | 77.78 | 80.01 |
| | | Interpretation | Quite Effective | Effective | Effective |
| | B | N-Gain Index | 0.54 | 0.77 | 0.75 |
| | | Classification | Medium | High | High |
| | | N-Gain (%) | 54.08 | 76.65 | 75.02 |
| | | Interpretation | Quite Effective | Effective | Effective |
| | C | N-Gain Index | 0.67 | 0.80 | 0.83 |
| | | Classification | Moderate | High | High |
| | | N-Gain (%) | 67.22 | 80.01 | 82.61 |
| | | Interpretation | Effective | Effective | Effective |
| Control | A | N-Gain Index | 0:33 | 0:47 | 0:52 |
| | | Classification | Low | Medium | Medium |
| | | N-Gain (%) | 33.34 | 47.37 | 52.39 |
| | | Interpretation | Ineffective | Less Effective | Less Effective |
| | B | N-Gain Index | 0:31 | 0:47 | 0:55 |
| | | Classification | Low | Medium | Medium |
| | | N-Gain (%) | 31.33 | 47.37 | 55.39 |
| | | Interpretation | Ineffective | Less Effective | Less Effective |
| | C | N-Gain Index | 0:33 | 0 .48 | 0.50 |
| | | Classification | Low | Medium | Medium |
| | | N-Gain (%) | 33.34 | 47.83 | 50.01 |
| | | Interpretation | Ineffective | Less Effective | Less Effective |

Furthermore, the students were also able to conduct mixed-method research and combine online and offline learning. The project-based laboratory experimental approach in research requires students to evaluate each stage of learning. The most effective HOTS indicator is creating (N-gain = 79.21), which illustrates the success of the blended model in researching the ability of pre-service biology teacher students to create or produce products targeted in research projects.

Mucosal immune system material in the immunology course designed with blended PjBL could improve students' ability to evaluate and understand the function of probiotics against immune stimulation. It is also in line with several other studies regarding the function of probiotics as an immunomodulator (Sumarno et al., 2011; Evrard et al., 2011), immunostimulators (Yan & Polk, 2011; Sumarno et al., 2015), and immunoregulators (Sumarno et al., 2012; Wibowo et al., 2014). Retnowati (2020) stated that thinking skills would be manifested in a more active attitude in processing and evaluating new, more relevant information.

The implementation of blended PjBL integrated with 21st-century skills on HOTS based

on the indicator of analyzing the concept of immunology, and the body's integrity system is considered quite effective. In contrast, the indicators of evaluating and creating have a higher score and are effective in increasing students' HOTS. The increase in students' HOTS is very significant in the aspect of creating, in line with the basic principles of PjBL integrated with 21st-century skills, which direct students as a generation capable of producing scientific-based products that they have (Chu et al., 2017; Akhdinirwanto et al., 2020; Parno et al., 2020). The increase in students' HOTS on creating indicators through the blended PjBL learning emphasizes producing protein-based supplementation. Darling et al. (2020) emphasized that the ability to organize information into interrelated units that later become new information manifests the development of thinking skills.

The increase in HOTS was evident in the experimental group compared to the control group for students in the high-level class (Table 5). It showed that the innovative PjBL model integrated with 21st-century skills can significantly influence HOTS in students in high-level classes but is also considered quite effective in low-level and medium-level class groups. The increase in HOTs in the aspect of creative skills in the experimental class is influenced by the treatment of the learning model in the experimental class, which emphasizes the combination of the PjBL model integrated 21-century skills. The data in table 5 confirms the effectiveness of integrated PjBl blended 21st-century skills to improve students 'HOTs skills, where PjBL is integrated with models or other variables that affect students' thinking skills. The significance of this effect can be seen in the results of the paired sample test (Sig. 2-tailed 0.000<0.05), where the value of t (-26.073>1.59)/ df=104 in the experimental class and the value of t (-15,402> 1.66)/df =94 in the control class. The standard deviation in the experimental class (pretest=7.134, and posttest=7.496) is lower than the control class (pretest = 6.487, and posttest =9.385). The lower the standard deviation value, the more homogeneous the data. However, the df value representing the number of samples in the two classes of research is different, Sig. 2-tailed 0.000 <0.05, so it can be concluded that the blended PjBL model integrated with 21st-century skills significantly affects students' HOTS (Table 8).

**Table 8.** The Results of Paired Analysis of Test Samples

| Student HOTs | | N | Std. Deviation | Std. Error Mean | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| Pair 1 (Experiment) | Pre-Test | 105 | 7,134 | 1,261 | -26,073 | 104 | .000 |
| | Post-Test | 105 | 7,496 | 1,325 | | | |
| Pair 2 (Control) | Pre-Test | 95 | 6,487 | 1,297 | -15,402 | 94 | .000 |
| | Post-Test | 95 | 9,385 | 1,877 | | | |

It can be seen from the comparison of the mean in the experimental group and the control group based on the independent sample T-test analysis results to find out how much influence the blended PjBL model integrated with 21st-century skills toward students HOTS (Figure 3).

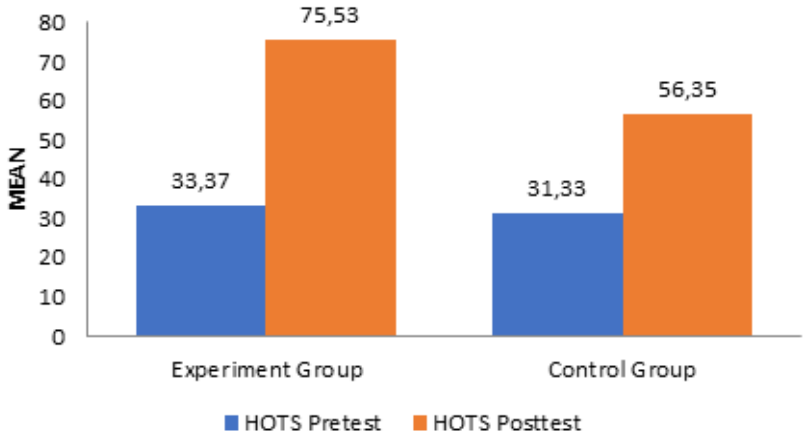



**Figure 3.** The Results of the Analysis based on the Independent T-test

The comparison of the mean score in Figure 3 showed an increase in students' HOTS after implementing the blended PjBL model integrated with 21st-century skills, which is more significant in the experimental group (75.53) than in the control group (56.35). The mean score proves how much influence the blended PjBL model integrated with 21st-century skills has on students' HOTS at low-level, medium, and high-level classes. The significance of the influence of the blended PjBL model integrated with 21st-century skills on students' HOTS is also supported by the results of observations of self-assessment and peer assessment of lecturers (97.5%) and students (92.5%), in the good category. Integrating the innovative learning model in this study is appropriately implemented, making it easier to achieve the learning objectives designed before. Implementing this innovative model can increase active attitudes in developing students' HOTS and improve the quality of learning.

The N-Gain index in the experimental group was higher than in the control group. It proves the effectiveness of the blended PjBL integrated with 21st-century skills in increasing HOTS. Using the blended PjBL integrated with 21st-century skills as an innovative learning model in the experimental group made students more focused on learning, more active in expressing ideas and thoughts, and was jointly involved by lecturers in designing projects. The control group used a practicum-based learning model in this study, which focused more on the lecturer as the information provider. The teacher-centered learning model has the lecturer or teacher as the primary source of information and is considered a person with broader knowledge (Zainudin, 2017). The successful implementation of blended learning in this study is not a substitute for the conventional learning model but enriches the previous learning model. Castro (2019) emphasized that blended learning cannot completely replace conventional learning, but blended learning adds and reinforces an innovative learning model.

HOTS indicators in this study used analytical skills because the analyzing ability is the basis for the critical thinking process. When thinking skills develop optimally, they generate ideas, create, imagine, and encourage problem-solving (Kenedy et al., 2012). Problem-solving in the blended PjBL integrated with 21st-century skills put students as pre-service biology teachers to work collaboratively in teams. The collaborative ability formed is intended so that students can take care of each other independently (Sutarto et al., 2018; Thambu et al., 2020). Therefore, critical, creative, and high-order thinking skills are essential for developing 21st-century skills. Through this blended learning, pre-service biology teachers are directed to follow scientific developments to critically analyze any problems that arise because scientific developments require pre-service teachers to think at a higher level. Besides, pre-service teachers are also required to have self-regulated learning (SRL) in overcoming the problems they will face in real life (Alibakhshi & Zare, 2010; Sutarto et al., 2018; Tarchi et al., 2022). SRL theory is used as a framework that combines motivation, metacognitive awareness, cognitive skills, and beliefs about learning (Hartley et al., 2020).

One of the efforts to improve the quality of teaching is by exploring innovative models of learning. The characteristics of project-based learning require students to think critically (Mataniari et al., 2020) and think at higher levels (Facione, 2011; Stanley & Moore, 2013). Therefore, the results of this study confirmed that project-based learning is highly recommended for use in science learning. Project-based learning carried out in this study refers to a driving question (Bender, 2012), which is closely related to the immune system material and its potential for infection. The arranged questions are contextual and based on local issues regarding the mechanism of the body's defense system against infection with microorganisms, especially in the current pandemic era.

Local and contextual issues presented in the learning material are in the form of questions that are not specific to one aspect only but are more straight-forward and broad to encourage students to think critically (Hudha & Batlolona, 2017; Pursitasari et al., 2020), develop the ability to find solutions actively and collaboratively (Ramos et al., 2013; Raiyn, 2016). Students must make discoveries and innovations by adding questions to complete the project more specifically (Bender, 2012). The preliminary information presented in this study used several articles related to microorganism infection as initial references. Furthermore, students are encouraged to identify problems, develop, and design solutions based on the design of the probiotic supplementation practicum. The project is designed to prove the potential of probiotic supplementation in producing the secretion of immunoglobulin A in serum as the body's defense system against infection by microorganisms. During this pandemic, the immune system and infection are contextual and factual problems, thus stimulating thinking skills to be more developed (Bustami et al., 2018).

The PjBL model integrated with 21st-century skills requires students to be cooperative and collaborative, so teamwork is formed in completing planned projects (Raiyn, 2016). Projects undertaken involve the active role of students directly so that the material is easier to understand. Collaboration in heterogeneous groups requires students to work together positively in solving learning problems faced as a characteristic of 21st-century skills (Bertoncelli et al., 2016; Rahardjanto, 2019). In such situations, students are stimulated to control emotions, have teamwork skills, think creatively (Chu et al., 2017), be confident, have courage in making decisions, and respect the opinions of their group members (Tsybulsky & Muchnik-Rozanov, 2019).

The findings of this study are the implementation of the innovative blended PjBL model integrated with 21st-century skills, which have a significant effect in increasing students' HOTS as evidenced by the results of the hypothesis paired sample test (Sig. 2-tailed 0.000<0.05), comparison of the mean score, and the N-gain value (%). The increase in HOTS of students in the experimental class implemented by the blended PjBL model integrated with 21st-century skills was higher than that of the control class using practicum-based learning models. The effectiveness of the blended model PjBl integrated with 21st-century skills as evidenced by the significant increase in HOTS (the ability to analyze, evaluate, and create) to become a reference for innovative learning models for pre-service biology teachers on immunology material. The practicum-based learning model is a learning method that is still centered on lecturers as the focus of knowledge, so students have a tendency not to be strongly motivated to develop thinking skills that are owned empirically (Carter et al., 2016; Zainudin, 2017).

The findings of this study are in line with previous research that blended learning can improve concept mastery and emphasizes more on students' procedural attitudes (Fuad et al., 2017). Concept mastery is better in groups of students who are given a blended learning model than other direct learning. The PjBL model can improve student cognitive learning outcomes and HOTS (Anazifa & Djukri, 2017). Besides, that blended learning can enhance physical reasoning (Heong et al., 2012), increase learning motivation (Chu et al., 2017), make decisions through a systematic framework (Tsybulsky & Muchnik-Rozanov, 2019), find complete solutions to the problems given (Maries & Singh, 2017), and be independent in designing activity processes (Rahardjanto, 2019). Those previous studies reinforce the findings of this study that the blended PjBL model integrated with 21st-century skills is influential and effective in increasing students' HOTS. The results of this study can be a solution to the needs of an innovative learning model with integrative blended learning in preparing pre-service teachers who have higher-order thinking skills following the demands of 21st-century skills.

## CONCLUSION

The results proved that the blended PjBL integrated with 21st-century skills significantly affects students' HOTS based on the paired sample test (Sig. 2-tailed 0.000<0.05). The mean scores in the experimental group (75.53) and the control group (56.35) strengthen the data significance of students' higher-order thinking skills based on the N-Gain value and the independent sample T-test. The blended PjBL integrated with 21st-century skills to improve student HOTS is more effectively implemented at high-level classes compared to low-level classes and medium-level classes. The findings of this study are that the blended PjBL integrated with 21st-century skills can increase students' HOTS, and this integration model can be used as an innovative learning model for pre-service biology teachers to improve HOTS following the demands of 21st-century skills.

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