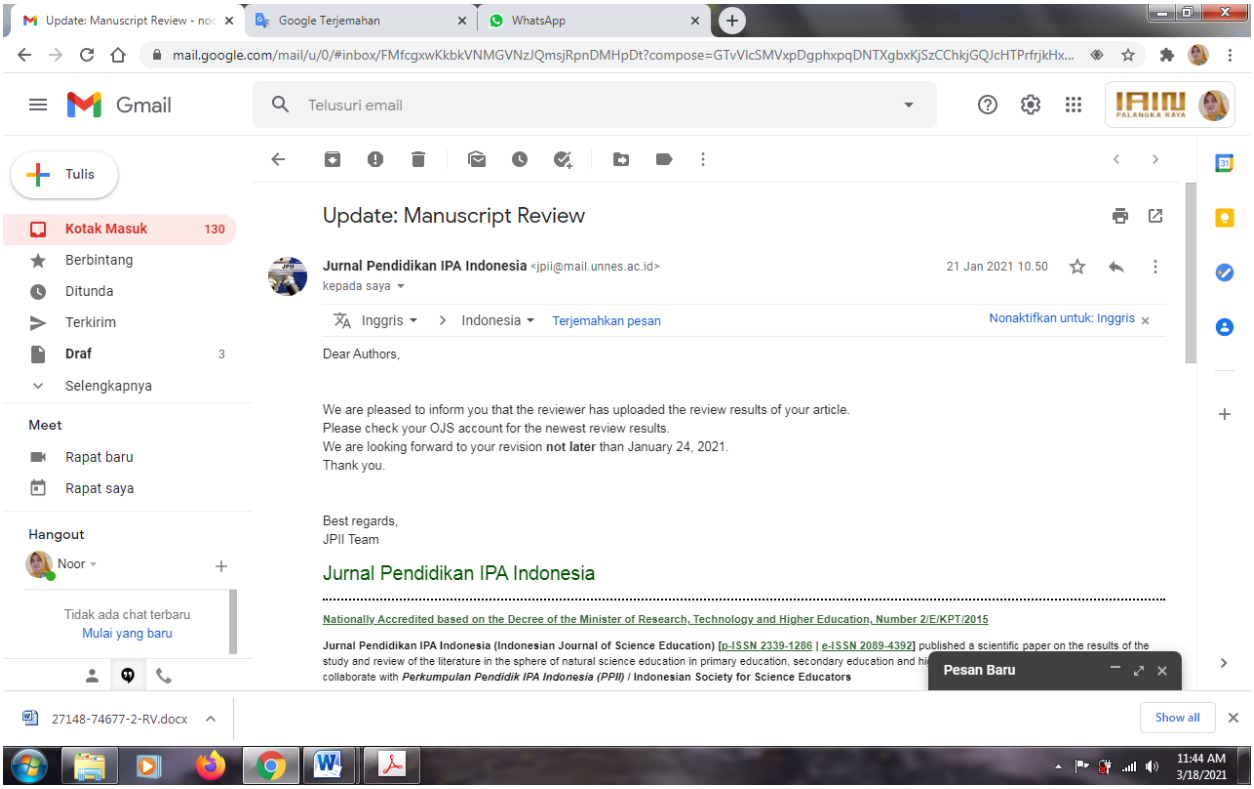
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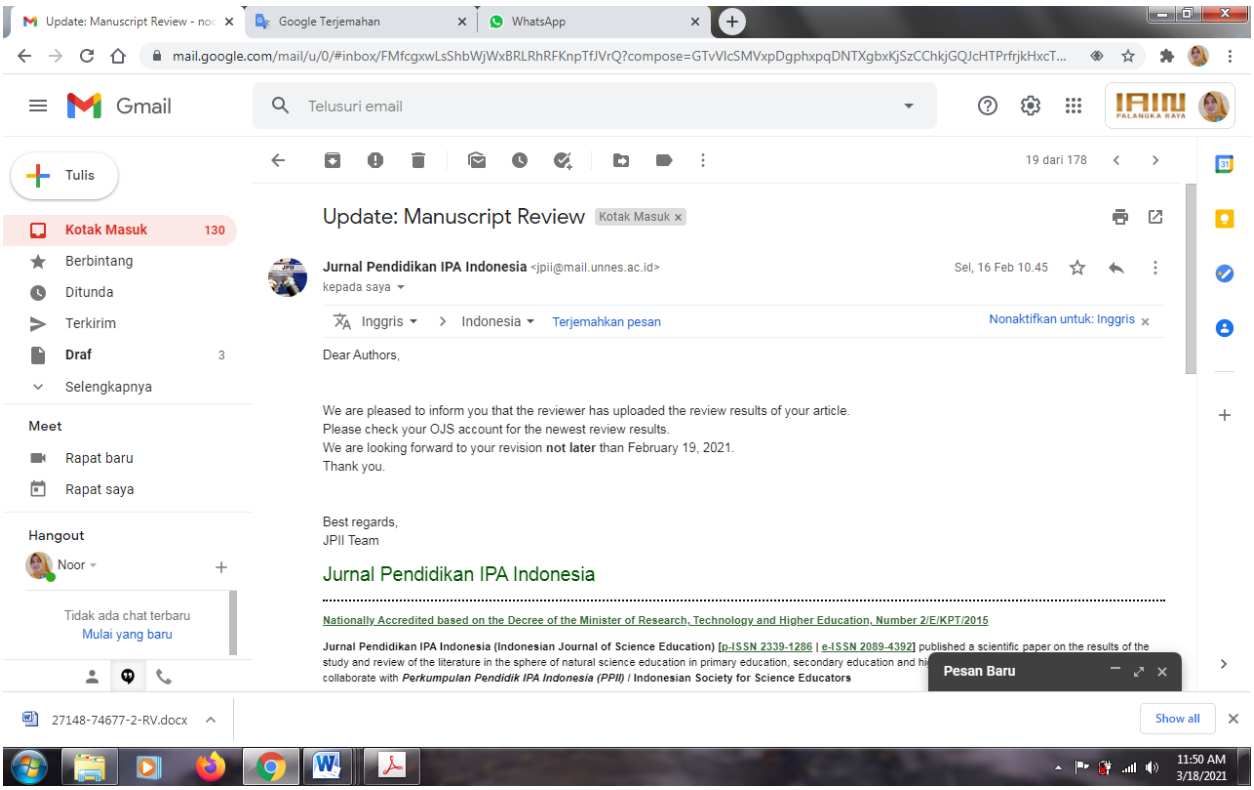
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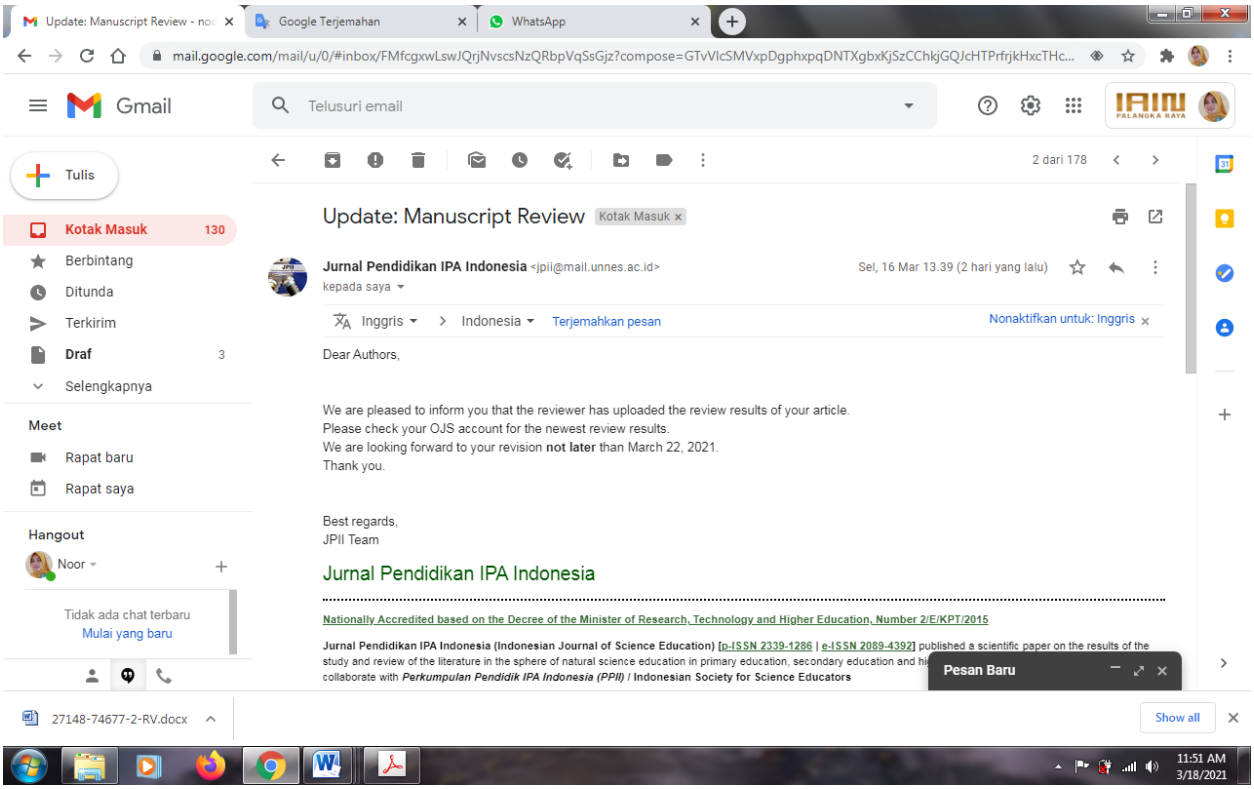
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The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

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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 material. This research is an experimental study with a mixed method, in which the qualitative observation data uses 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. Prospective pre-service teacher to in this study are students who are still studying at educational institutions, especially teacher faculty. so they are categorized as pre-service teachers. The research sample was students who were taking the immunology course in the Biology Education Study Program, namely 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 results of the study proved that the blended PjBL integrated with 21st-century skills had a significant effect on students' high-level thinking skills based on the paired sample test (Sig. 2-tailed 0.000<0.05). The value of mean 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 the Higher Order Thinking Skill of pre-service biology teachers according to the demands of 21st-century skills.

Keywords: Blended Learning, Project-Based Learning, 21st-Century Skills, Immunology

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INTRODUCTION

The 21st-century skills are the primary targets in the curriculum of educational institutions around the world (Alismail & McGuire, 2015; Mohammed, 2017; Wang et al., 2018; Haviz et al., 2018), because a good skill in the 21st Century becomes 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 life for 21st century. One of the main ability in 21st century skills is creative, critical and problem solving, or known as higher order thinking skill (Laar et al., 2017; Rahman, 2019). Critical thinking really needs to be developed for students, because it is a cognitive thinking process (Sardone & Devlin-Scherer, 2010). The capability of thinking creatively is to produce products through new ideas (Hasanah, 2017), to produce ideas, changing thinking flexibly, and able to develop ideas to find problem solutions (Mayes et al., 2015). Problem-solving ability is a thought process that stimulates students to treat a problem and analyze it which aims 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 activity in the learning process. The problem-solving ability needs to be developed for each individual (López et al., 2011; Basilotta et al., 2017). The development of HOTS can be done in the learning process including biology learning.

Educators have prepared various strategies in preparing students and pre-service teachers with the demands of the 21st-century (Teo, 2019). Quality education is one of the factors that determine the progress of a nation. Therefore, educational institutions are required to prepare the nation's generation with special skills, otherwise known as 21st-century skills (Geisinger, 2016). 21st-century skills, or known as 4C, includes Critical Thinking and Problem Solving, Creativity and Innovation, Communication, Collaboration. Overall, these competencies are very much needed to survive in facing global problems (Jia et al., 2016; Greiff & Kyllonen, 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 that are 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 that is expected to produce graduates who can follow scientific developments in the fields of science and technology. The teacher, as a human resource, has 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.

The 21st-century skills are needed by aspiring teachers to compete in the 21st century. Research by Haviz et al. (2020a) 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 a variety of analysis, assessments, evaluations, reconstruction, decision-making that leads 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 behaviour (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 is positively correlated 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 to the study reported that there was a significant increase in the thinking skills and perspectives of biology teachers in learning chromosome inheritance material through online discussion forums. Result of this research illustrates the importance of integrating science learning with 21st-century skills. Integrated

learning is more focused on competency content so that exploration of 21st-century skills and thinking skills in learning more broadly is essential (Zainuddin & Attaran, 2016).

Strengthening higher-order thinking skills (HOTS) is not only student-centred but also influenced by strategies and innovative models of learning (Haviz et al., 2020a). This 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, the exploration of learning models is essential to improve thinking skills (Maryuningsih et al., 2019; Fitriani 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 the knowledge they have practised 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). 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 carry out exploration, assessment, interpretation, synthesis, 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 be used to analyze solutions to complex problems in investigating. The blended PjBL, which is integrated with 21st-century skills, is expected to have a better influence on the HOTS of pre-service biology teachers (Haviz et al., 2020b). Through this research, it is hoped 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; Wibowo 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 that it can stimulate students' thinking and analysis skills. The target of learning outcomes in the immunology course is so that students can 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 various problems that develop in the environment as an implementation of the concept in the field of immunology and communicate the results of applying the basic concepts of immunology based on scientific written observations. Immunoglobulin A (IgA) as a protein secreted by plasma cells that binds to antigens and functions as an effector of the humoral immune system, is essential to understand more explicitly concerning its function for 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 so that it can be understood optimally. The blended PjBL integrated 21st-century skills are recommended as an innovative learning model that has characteristics that are 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., (2020b) to give a significant contribution to the improvement of thinking skills of prospective biology teachers. The results of Haviz's study becomes 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, namely 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 the form of group projects. The probiotic lactobacillus reuteri supplementation in this project against Balb/c mice given for 1 month. 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 be able 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 uses modified practicum-based learning tools, both in terms of material and assessment aspects. Blended learning is expected to show significant results as one of the results of the exploration of 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 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 supported by various sources of literature, which can be accessed via the internet (online). The information collected is discussed through an offline face to face meeting, and become a discussion material for the experimental project. The implementation stages of blended learning in this study referes 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 activities of the mucosal immune system practicum. There were three research locations, namely 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 difference tests and score analysis of each indicator. The research subjects used as research samples were Biology Education undergraduate students taking Immunology courses, namely 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 who were studying at the teacher training faculty, so that 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 'HOTs' initial abilities, namely 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 ability of 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 implementation of the used 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.

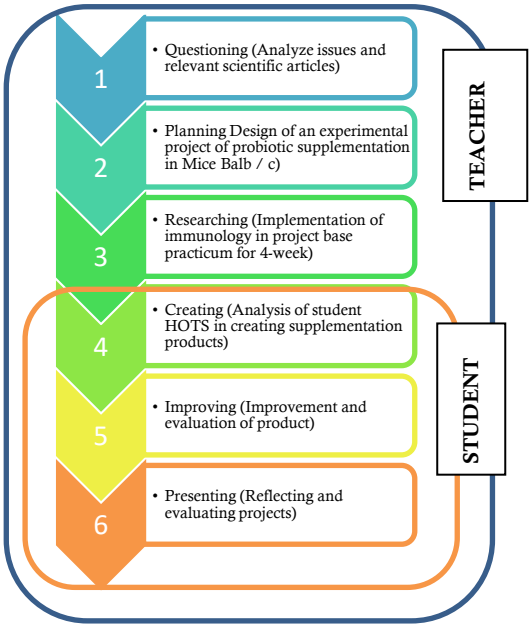


Figure 1 Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020).

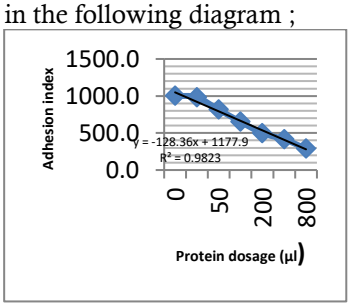
The research stages in Figure 1 begin with; 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of preparing questions or project assignments that come from 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 project group, namely the problem of 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, as well as 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 to evaluate the achievement of the project; 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 that raised in the experimental project include the ability to analyze the concept of immunology and the body's integrity system, the ability to evaluate the immunomodulatory mechanisms and immunoregulators, and the ability to create basic concepts for protein-based supplementation products.

Yustina et al (2020) combines PjBL with PBL on pre-service creative thinking skills, so it is important that researchers 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 use of online and offline methods in this study also refers to the research. Modifications were made after the quetioning (issue analysis) and planning stages, because the integrated 21st century PjBL blended learning was continued with the researching 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 methods of 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.

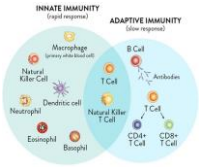
The instrument in this study are :

1. Look at the results of the 65kDa protein adhesion test sub unit 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 has an effect on the adhesion index

   From the four statements above, which is the correct statement based on the observed data:
   a. 1 dan 3
   b. 2 dan 3
   c. 1 dan 4
   d. 3 only
2. Look at the following picture:



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

a. The interaction between non-specific and specific immune responses is linked by receptors in recognizing pathogens
b. There is activation of T cells and NK cells which then 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

The Blended Project-Based Learning Integrated with 21st-Century Skills

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

Data analysis

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 test, paired test, and Independent T-test assisted by the SPSS-22 program. The N-gain score is the difference between the pre-test value (before treatment) and the post-test value (after treatment). The difference in value between the pre-test and post-test is an indicator of 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 is to determine 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 group and the control group.

Learning outcomes increase if the students' posttest results (X_2) are 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 range of acquisition. 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 (%) can be used as an indicator of the effectiveness of the treatment obtained from the percentage of the N-Gai score, in the following formula :

$$N - Gain\ score\ (\%) = \frac{Post\ test\ score - pre\ test\ score}{100 - pre\ test\ score} x\ 100$$

The difference in value between the pre-test and post-test in the form of an N-Gain score (%) is an indicator of the effectiveness of the treatment in the study, so that the level of effectiveness is interpreted in the category of the effectiveness of the N-Gain score 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 in understanding the immune system and its potential for body integrity against infection. Data on students' initial abilities were obtained from the pre-test given to all research samples using the online test. The blended learning stage begins with the questioning stage, where Analyze issues and relevant scientific articles also use the online method. The experimental stage is designed based on an integrated project in quasi-experimental research which is implemented in the PjBL model through practicum activities using the offline method. Posttest data was carried out to determine the achievement of students' understanding of the material which was carried out offline. The results of the pretest and posttest 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 5 showed an increase in the average value of pretest and posttest in the experimental group and the control group. 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 gorup (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 gorup (A = 73.05, B = 73.34, C = 72.54) is higher than the control gorup (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 the implementation of the innovative PjBL model integrated with 21st-century skills is effective in increasing students' HOTS. The effectiveness of using the learning model can be seen through the N-Gain score obtained (Mayub et al., 2020). The effectiveness of the implementation of the learning model is supported by the N-Gain score (%) in Figure 2.

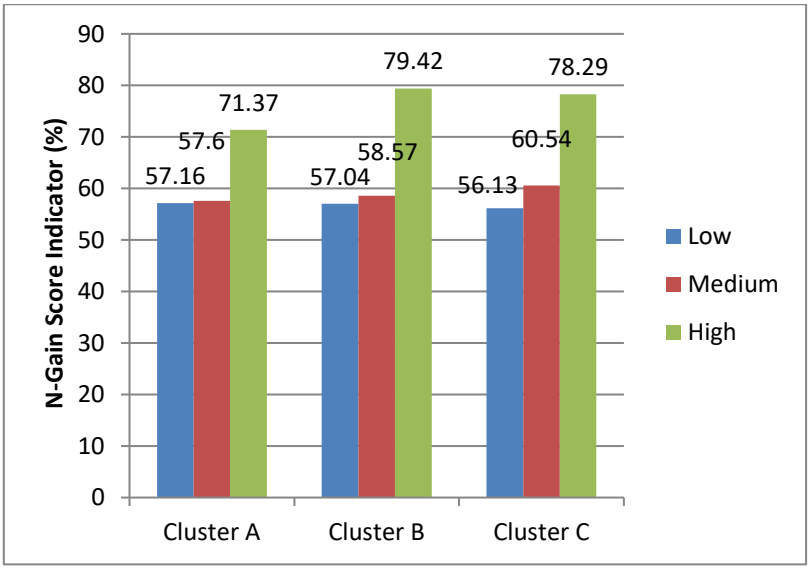


Figure 2. The Analysis Result of Average of the HOTS Increase based on the N-Gain Score (%)

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 compared to low-level classes and medium level classes.

Thinking skills are the application of thinking processes in complex situations, where higher-order thinking processes need encouragement and enthusiasm (Zulfiani et al., 2020). HOTS at high-level class has better thinking skill than low-level class and medium level class. HOTS at the high-level class has a higher ability 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 new 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 the critical analysis of students 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 below.

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

Group	Cluster	HOTS	Analyzes to	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 is	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 is	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

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 6 shows the increase in the students' HOTS N-gain score. It indicates the improvement of students' analysis skills such as the ability to discuss, identify problems, formulate problems, conduct studies of relevant literature, design project frameworks, present hypotheses, determine research parameters and instruments, use appropriate data collection techniques, and use appropriate data analysis method. All components in the analytical ability indicator provide a strong basis for students as pre-service biology teachers in responding to actual problems, 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 aspect of the ability to evaluate becomes a barometer of pre-service biology teachers' ability to utilize technology. Furthermore, the students were also able to conduct mixed-method research and to combine online and offline learning. The project-based laboratory experimental approach in research requires students to be able to evaluate each stage of learning. The most effective HOTS indicator is in the aspect of 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 (Fang & 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 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; Parno et al., 2020; Akhdinirwanto 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 becomes new information is a manifestation of 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 have a more significant influence on HOTS in students in high-level class but is also considered quite effective in groups of students who are low-level class and medium-level class. 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 (pre test =7.134, and post test =7.496) is lower than the control class (pre test = 6.487, and post test =9.385). The lower the standard deviation value, the more homogeneous the data will be. Although the df value which represents 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 has a significant effect on 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 results of the independent sample T-test analysis to find out how much influence the blended PjBL model integrated with 21st-century skills toward students' HOTS (Figure 3).

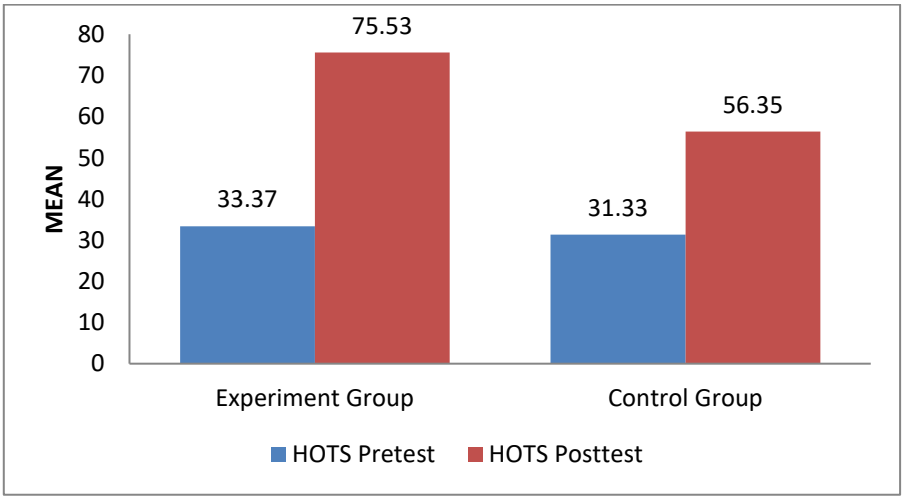


Figure 3. The Results of the Analysis based on the Independent T-test

The comparison of the mean score in Figure 3 showed an increase in students' HOTS after the implementation of 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 class, medium level class, and high-level class. 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. The step of integrating the innovative learning model in this study is appropriately implemented, making it easier to achieve the learning objectives designed before. The implementation of this innovative model can increase active attitudes in developing students' HOTS and can improve the quality of learning.

The N-Gain index in the experimental group was higher than 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-centred learning model has the lecturer or teacher as the primary source of information and is considered a person who has broader knowledge (Zainudin, 2017). The successful implementation of blended learning in this study is not a substitute for the conventional learning model but rather enriches the previous learning model. Bender et al. (2012) emphasized that blended learning cannot completely replace conventional learning, but blended learning is an add-on and reinforcement of 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 will 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; Nadarajan et al., 2020). Therefore, critical, creative, and high-order thinking skills are essential factors for developing 21st-century skills. Through this blended learning pre-service biology teachers are directed to follow scientific developments with the ability 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; Cheng, 2011; Sutarto et al., 2018). 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 (Moore & Stanley, 2010; Facione, 2011). 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 questions that are arranged are contextual and based on local issues regarding the mechanism of the body's defence 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 are required to make discoveries and innovations by adding questions to make the project more specific (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 defence system against infection by microorganisms. The immune system and infection during this pandemic 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 with each other, so that 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 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 centred 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 compared to other direct learning. The PjBL model can improve student cognitive learning outcomes and HOTS (Anazifa & Djukri, 2017). Besides, that blended learning can improve physical reasoning (Heong et al., 2012), increase learning motivation (Chu et al., 2017), make decisions through a systematic framework (Tsybulsky & Muchnik-Rozanov, 2019), find unlimited 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 findings 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 have a significant effect on 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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The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

Parts of review	Guidelines	Yes	Partly	No	Reviewer's note for improvement	Author's responds (highlight of revision)
Title	• Does the subject matter fit within the scope of journal?	√				
	• Does the title clearly and sufficiently reflect its contents?		√			
Abstract	• Does the abstract contain informative, including Background, Methods, Results and Conclusion?	√				
Back-ground	• Is the background informative and sufficient (include the background problem and objectives)?	√				
	• Is research question of the study clear and understandable?	√				

	• Does the rationale of the study clearly explained using relevant literature?	√				
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Methods	• Is the methodology chosen suitable to the nature of the topic studied?		√			
	• Is the methodology of the research described clearly?(including study design, location, subjects, data collection, data analysis)		√			
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Results & Discussion	• Are the tables, graphs and pictures understandable, well presented and numbered consecutively?	√				
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	• Are the findings discussed adequately considering the research question(s), sub-question(s) or hypothesis?	√				
Conclusion	• Is the conclusion clear and in the form of a narration instead of pointers?	√				
	• Isn’t the conclusion a summary and consistent between problems, objectives and conclusion?	√				
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Quality Criteria	• Do the title, problem, objectives, methods and conclusion are in line? Is it well organized?	√				
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The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

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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 material. This research is an experimental study with a mixed method, in which the qualitative observation data uses 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. Prospective pre-service teacher to in this study are students who are still studying at educational institutions, especially teacher faculty. The research sample was students who were taking the immunology course in the Biology Education Study Program, namely 57 students from IAIN Palangka Raya, 60 students from Universitas Palangka Raya, and 83 students from Universitas Negeri Malang. 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 results of the study proved that the blended PjBL integrated with 21st-century skills had a significant effect on students' high-level thinking skills based on the paired sample test (Sig. 2-tailed 0.000<0.05). The value of mean 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 the Higher Order Thinking Skill of pre-service biology teachers according to the demands of 21st-century skills.

Keywords: Blended Learning, Project-Based Learning, 21st-Century Skills, Immunology

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INTRODUCTION

The 21st-century skills are the primary targets in the curriculum of educational institutions around the world (Alismail & McGuire, 2015; Mohammed, 2017; Wang et al., 2018; Haviz et al., 2018), because a good skill in the 21st Century becomes 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 life for 21st century. One of the main ability in 21st century skills is creative, critical and problem solving, or known as higher order thinking skill (Laar et al., 2017; Rahman, 2019). Critical thinking really needs to be developed for students, because it is a cognitive thinking process (Sardone & Devlin-Scherer, 2010). The capability of thinking creatively is to produce products through new ideas (Hasanah, 2017), to produce ideas, changing thinking flexibly, and able to develop ideas to find problem solutions (Mayes et al., 2015). Problem-solving ability is a thought process that stimulates students to treat a problem and analyze it which aims 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 activity in the learning process. The problem-solving ability needs to be developed for each individual (López et al., 2011; Basilotta et al., 2017). The development of HOTS can be done in the learning process including biology learning.

Educators have prepared various strategies in preparing students and pre-service teachers with the demands of the 21st-century (Teo, 2019). Quality education is one of the factors that determine the progress of a nation. Therefore, educational institutions are required to prepare the nation's generation with special skills, otherwise known as 21st-century skills (Geisinger, 2016). 21st-century skills, or known as 4C, includes Critical Thinking and Problem Solving, Creativity and Innovation, Communication, Collaboration. Overall, these competencies are very much needed to survive in facing global problems (Jia et al., 2016; Greiff & Kyllonen, 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 that are 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 that is expected to produce graduates who can follow scientific developments in the fields of science and technology. The teacher, as a human resource, has 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.

The 21st-century skills are needed by aspiring teachers to compete in the 21st century. Research by Haviz et al. (2020a) 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 a variety of analysis, assessments, evaluations, reconstruction, decision-making that leads 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 behaviour (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 is positively correlated 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 to the study reported that there was a significant increase in the thinking skills and perspectives of biology teachers in learning chromosome inheritance material through online discussion forums. Result of this research illustrates the importance of integrating science learning with 21st-century skills. Integrated learning is more focused on competency content so that exploration of 21st-century skills and

thinking skills in learning more broadly is essential (Zainuddin & Attaran, 2016).

Strengthening higher-order thinking skills (HOTS) is not only student-centred but also influenced by strategies and innovative models of learning (Haviz et al., 2020a). Therefore, the exploration of learning models is essential to improve thinking skills (Maryuningsih et al., 2019; Fitriani 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 the knowledge they have practised 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). 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 carry out exploration, assessment, interpretation, synthesis, 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 be used to analyze solutions to complex problems in investigating. The blended PjBL, which is integrated with 21st-century skills, is expected to have a better influence on the HOTS of pre-service biology teachers (Haviz et al., 2020b). Through this research, it is hoped 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; Wibowo 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. These characteristics of the material require higher-order thinking skills and better critical analysis skills. The target of learning outcomes in the immunology course is so that students can 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 various problems that develop in the environment as an implementation of the concept in the field of immunology and communicate the results of applying the basic concepts of immunology based on scientific written observations. Immunoglobulin A (IgA) as a protein secreted by plasma cells that binds to antigens and functions as an effector of the humoral immune system, is essential to understand more explicitly concerning its function for 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 so that it can be understood optimally. The blended PjBL integrated 21st-century skills are recommended as an innovative learning model that has characteristics that are 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., (2020b) to give a significant contribution to the improvement of thinking skills of prospective biology teachers. The results of Haviz's study becomes 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, namely 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.

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 uses modified practicum-based learning tools, both in terms of material and assessment aspects. Blended learning is expected to show significant results as one of the results of the exploration of 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 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 supported by various sources of literature, which can be accessed via the internet (online). The information collected is discussed through an offline face to face meeting, and become a discussion material for the experimental project. The implementation stages of blended learning in this study referes 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 activities of the mucosal immune system practicum. There were three research locations, namely 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 difference tests and score analysis of each indicator. The research subjects used as research samples were Biology Education undergraduate students taking Immunology courses, namely 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 who were studying at the teacher training faculty, so that the students were referred to as pre-service teachers. The whole research subjects were grouped into three groups based on their basic skills, namely 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 ability of 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 implementation of the used 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.

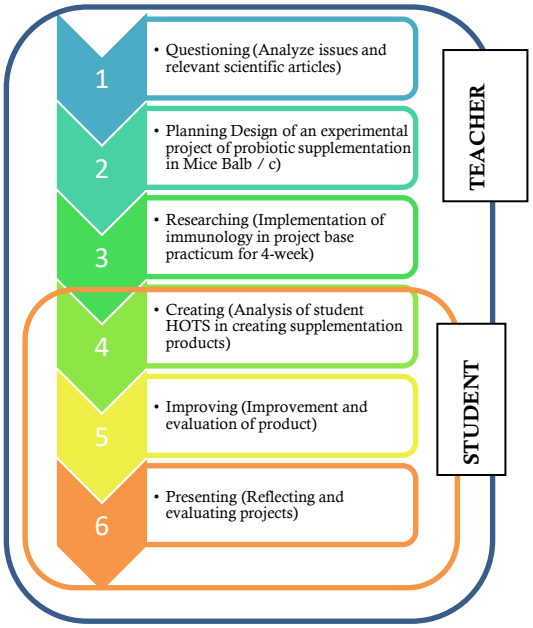


Figure 1 Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020).

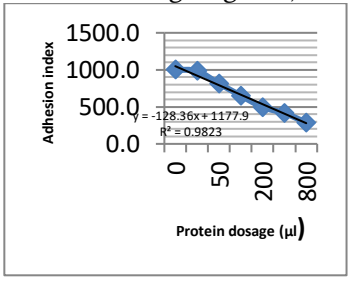
The research stages in Figure 1 begin with; 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of

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 project group, namely the problem of 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, as well as 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 to evaluate the achievement of the project; 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 that raised in the experimental project include the ability to analyze the concept of immunology and the body's integrity system, the ability to evaluate the immunomodulatory mechanisms and immunoregulators, and the ability to create basic concepts for protein-based supplementation products.

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 methods of 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 :

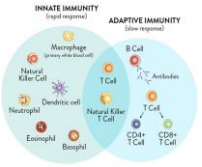
1. Look at the results of the 65kDa protein adhesion test sub unit Yersinia enterocolitica in the following diagram ;



   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 has an effect on the adhesion index

   From the four statements above, which is the correct statement based on the observed data:
   a. 1 dan 3
   b. 2 dan 3
   c. 1 dan 4
   d. 3 only

2. Look at the following picture:



   What conclusions can be concluded to reinforce the above regulatory facts ...
   a. The interaction between non-specific and specific immune responses is linked by receptors in recognizing pathogens
   b. There is activation of T cells and NK cells which then 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

The Blended Project-Based Learning Integrated with 21st-Century Skills

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

Data analysis

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 test, paired test, and Independent T-test assisted by the SPSS-22 program. The N-gain score is the difference between the pre-test value (before treatment) and the post-test value (after treatment). The difference in value between the pre-test and post-test is an indicator of 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 is to determine 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 group and the control group.

Learning outcomes increase if the students' posttest results (X_2) are 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 range of acquisition. 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 (%) can be used as an indicator of the effectiveness of the treatment obtained from the percentage of the N-Gai score, in the following formula :

$$N - Gain\ score\ (\%) = \frac{Post\ test\ score - pre\ test\ score}{100 - pre\ test\ score} x\ 100$$

The difference in value between the pre-test and post-test in the form of an N-Gain score (%) is an indicator of the effectiveness of the treatment in the study, so that the level of effectiveness is interpreted in the category of the effectiveness of the N-Gain score 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 in understanding the immune system and its potential for body integrity against infection. Data on students' initial abilities were obtained from the pre-test given to all research samples using the online test.

The blended learning stage begins with the questioning stage, where Analyze issues and relevant scientific articles also use the online method. The experimental stage is designed based on an integrated project in quasi-experimental research which is implemented in the PjBL model through practicum activities using the offline method. Posttest data was carried out to determine the achievement of students' understanding of the material which was carried out offline. The results of the pretest and posttest 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 5 showed an increase in the average value of pretest and posttest in the experimental group and the control group. 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 gorup (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 gorup (A = 73.05, B = 73.34, C = 72.54) is higher than the control gorup (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 the implementation of the innovative PjBL model integrated with 21st-century skills is effective in increasing students' HOTS. The effectiveness of using the learning model can be seen through the N-Gain score obtained (Mayub et al., 2020). The effectiveness of the implementation of the learning model is supported by the N-Gain score (%) in Figure 2.

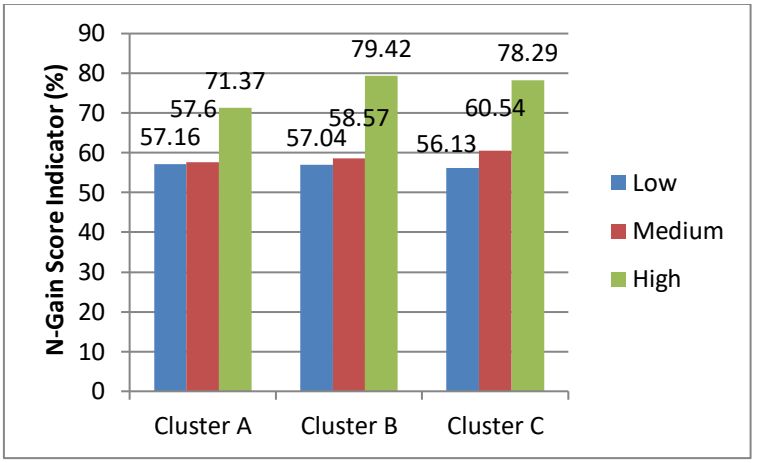


Figure 2. The Analysis Result of Average of the HOTS Increase based on the N-Gain Score (%)

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 compared to low-level classes and medium level classes.

Thinking skills are the application of thinking processes in complex situations, where higher-order thinking processes need encouragement and enthusiasm (Zulfiani et al., 2020). HOTS at high-level class has better thinking skill than low-level class and medium level class. HOTS at the high-level class has a higher ability 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 new 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 the critical analysis of students 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 below.

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

Group	Cluster	HOTS	Analyzes to	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 is	Ineffective	Less Effective	Less Effective
	N-Gain Index	0:31	0:47	0:55
B	Classification	Low	Medium	Medium
	N-Gain (%)	31.33	47.37	55.39
	interpretation is	Ineffective	Less Effective	Less Effective
	N-Gain Index	0:33	0 .48	0.50
C	Classification	Low	Medium	Medium
	N-Gain (%)	33.34	47.83	50.01
	Interpretation	Ineffective	Less Effective	Less Effective

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 6 shows the increase in the students' HOTS N-gain score. It indicates the improvement of students' analysis skills such as the ability to discuss, identify problems, formulate problems, conduct studies of relevant literature, design project frameworks, present hypotheses, determine research parameters and instruments, use appropriate data collection techniques, and use appropriate data analysis method. All components in the analytical ability indicator provide a strong basis for students as pre-service biology teachers in responding to actual problems, 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 aspect of the ability to evaluate becomes a barometer of pre-service biology teachers' ability to utilize technology. Furthermore, the students were also able to conduct mixed-method research and to combine online and offline learning. The project-based laboratory experimental approach in research requires students to be able to evaluate each stage of learning. The most effective HOTS indicator is in the aspect of 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 (Fang & 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 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; Parno et al., 2020; Akhdinirwanto 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 becomes new information is a manifestation of 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 have a more significant influence on HOTS in students in high-level class but is also considered quite effective in groups of students who are low-level class and medium-level class. 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 (pre test =7.134, and post test =7.496) is lower than the control class (pre test = 6.487, and post test =9.385). The lower the standard deviation value, the more homogeneous the data will be. Although the df value which represents 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 has a significant effect on 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 results of the independent sample T-test analysis to find out how much influence the blended PjBL model integrated with 21st-century skills toward students' HOTS (Figure 3).

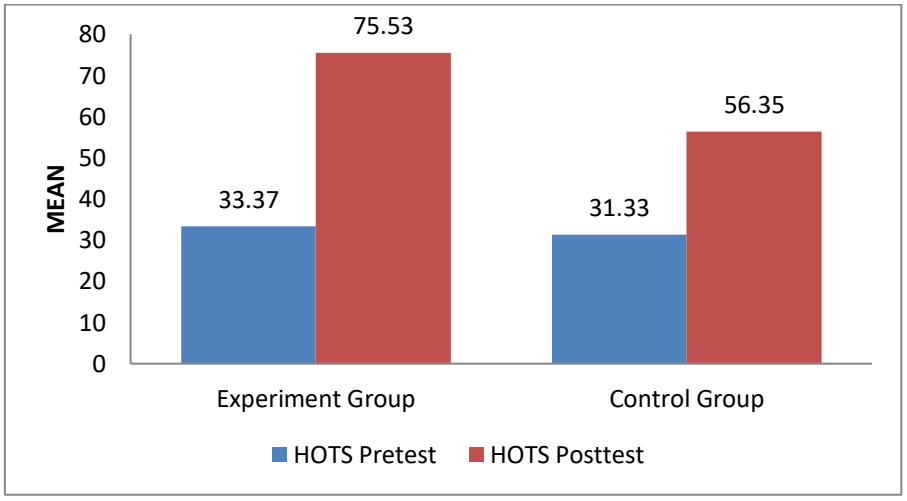


Figure 3. The Results of the Analysis based on the Independent T-test

The comparison of the mean score in Figure 3 showed an increase in students' HOTS after the implementation of 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 class, medium level class, and high-level class. 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. The step of integrating the innovative learning model in this study is appropriately implemented, making it easier to achieve the learning objectives designed before. The implementation of this innovative model can increase active attitudes in developing students' HOTS and can improve the quality of learning.

The N-Gain index in the experimental group was higher than 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-centred learning model has the lecturer or teacher as the primary source of information and is considered a person who has broader knowledge (Zainudin, 2017). The successful implementation of blended learning in this study is not a substitute for the conventional learning model but rather enriches the previous learning model. Bender et al. (2012) emphasized that blended learning cannot completely replace conventional learning, but

blended learning is an add-on and reinforcement of 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 will 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; Nadarajan et al., 2020). Therefore, critical, creative, and high-order thinking skills are essential factors for developing 21st-century skills. Through this blended learning pre-service biology teachers are directed to follow scientific developments with the ability 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; Cheng, 2011; Sutarto et al., 2018). 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 (Moore & Stanley, 2010; Facione, 2011). 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 questions that are arranged are contextual and based on local issues regarding the mechanism of the body's defence 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 are required to make discoveries and innovations by adding questions to make the project more specific (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 defence system against infection by microorganisms. The immune system and infection during this pandemic 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 with each other, so that 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 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 centred 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 compared to other direct learning. The PjBL model can improve student cognitive learning outcomes and HOTS (Anazifa & Djukri, 2017). Besides, that blended learning can improve physical reasoning (Heong et al., 2012), increase learning motivation (Chu et al., 2017), make decisions through a systematic framework (Tsybulsky & Muchnik-Rozanov, 2019), find unlimited 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 findings 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 have a significant effect on 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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Paper title:
The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

Parts of review	Guidelines	Yes	Partly	No	Reviewer's note for improvement	Author's responds (highlight of revision)
Title	• Does the subject matter fit within the scope of journal?	√				
	• Does the title clearly and sufficiently reflect its contents?		√			
Abstract	• Does the abstract contain informative, including Background, Methods, Results and Conclusion?	√				
Back-ground	• Is the background informative and sufficient (include the background problem and objectives)?	√				
	• Is research question of the study clear and understandable?	√				
	• Does the rationale of the study clearly explained using relevant literature?	√				
	• Is the "aim" of the manuscript clear and understandable?	√				
Methods	• Is the methodology chosen suitable to the nature of the topic studied?		√			
	• Is the methodology of the research described clearly?(including study design, location, subjects, data collection, data analysis)		√			
	• Is there adequate information about the data collection tools used? (only for empirical studies)		√			
	• Are the validity and reliability of data collection tools established? (only for empirical studies)	√				
	• Are the data collection tools suitable for the methodology of the study? (only for empirical studies)	√				
Results & Discussio n	• Are the tables, graphs and pictures understandable, well presented and numbered consecutively?	√				
	• Do the data analysis and the interpretation appropriate to the problem and answer the objectives?		√			

	• Does the “discussion” section of the manuscript adequately relate to the current and relevant litarature?	√				
	• Are the findings discussed adequately considering the research question(s), sub-question(s) or hypothesis?	√				
Conclusion	• Is the conclusion clear and in the form of a narration instead of pointers?	√				
	• Isn’t the conclusion a summary and consistent between problems, objectives and conclusion?	√				
References	• Do the references and citations match?	√				
	• Are the writing of references correct?	√				
Quality Criteria	• Do the title, problem, objectives, methods and conclusion are in line? Is it well organized?	√				
	• The quality of the language is satisfactory	√				
	• The work relevant and novel	√				
	• Are there strong consistencies among the parts of the manuscript? (introduction, methods, results and discussion, and conclusion)		√			

AUTHOR'S RESPONDS

Paper title:

The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

PARTS OF REVIEW	GUIDELINES	AUTHOR'S RESPONDS
Title	Could you explain more "Blended Project-based Learning Integrated with 21st-Century Skills" ?	The explain has been added in method
INTRODUCTION	In the introduction part, I cannot find the research gap of this study and why this study is important?	The urgency and research gap this study, and why this study is important has been added on paragraph 1, page 248
	What is this? Page 249. line 3 "21 4C century skills,"	This has been revisied and added on page 249
	Provide citation of this statement Paragraph 2 "The material of the mucosal immune system in immunology courses is abstract"	This has been revisied page 249 .
METHODS	METHODS should Could you explain more "Blended Project-based Learning Integrated with 21st-Century Skills" in the method?	This has been added page 250
	Could you provide an example of instruments to measure HOTS abilities? "where students whose score less than 0.65 are categorized in low-level group, a score between 0.65 and 0.80 is considered a medium level group, and a score greater than 0.80 is categorized as a high-level group". Form these statements, I am wondering if you can provide categorization. (if I see at Table 4, the category is different"	The example instrumens to measure HOTS abilities has been added on page 250 This has been revised and the student class grouping category table is added to table 1 page 250
	Check. "planning.".	This has been revised page 250

	Each stage is explained and analyzed by what method Better to change the symbol. For instance O1, O2, O3, and O4.	This has been revised page 251.
	What if the N gain is 0.7? include in which category? Tabel 2	This has been revised page 251 table 3
RESULTS AND DISCUSSION	What is the function of Min and Max value in Table 5?	This has been revised page 250
	In this figure, check again if you still use Indonesia language. Fig. 2	This has been revised page 253
	What is the meaning of this number? Table 7 (df=94)	This has been revised page 254-255
	Provide different color of this figure. **Figure. 3**	This has been revised page 255

AUTHOR'S RESPONDS

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PARTS OF REVIEW	GUIDELINES	AUTHOR'S RESPONDS
INTRODUCTION	INTRODUCTION should: 1. Contain urgency (importance) to research	The urgency (importance) of the suggested research has been strengthened on page. 248 paragraph 2, lines 18 to 20.
	2. Contain a carrying capacity in the form of supporting data and facts	Supporting capacity in the form of data and facts as suggested support has been added to page 248 paragraphs 4, lines 12 to 20
	3. Contain a preliminary study as a basis for the importance of the research conducted	A preliminary study in the form of an analysis of learning outcomes as a basis for the importance of research being carried out has been added to page 249 paragraph 2, line 4 to 11.
	4. Contain a GAP ANALYSIS Departing from the preliminary study, analysis of published articles formulated in the Gap analysis. GAP ANALYSIS refers to articles published in various internationally reputable journals to emphasize the novelty of research.	GAP ANALYSIS from the preliminary study was added on page. 249 paragraph 2, lines 4 to 11. Analysis of articles published to formulate a gap analysis has been presented on page. 248 paragraph 4, lines 1 to 10, and reinforced at page. 249 paragraph 2, lines 11 to 23 GAP ANALYSIS refers to published articles to emphasize the novelty of research has been added to p. 249 2nd paragraph, and the renewal is clarified in the paragraph 3, line 5 to 11.
	5. Clear limitation of research objectives	The boundaries of the research objectives have been clearly stated on page. 249 paragraph 3, lines 1 to 5.
METHODS	METHODS should 1. Contain detailed research stages	The research stages have been added and detailed on page 250 of the paragraph 2, accompanied by an illustration on Figure 1
	2. Each stage is explained and analyzed by what method	It is clear that each stage of the research has been added and detailed on page 250 paragraph 2, lines 1 to 42. The analytical method has been added in page 250 paragraph 1 to 5
	3. Data analysis must be with clear references	Reference for data analysis has been added on page 251, line 4
	4. The research instruments used were elaborated to the data analysis technique	The research instrument used and data analysis techniques have been added to page 250 paragraphs 1 to 5

	5. It is hoped that there will be a modification in the stages of research from sources referred by the researcher	The modification of the research stages from the source referred to by the researcher has been illustrated in Figure 1 page. 250
Page	1. (each picture/table is preceded by an introduction to the description, and after the picture/table is given a description of the results shown.	Introduction / description before table as table explanation has been added on page 251 before table 3.
	2. The pictures/tables must not be consecutive	Figure / table has been improved (Table 2 and Table 3)
RESULTS AND DISCUSSION	1. Tables or graphs (one selected) must represent different results	Tables or graphs have been adjusted to represent different results
	2. The results of data analysis must be strong in answering the analysis gap	Data analysis in answering the analysis gap has been added to page. 253 paragraphs 4, lines 10 to 16. Page 254 lines 1 to 8. Lines 13 to 27
	3. Display of results other than those narrated in table-graph-image-modeling	There is no display of results other than those narrated in the table-graph-picture model in writing
	4. The research novelty has not been clear enough	The novelty of research has been emphasized on p. 256 paragraphs 3, lines 13 to 18
	5. It is recommended not to repeat the references in the introduction, using previous research findings.	References in the introduction using the results of previous studies have been adjusted so that there is no repetition of references.
	6. References used should be taken from reputable journals.	The references used have been taken from reputable journals
	7. It is necessary to explain the specifications of the findings in this study that show	Specification of findings in this study on page 256 paragraph 2
References	1. Please provide at least 30 references which 80% of them are taken from the last 10 years (>2011) articles of no-predatory journals, written in accordance with the APA Standard. You may go to Google Scholar and find the right format for APA Style provided.	The references used have been as recommended, not included in predatory journals, and have been adjusted to the APA style
	2. For books, please refer to the original/primary book reference no matter the date.	Book references have been adjusted
	3. All of the listed references must be cited in the body of the article, and vice versa.	Has been adjusted as suggested

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The Effect of Blended Project-based Learning Integrated with 21st-Century Skills on Higher-order Thinking Skills of Pre-Service Biology Teachers

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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 material. This research is an experimental study with a mixed method, in which the qualitative observation data uses 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. The research sample was students who were taking the immunology course in the Biology Education Study Program, namely 57 students from IAIN Palangka Raya, 60 students from Universitas Palangka Raya, and 83 students from Universitas Negeri Malang. 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 results of the study proved that the blended PjBL integrated with 21st-century skills had a significant effect on students' high-level thinking skills based on the paired sample test (Sig. 2-tailed 0.000<0.05). The value of mean 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 the Higher Order Thinking Skill of pre-service biology teachers according to the demands of 21st-century skills.

Keywords: Blended Learning, Project-Based Learning, 21st-Century Skills, Immunology

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INTRODUCTION

21st-century skills are the primary targets in the curriculum of educational institutions around the world (Alismail & McGuire, 2015; Mohammed, 2017; Wang et al., 2018; Haviz et al., 2018), because a good skill in the 21st Century becomes 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 life for 21st century. One of the main ability in 21st century skills is creative, critical and problem solving, or known as higher order thinking skill (Laar et al., 2017; Rahman, 2019). Critical thinking really needs to be developed for students, because it is a cognitive thinking process (Sardone & Devlin-Scherer, 2010). The capability of thinking creatively is to produce products through new ideas (Hasanah, 2017), to produce ideas, changing thinking flexibly, and able to develop ideas to find problem solutions (Mayes et al., 2015). Problem-solving ability is a thought process that stimulates students to treat a problem and analyze it which aims 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 activity in the learning process. The problem-solving ability needs to be developed for each individual (López et al., 2011; Basilotta et al., 2017). The development of HOTS can be done in the learning process including biology learning.

Educators have prepared various strategies in preparing students and pre-service teachers with the demands of the 21st-century (Teo, 2019). Quality education is one of the factors that determine the progress of a nation. Therefore, educational institutions are required to prepare the nation's generation with special skills, otherwise known as 21st-century skills (Geisinger, 2016). 21st-century skills, or known as 4C, includes Critical Thinking and Problem Solving, Creativity and Innovation, Communication, Collaboration. Overall, these competencies are very much needed to survive in facing global problems (Jia et al., 2016; Greiff & Kyllonen, 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 that are 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 that is expected to produce graduates who can follow scientific developments in the fields of science and technology. The teacher, as a human resource, has 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.

21st-century skills are needed by aspiring teachers to compete in the 21st century. Research by Haviz et al. (2020a) 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 a variety of analysis, assessments, evaluations, reconstruction, decision-making that leads 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 behaviour (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 is positively correlated 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 to the study reported that there was a significant increase in the thinking skills and perspectives of biology teachers in learning chromosome inheritance material through online discussion forums. Result of this research illustrates the importance of integrating science learning with 21st-century skills. Integrated learning is more focused on competency content so that exploration of 21st-century skills and

thinking skills in learning more broadly is essential (Zainuddin & Attaran, 2016).

Strengthening higher-order thinking skills (HOTS) is not only student-centred but also influenced by strategies and innovative models of learning (Haviz et al., 2020a). Therefore, the exploration of learning models is essential to improve thinking skills (Maryuningsih et al., 2019; Fitriani 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 the knowledge they have practised 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). 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 carry out exploration, assessment, interpretation, synthesis, 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 be used to analyze solutions to complex problems in investigating. The blended PjBL, which is integrated with 21st-century skills, is expected to have a better influence on the HOTS of pre-service biology teachers (Haviz et al., 2020b). Through this research, it is hoped 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; Wibowo 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. These characteristics of the material require higher-order thinking skills and better critical analysis skills. The target of learning outcomes in the immunology course is so that students can 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 various problems that develop in the environment as an implementation of the concept in the field of immunology and communicate the results of applying the basic concepts of immunology based on scientific written observations. Immunoglobulin A (IgA) as a protein secreted by plasma cells that binds to antigens and functions as an effector of the humoral immune system, is essential to understand more explicitly concerning its function for 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 so that it can be understood optimally. The blended PjBL integrated 21st-century skills are recommended as an innovative learning model that has characteristics that are 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., (2020b) to give a significant contribution to the improvement of thinking skills of prospective biology teachers. The results of Haviz's study becomes 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, namely 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.

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 blended model in this study uses modified practicum-based learning tools, both in terms of material and assessment aspects. Blended learning is expected to show significant results as one of the results of the exploration of 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 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 supported by various sources of literature, which can be accessed via the internet (online). The information collected is discussed through an offline face to face meeting, and become a discussion material for the experimental project. The implementation stages of blended learning in this study referes 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 activities of the mucosal immune system practicum. There were three research locations, namely 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.

21st-century skills to improve students' HOTS were analyzed from difference tests and score analysis of each indicator. The research subjects used as research samples were Biology Education undergraduate students taking Immunology courses, namely 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 whole research subjects were grouped into three groups based on their basic skills, namely 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).The grouping aims to determine the effectiveness of the implementation of the used 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.

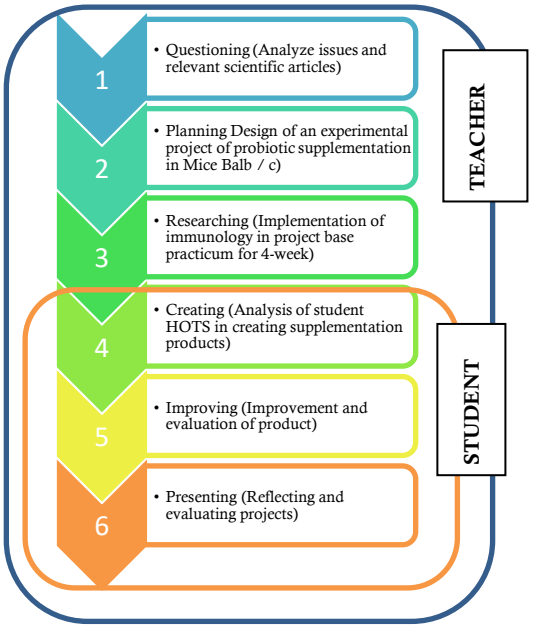


Figure 1 Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020).

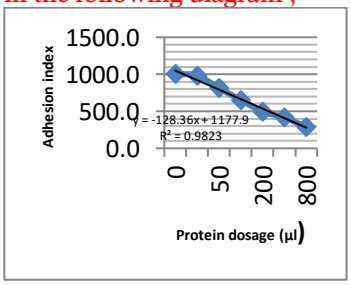
The research stages in Figure 1 begin with; 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of preparing questions or project assignments that come from 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 project group, namely the problem of 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, as well as 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 to evaluate the achievement of the project; 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 that raised in the experimental project include the ability to analyze the concept of immunology and the body's integrity system, the ability to evaluate the immunomodulatory mechanisms and immunoregulators, and the ability to create basic concepts for protein-based supplementation products.

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 methods of 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 :

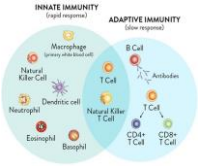
1. Look at the results of the 65kDa protein adhesion test sub unit Yersinia enterocolitica in the following diagram ;



   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 has an effect on the adhesion index

   From the four statements above, which is the correct statement based on the observed data:
   a. 1 dan 3
   b. 2 dan 3
   c. 1 dan 4
   d. 3 only

2. Look at the following picture:



   What conclusions can be concluded to reinforce the above regulatory facts ...
   a. The interaction between non-specific and specific immune responses is linked by receptors in recognizing pathogens
   b. There is activation of T cells and NK cells which then 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

The Blended Project-Based Learning Integrated with 21st-Century Skills

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

Data analysis

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 test, paired test, and Independent T-test assisted by the SPSS-22

program (Sugiyono, 2010). The N-gain test is to determine the effectiveness of implementing innovative models in the experimental group and the control group (low-level class, medium level class, and high-level class). The paired test is to determine 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 group and the control group.

Learning outcomes increase if the students' posttest results (X_2) are 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 range of acquisition. 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	$g \geq 0.7$	High
Increased	$0.3 < g < 0.7$	Medium
Quite increased	$g \leq 0.3$	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 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 in understanding the immune system and its potential for body integrity against infection. At the end of the project-based learning, a posttest is carried out to find out the achievement of student understanding of the material. The results of the pretest and posttest 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 5 showed an increase in the average value of pretest and posttest in the experimental group and the control group. 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 gorup (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 gorup (A = 73.05, B = 73.34, C = 72.54) is higher than the control gorup (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 the implementation of the innovative PjBL model integrated with 21st-century skills is effective in increasing students' HOTS. The effectiveness of using the learning model can be seen through the N-Gain score obtained (Mayub et al., 2020). The effectiveness of the implementation of the learning model is supported by the N-Gain score (%) in Figure 2.

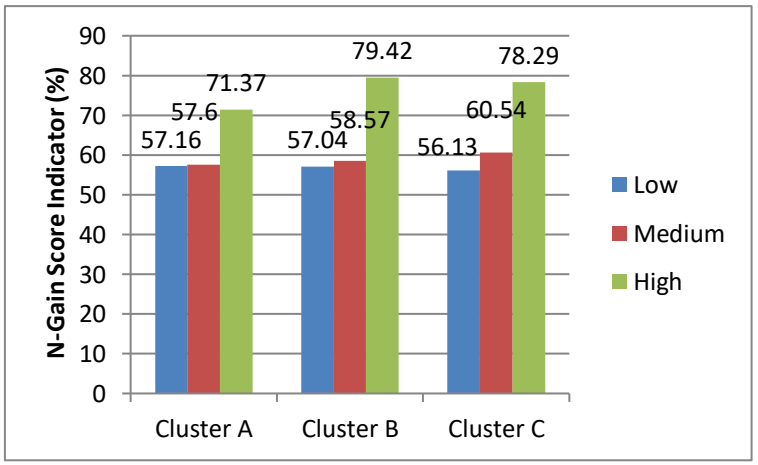


Figure 2. The Analysis Result of Average of the HOTS Increase based on the N-Gain Score (%)

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 compared to low-level classes and medium level classes.

Thinking skills are the application of thinking processes in complex situations, where higher-order thinking processes need encouragement and enthusiasm (Zulfiani et al., 2020). HOTS at high-level class has better thinking skill than low-level class and medium level class. HOTS at the high-level class has a higher ability 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 new 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 the critical analysis of students 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 below.

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

Group	Cluster	HOTS	Analyzes to	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
		The 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 is	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 is	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

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 6 shows the increase in the students' HOTS N-gain score. It indicates the improvement of students' analysis skills such as the ability to discuss, identify problems, formulate

problems, conduct studies of relevant literature, design project frameworks, present hypotheses, determine research parameters and instruments, use appropriate data collection techniques, and use appropriate data analysis method. All components in the analytical ability indicator provide a strong basis for students as pre-service biology teachers in responding to actual problems, 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 aspect of the ability to evaluate becomes a barometer of pre-service biology teachers' ability to utilize technology. Furthermore, the students were also able to conduct mixed-method research and to combine online and offline learning. The project-based laboratory experimental approach in research requires students to be able to evaluate each stage of learning. The most effective HOTS indicator is in the aspect of 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 (Fang & 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 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; Parno et al., 2020; Akhdinirwanto 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 becomes new information is a manifestation of 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 have a more significant influence on HOTS in students in high-level class but is also considered quite effective in groups of students who are low-level class and medium-level class. 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. Although the df value which represents 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 has a significant effect on 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 results of the independent sample T-test analysis to find out how much influence the blended PjBL model integrated with 21st-century skills toward students' HOTS (Figure 3).

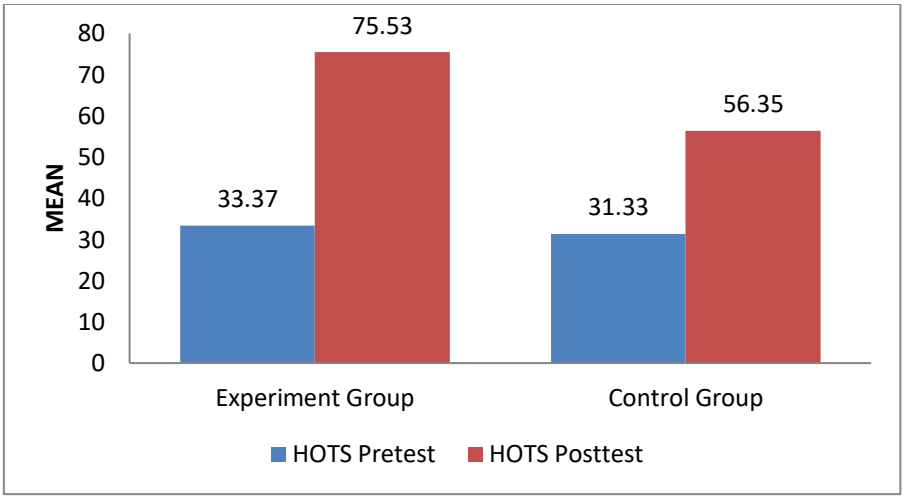


Figure 3. The Results of the Analysis based on the Independent T-test

The comparison of the mean score in Figure 3 showed an increase in students' HOTS after the implementation of 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 class, medium level class, and high-level class. 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. The step of integrating the innovative learning model in this study is appropriately implemented, making it easier to achieve the learning objectives designed before. The implementation of this innovative model can increase active attitudes in developing students' HOTS and can improve the quality of learning.

The N-Gain index in the experimental group was higher than 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-centred learning model has the lecturer or teacher as the primary source of information and is considered a person who has broader knowledge (Zainudin, 2017). The successful implementation of blended learning in this study is not a substitute for the conventional learning model but rather enriches the previous learning model. Bender et al. (2012) emphasized that blended learning cannot completely replace conventional learning, but blended learning is an add-on and reinforcement of 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 will 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; Nadarajan et al., 2020). Therefore, critical, creative, and high-order thinking skills are essential factors for developing 21st-century skills. Through this blended learning pre-service biology teachers are directed to follow scientific developments with the ability 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; Cheng, 2011; Sutarto et al., 2018). 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 (Moore & Stanley, 2010; Facione, 2011). 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 questions that are arranged are contextual and based on local issues regarding the mechanism of the body's defence 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 are required to make discoveries and innovations by adding questions to make the project more specific (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 defence system against infection by microorganisms. The immune system and infection during this pandemic 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 with each other, so that 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 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 centred 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 compared to other direct learning. The PjBL model can improve student cognitive learning outcomes and HOTS (Anazifa & Djukri, 2017). Besides, that blended learning can improve physical reasoning (Heong et al., 2012), increase learning motivation (Chu et al., 2017), make decisions through a systematic framework (Tsybulsky & Muchnik-Rozanov, 2019), find unlimited 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 findings 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 have a significant effect on 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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Parts of review	Guidelines	Yes	Partly	No	Reviewer's note for improvement	Author's responds (highlight of revision)
Title	• Does the subject matter fit within the scope of journal?	√				
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Abstract	• Does the abstract contain informative, including Background, Methods, Results and Conclusion?	√				
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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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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: Blended Learning, Project-Based Learning, 21st-Century Skills, Immunology

INTRODUCTION

The 21st-century skills are the primary targets in the curriculum of educational institutions around the world (Alismail & McGuire, 2015; Mohammed, 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 (Laar et al., 2017; Rahman, 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 (Hasanah, 2017), create ideas, change thinking flexibly, and develop ideas to find problem solutions (Mayes et al., 2015). 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 (López et al., 2011; Basilotta et al., 2017). 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 (Jia et al., 2016; Greiff & Kyllonen, 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. (2020a) 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., 2020a). 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 (Maryuningsih et al., 2019; Fitriani 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). 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., 2020b). 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; Wibowo 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. (2020b) 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

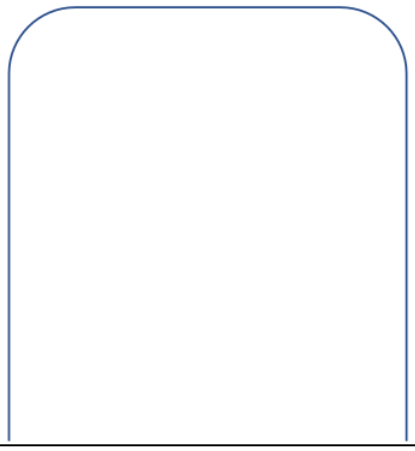
This research used mixed-method, in which experimental research is integrated into educational research through a project-based blended learning model (Creswell, 2016). The mix method in blended PjBL integrated with 21st-Century Skills" in this research is a combination or mixture of online and offline 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.



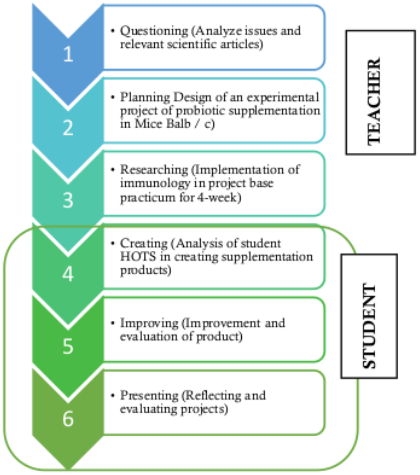


Figure 1. Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020).

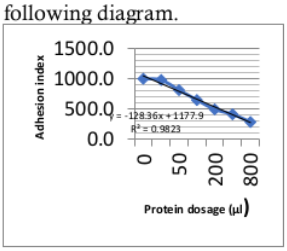
The research stages in Figure 1 begin with 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of preparing questions or project assignments that come from 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

The Blended Project-Based Learning Integrated with 21st-Century Skills

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

Data analysis

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 5 showed 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.

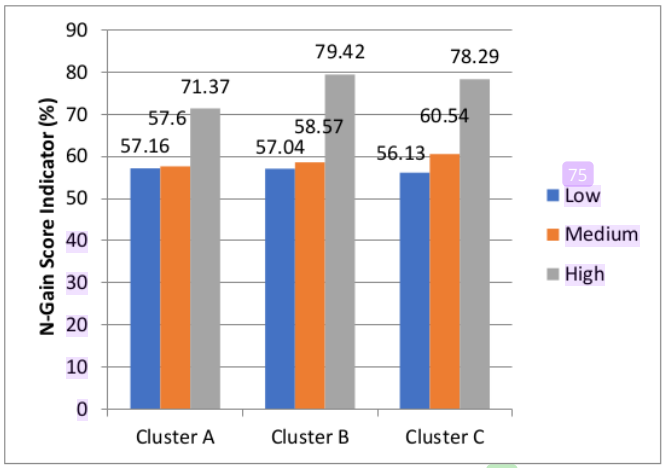


Figure 2. The Analysis Result of Average of the HOTS Increase based on the N-Gain score (%)

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.

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

Group	Cluster	HOTS	Analyzes to	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 is	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 is	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

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 6 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.

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 (Fang & 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; Parno et al., 2020; Akhdinirwanto 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).

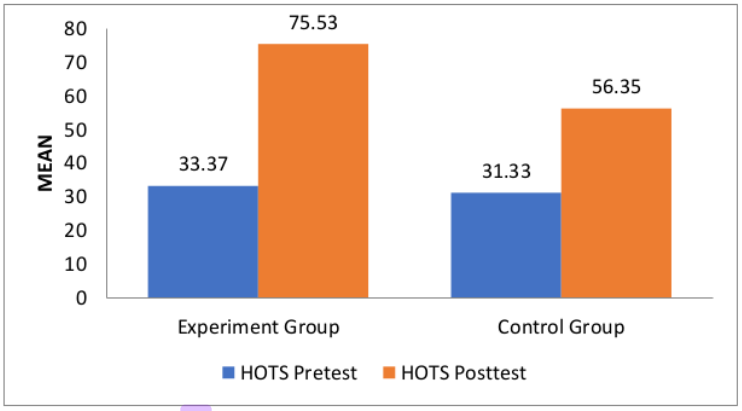


Figure 3. The Results of the Analysis based on the Independent T-test

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. Bender et al. (2012) 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; Nadarajan 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; Cheng, 2011; Sutarto et al., 2018). 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 (Moore & Stanley, 2010; Facione, 2011). 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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THE EFFECT OF BLENDED PROJECT-BASED LEARNING INTEGRATED WITH 21ST-CENTURY SKILLS ON PRE-SERVICE BIOLOGY TEACHERS' HIGHER-ORDER THINKING SKILLS

written by

Noor Hujjatusnaini, A Duran Corebima, Sumarno Reto Prawiro, Abdul Gofur

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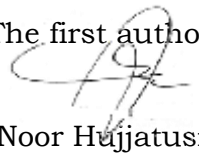

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authors :

1. **Noor Hujjatusnaini**
2. **A Duran Corebima**
3. **Sumarno Reto Prawiro**
4. **Abdul Gofur**

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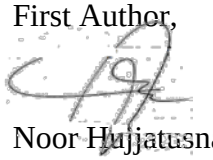

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THE EFFECT OF BLENDED PROJECT-BASED LEARNING INTEGRATED WITH 21ST-CENTURY SKILLS ON PRE-SERVICE BIOLOGY TEACHERS' HIGHER-ORDER THINKING SKILLS

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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.

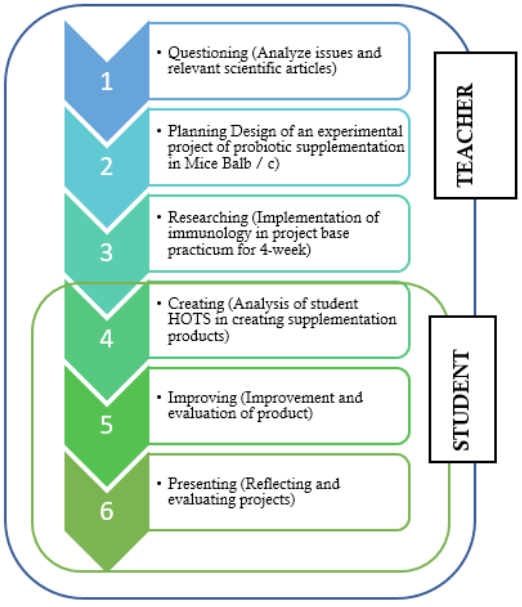


Figure 1. Steps for Blended Project-Based Learning Integrated with 21st-Century Skills (Modification from Yustina et al., 2020)

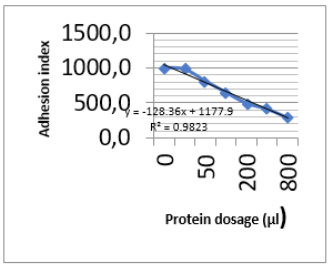
The research stages in Figure 1 begin with 1) the preparatory stage, consisting of the process of constructing instruments and designing a blended research design for the PjBL learning model that integrates 21st-century skills; 2) the stage of preparing questions or project assignments that come from 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.

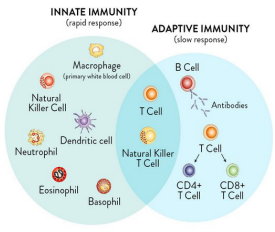


1. The higher the dose, the greater the adhesion index
2. The dose of protein adhesin 65kDa pili sub unit Yersinia enterocolitica is directly proportional to the adhesion index
3. There is no effect on the percentage of the adhesion index with the treatment of the dose of adhesin protein 65kDa pili sub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

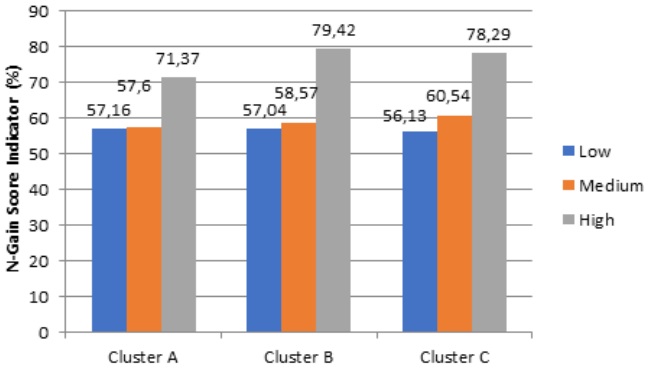


Figure 2. The Analysis Result of Average of the HOTS Increase based on the N-Gain Score (%)

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).

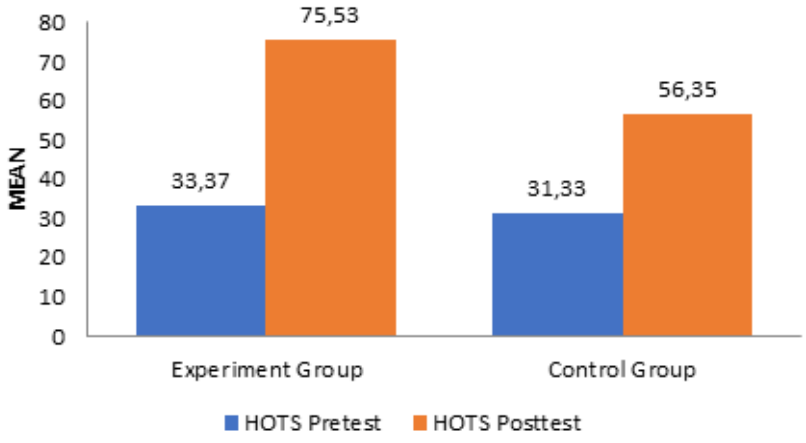


Figure 3. The Results of the Analysis based on the Independent T-test

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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