Agile Management Skills of Sports Coaches: An In-Depth Assessment

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Abstract — Problem-solving, time management, communication, and honing athletes' talents are all vital aspects of agile management. This study examines the agile management skills of sports coaches in the Higher Education Institutions of Tarlac Province, along with forming, norming, and performing. It also identified the profile of the coaches and used the descriptive-correlation research method with thirty-two respondents. Frequency counts, percentages, weighted mean, Pearson-r, and t-test were utilized to treat the study's data.

Particular interest is gained in the analyses of the profile of the coaches, agile management skills, and the innovative work plan as an output. Further, the study concluded that most of the sports coaches are 23-34 years old, male, have a monthly family income of 23,382.00-46,761.00, with MA units, 6-15 years of service, have 1-3 memberships to professional organizations, received gold medals and handling badminton sports. The level of agile management skills of sports coaches is relatively exercised. Age, highest educational attainment, years of service, training, and sports they handle are linked with their abilities. By and large, there is a vigorous indication to suggest intensive provision of a higher level of experience and training for all the coaches because these are correlated with their agile management skills.

Moreover, this literature frame suggests that interest in boosting more of sports coaches' desires, awareness, and yearnings to manage their skills should be strengthened. Coaches may be constantly offered opportunities for their development at all levels of the coaching landscape.

Keywords — Forming, Norming, Performing,

I. Introduction

The significance of sports and sporting ethics has been qualified since ancient times, particularly those advocated through human values that help the development of a cohesive personality. In this setting, developing rules and establishing a regulatory framework to safeguard and promote sport's principles and objectives became important. Aside from that, sports and physical education are both fundamental human rights. Sport has evolved into a global phenomenon in which people participate in various aspects of it by watching video sporting events

or actively participating in the administration or management of these events, and as the number of professional athletes and coaches grows exponentially, a plethora of relationships that necessitate legislation emerges. Sports law investigates the notions of sport, sports activities, and sporting life, as well as the issues that occur within the sports scene and the legal practices that apply to them. (Sportiva, 2021)

The Philippine Sports Commission was created and established under Republic Act No. 6847, which defined its powers, functions, and responsibilities. The Philippine Sports Commission adopted the policy of promoting physical education, encouraging and sustaining the empowerment of sports in the Philippines to foster physical fitness, self-discipline, teamwork, and excellence for the development of a healthy and vigilant citizenry through a unified national sports promotion and development.

Likewise, The Department of Education shall implement Republic Act No. 5708, denoting an act providing for the fostering and financing of an Integrated Physical Education and Sports Development Program for Philippine Schools, which was founded on an integrated physical education and sports development program in all Philippine schools, by the following guiding principles. (1) Physical education aims to instill a proper understanding of the value of physical and mental development in individual and social activities. These things can be accomplished through a coach's innovative qualities and the coaching process' creative process.

Brent (2018) noted that organizations that want to capitalize on knowledge-based personnel need to change their management style to one that is consultative and participatory. Coaching is a common term for this approach. Managers must shift from a typical function of managing and monitoring employee performance to a more consultative role when coaching. Coaching is a method of forming a relationship between management and an employee, resulting in a shared understanding of what needs to be attained and how the coach should accomplish it.

By giving timely feedback, recognition, clarity, and support, coaching promotes a motivating climate for performance, improves the match between an employee's actual and expected performance, and raises the possibility of an employee's success. Coaching is a term used in the performance management cycle to provide continuous feedback and support to an employee throughout the year. Coaching allows employees to hear about parts of their performance in "real-time" and participate in determining how to adapt or adjust their behavior for success effectively. (Johnson, 2020)

Simply, coaching is a practice that focuses on the now and now rather than the distant past or future to improve performance. While there are different coaching approaches, we focus as a facilitator of learning rather than as an expert. There is a significant distinction between teaching and assisting someone in learning. Fundamentally, the coach's role is to assist the individual in improving their performance: in other words, to assist them in education. Good coaches believe each person has the answer to their difficulties but recognize that they may require assistance in

finding it. Coaching is established on the premise that each person has the solutions to their challenges. The coach is not a subject expert; instead, they are concerned with assisting the individual in realizing their full potential. The emphasis is on the athlete and what is going on within their heads. A coach does not have to be a specific person; anyone, whether peers, subordinates, or superiors, can use a coaching technique with others (Skillsyouned.com, 2021)

Coaches have an important role in sports, performing instructional, organizational, strategic, and social connection functions, and their interactions with athletes impact both skill development and psychosocial consequences. (Smith et al., 2017)

Athletes are assisted in reaching their most significant potential by sports coaches. They are in charge of coaching athletes in a sport by analyzing their performances, teaching relevant skills, and encouraging them. However, you also control the athlete's life and sport-related guidance. As a result, the coach will play a wide range of roles, including instructor, assessor, friend, mentor, facilitator, chauffeur, demonstrator, adviser, supporter, fact seeker, motivator, counselor, organizer, planner, and the Fountain of All Knowledge. In sports, the coach's job is to provide the ideal learning environment and develop ways to motivate the athletes. Most athletes are highly motivated, so the challenge is to keep them motivated while generating excitement and enthusiasm. (Topendsports, 2020)

Gedge (2016) outlined essential aspects of agile coaching to assist teams in learning to self-organize and employees in shifting their attitudes away from command and control and up-front planning and toward accepting uncertainty, devolving responsibility, and valuing experimentation and learning. Agile coaches contribute unique expertise to organizations and assist in the training of corporate teams in the agile approach, as well as managing the development of agile teams to ensure organizational goals are fulfilled, and tasks are fulfilled quickly. In a word, agile coaches assist a company in transitioning from command-and-control management to collaborative, team-based workplaces. (Tokdemir, 2019)

An internal coach or an external coach can both undertake agile coaching. An external coach might be someone from outside the team but from the same organization or an external consultant. An internal coach is someone who works in the same team. When mentoring people, an agile coach must take into account a variety of elements. The agile coach must have a balanced perspective when dealing with many teams. They should not have strong opinions and should stick to their core ideals. Every prospect requires the agile coach to comprehend the complexity within the group – social, psychological, and political. The coach must have models that are models and make sense, as well as a map of the current state of the team. Agile coaching should ideally be non-intrusive. Therefore the agile coach should aim to guide the section on the proper path without interfering. Finally, coaching should be practiced for the benefit of the group, with an emphasis on individual development (Reeva, 2019)

According to Madan (2020), Agile coaching possesses expert-level knowledge of current lean-agile processes, is highly experienced in team facilitation, and has acquired professional coaching and mentoring skills. Coaches with these talents are likely to work with numerous teams or a program, begin new groups, mentor others, or spend a lot of time working with management and dealing with obstacles that aren't directly within their control.

Hawks (2015) also mentioned that organizations embracing agile development or having difficulties with it frequently seek assistance from an agile coach. Agile coaches usually have more excellent experience with agile processes and procedures and can help the team get through difficult spots until they can figure things out on their own. An agile coach may demonstrate how agile practices work for inexperienced teams or engage in more listening and questioning to improve the team. Agile coaches, on the other hand, traditionally spend all of their time focused on the coaching job and are not team members. A different variant, the player-coach, addresses this constraint.

Tengberg's (2015) study concluded that many doctoral students fail to complete their studies within the allowable time frame, partly because of problems related to the research and supervision process. Surveys showed that most post-graduate students are generally satisfied with their dissertation supervision. However, these studies also reveal some students think their supervisors meet with them too infrequently, lack motivation in their dissertation topics, and provide poor practical assistance. Furthermore, most countries witnessed a significant turnover in the labor market as people near retirement. Because this is the case at higher educational institutions, the belief is that there will be teaching and research opportunities. Therefore, the new doctoral students who plan to enter academia after earning their post-graduate degree are necessitated. In response to these complaints, this conceptual paper examined the use of the **agile approach**–which has achieved awards and approval in software development–in the post-graduate dissertation process. In the teaching/learning sphere, coaches can use the agile approach in iterative meetings between post-graduate students and supervisors for dissertation planning, direction, and evaluation. The focus of the emphasis, the so-called Sprints, is on communication and response throughout the whole process. The paper was based on theories on teaching/learning and the author's personal experience with the agile approach. The use of the agile approach and technique, which can lessen the time required for doctoral studies, may thus add to the number of graduates with doctoral degrees.

The agile coach is a catalyst, change agent, and servant leader who provides professional facilitation, teaching, and mentoring to help organizations realize their plan and achieve excellent results for organizations, teams, managers, and leaders who want to create a productive, empowered, high-performing, and adaptable culture. Unlike traditional project management and consulting, the agile coach lives and works by agile ideals, principles, and practices. They want to make clients less reliant on them. Finally, agile coaches achieve achievements by prioritizing the

development of people's and teams' capacities, over-optimizing for immediate, short-term results (Lopez, 2017)

LITERATURE REVIEW

Agile coaches are accustomed to the traits of innocent and defenseless sports organizations, such as. Agile coaching professionals owe it to athletes to explain what's best for them. This always includes assisting athletes in accepting complete accountability for their own learning. This typically requires the coach to pass up opportunities to take on more responsibility (Gedge, 2016).

In addition, successful game management is essential for any squad. Knowing when to use substitutes, timeouts, fouls, and other strategies can mean the difference between regaining momentum and succumbing to a 15+ point or 2+ goal run. The game plan must be managed by the coach. In an agile sports endeavor, the coach makes use of analytics and player feedback to develop and sell a strategy. The agile managers are in charge of compiling in-depth reports for forthcoming games and providing coaching advice while the game is in progress. They are in charge of keeping track of the game's data, which, when supplemented with an analytical or stats tool, can help the coaching staff make quick choices (Holman, 2018)

Nagarro.com (2022) revealed that as agile managers/coaches, it is crucial that we assist teams in creating their winning scenarios. This could entail accomplishing the strategic goals determined for the current year and increasing happiness. Each and every project management action includes coaching and mentoring. In coaching, team members' talents are developed and nurtured, and they are inspired to complete projects successfully. Similar to this, agile project management includes agile coaching as a key component, and agile adaptable leaders are accountable for it. The project manager or agile leader must act as both a coach and a mentor during agile coaching. Agile coaching involves enhancing a person's skills and inspiring them to adopt productive work practices. Here, the agile coach's job is to help the individual go to the next level by coordinating their goals with those of the organization. An internal coach or an external coach can both provide agile coaching. The difference between an external coach and an internal coach is that the latter might be either a consultant from outside the team or a member of the same organization. When mentoring people, an agile coach must take many things into account. A few of these are outlined here. While working with many teams, the agile coach must maintain a balanced viewpoint. They should not be opinionated and should adhere to their values. They also need to understand the team's complexities on all levels, including social, psychological, and political. They need to have models that make sense and map the team's current situation. Finally, they should ideally be non-intrusive, so they should try to steer the team in the right direction without getting in the way (Chandana, 2021)

According to Henriksen's (2016) research, there are several aspects that can influence the success of an agile project. It explicitly checked which agile practices are necessary for an agile project to succeed. A case study was undertaken among participants in agile projects in order to

figure this out. He went on to say that there has been a movement in project management, particularly in software project management, from traditional plan-based project management to agile event-driven project management. For a long time, agile project management has been viewed as the next big thing that will transform the software industry. Agile is a concept that has been around for a while, and while awareness and use are growing, agile is not always the best solution. It is critical to know when to adopt agile and which success elements to consider if you want to be successful.

The study of Khalil, et.al (2018) aimed at analyzing these questions through a longitudinal case study. The study has been conducted in a French telecommunication company that strives to become agile. It adopted a qualitative approach for collecting and analyzing data. The findings highlighted the reasons that drive organizations to become "agile" and stress on the contingency factors that affect the implementation of "agile" practices and tools in a "lightweight" organizational structure. The transition to an "agile" organization remains a challenge. Despite the increasing number of empirical research regarding "agile" software development, "agile" management and engineering practices are still difficult to implement within large organizations. There is no formal framework on which organizations may rely to become "agile" in terms of knowledge. The application of these emergent strategies appears to be constrained by a number of contextual constraints, such as team size and distribution.

Cojocaru, et al. (2022) evaluated the obstacles and opportunities of technology, as well as the benefits of adopting an Agile Management style to improve Physical Education and Sports teaching, learning, and practice (PES). Therefore, a survey was conducted on PES teachers and trainers, as they have a clear perspective on the field and their views are therefore very important and relevant to the study, even if they do not have solutions for all the challenges facing them. They were invited to provide their professional perspectives on the impact of digital methods and applications on performance sportsmen, athletes, and students' sporting results. The survey, which included 144 participants, largely consisted of multiple-choice questions with Likert scale ratings and open-ended questions that allowed respondents to provide solutions and openly express their opinions. The impact of Agile Management on the selection and deployment of PES technology was illustrated in this paper. Because of the COVID-19 pandemic, educational institutions and sports clubs have had to change their management strategies.

Adaptability is the first of five key concepts identified by Haak (2017). This method has the advantage of delivering benefits over the course of the project rather than all at once at the end. That pays off not just for the customer, but for the team’s morale and confidence. With agile, one zooms out to start, looks through a wide lens, and then adapts as one learns about new conditions and parameters. Second, there's effectiveness. In a similar vein, anyone on the agile project management team, as well as the client or customer, is empowered to recognize and respond to changing requirements or goals at any time, allowing the product to reach the market sooner. Agile project management has been described as continuous sprints in these circumstances, with

planning and execution occurring in waves. Third, with agile project management, all parties are given the opportunity to contribute to the end goal. Collaboration fosters trust and accountability, as well as ensuring that one bad idea does not go uncontested, jeopardizing the entire process. Disruption is the fourth element. Changing culture, priorities, and goals are all part of becoming agile. And that can be difficult. However, when all stakeholders are on board, management becomes much easier. Standards and expectations change, and it ultimately succeeds. The last option is straightforwardness.

II. Methodology

This is a quantitative analysis study. The descriptive research design was deemed relevant and appropriate because the main purpose is to determine the extent of the practice of the agile management skills of coaches at Higher Education Institutions in the Province of Tarlac along with forming, norming and performing.

The respondents of the study were the coaches of the Higher Education Institutions in the Province of Tarlac during the first semester of the SY 2021-2022.

Table 1. The Population of the Study

Higher Educational Institutions in the Province of Tarlac	**Number of Respondent-Coaches**
Tarlac State University	10
Dominican College of Tarlac	5
OLRA College Foundation	1
Tarlac Agricultural University	16
TOTAL	**32**

A researcher-made questionnaire was utilized in this study that was patterned from the related studies. It was made in accordance with the objectives of the present study. The questionnaire consisted of various parts: Part I of the instrument focused on the profile of the coaches in terms of their age, sex, monthly family income, highest educational attainment, years of experience as coach, no. of memberships to professional sports organizations and no. of relevant training/seminars attended, no. of awards as a coach and events/sports handled. Part II involved the agile management skills of coaches of Higher Education Institutions in the Province of Tarlac along with forming, norming and performing. The researcher sent a letter of intent to the University Presidents of the Institutions in the Province of Tarlac requesting permission to conduct the study. After receiving approval from the Presidents, the researcher sent a letter of intent to the respondents of the study.

The researcher then distributed the questionnaires to the participants using Google Forms. The agile management skills of coaches in the Higher Education Institutions were rated by

respondents. The data and results of the study were analyzed. The descriptive survey was employed to answer the questionnaire of data. Permission was obtained from the Offices of the President of the Higher Education Institutions and to all the Sports Directors for the floating of the questionnaire to gather the needed data. The data that was gathered was analyzed through the utilization of the Jeffreys's Amazing Statistics Program (JAPS). In determining the profile of respondents, the frequency counts and percentages were used.

$$\text{Percentage: } \% = \left(\frac{f}{N}\right) \times 100$$

where f = frequency, or the number of cases in any category
N = the number of cases in all categories

Likewise, the average weighted mean was utilized to determine the extent of the agile management skills of coaches at Higher Education Institutions in the Province of Tarlac, along with forming, norming, and performing.

$$x = \frac{\sum_{i=1}^{n} (x_i * w_i)}{\sum_{i=1}^{n} w_i}$$

Σ = summation

w = the weights.

x = the value.

The following scale was adopted:

Numerical Values	Mean Range	Scale	Descriptive Rating	Interpretation
4	3.26 – 4.00		Highly Practiced	If the skill is very well executed and extremely done at all times
3	2.51 – 3.25		Moderately Practiced	If the skill is executed and modestly done most of the times
2	1.76 – 2.50		Slightly Practiced	If the skill is slimly executed and sometimes done
1	1.00 – 1.75		Least Practiced	If the skill is sparingly executed and very seldom done

To identify the significant relationship between the extent of the practice of the agile management skills of coaches in their profile variables of coaches Higher Education Institutions in the Province of Tarlac chi-square and Cramer's V was used.

$$\chi^2 = \sum_{j=1}^{k} \frac{(f_{b_j} - f_{e_j})^2}{f_{e_j}}$$

whereby

k = the number of cells

fb = the observed absolute frequency within cell j

fe = the expected absolute frequency within cell j

The calculated chi-square test statistic is afterwards tested for significance.

$$\text{Cramer's V} = \sqrt{\frac{\chi^2}{n \cdot \min(r-1, c-1)}}$$

whereby

n = sample size

r = the number of rows

c = the number of columns

III. Results and Discussion

Profile of the Sports Coaches of the Higher Education Institutions in the Province of Tarlac

Age. The table shows that most of the coaches are 25-34 years old, as indicated by the frequency of 15 or 46.86%. Ten (10) or 31.25% are 35-49 years old, and four (4) or 12.50 % are 50 and above. Moreover, three (3) or 9.38% are 24 years old and below. The data imply that most of the coaches are young adults.

Sex. It is reflected on the table that most coaches are males, as indicated by the frequency of 27 or 84.38%, while only five (5) or 15.63% are females. The data suggest that although coaching is not gender-specific, males still undeniably dominate it. On this note, coaching is likely significant among males.

As affirmed by the data-driven research conducted by Leasca et al. (2020), within the women's sports arena, female intercollegiate coaches face stark biases that male coaches do not. Equally, the report produced by the Institute for Diversity and Ethics and Sport (TIDES), as cited by Elsesser (2019), that there has been little change and that men still dominate coaching when it comes to college sports.

Monthly Family Income. It is reflected in the table that most sports coaches have a monthly family income of 23,382.00-46,761.00, which has been categorized as lower middle class with a frequency of 12 or 37.50%. This is followed by those with an income of 11,691.00-23,381.00 (low income but not poor), as evidenced by the frequency of 7 or 21.88%. Six (6) or 18.75% have a monthly income of 46,762.00-81,832.00 (middle class), and four (4) or 12.50% belong to the poor as reflected with an income that ranges from 11,690.00 and below. Two (2) or 6.25% of the coaches' families have a family income of 81,833.00-140,284.00, categorized as upper middle income. However, one (1) coach or 3.13% enjoys a salary ranging from 140 285.00-233,806.00 (upper income but not rich).

Highest Educational Attainment. The table shows that most coaches are still on their way to completing their Masters' degrees, as indicated by the frequency of 13 or 40.63%. Seven (7) or 21.88% and five (5) or 15.63% are Master's Degree and DA/Ph.D./EdD graduates, respectively. However, four (4) or 12.50% are still pursuing their Doctoral degree, and three (3) or 9.38% are just graduates of Baccalaureate Degree.

Table 2. Profile of the Sports Coaches of the Higher Education Institutions in the Province of Tarlac

n=32

Profile	Categories	Frequency	Percentage
Age	**25-34 years old**	**15**	**46.86**
	35-49 years old	10	31.25
	50 and above	4	12.50
	24 years old and below	3	9.38
Sex	**Male**	**27**	**84.38**
	Female	5	15.63
Monthly Family Income	**23,382.00-46,761.00**	**12**	**37.50**
	11,691.00-23,381.00	7	21.88
	46,762.00-81,832.00	6	18.75
	11,690.00 and below	4	12.50
	81,833.00-140,284.00	2	6.25
	140, 285.00-233,806.00	1	3.13
	233, 807.00 and above	0	0.00
Highest Educational Attainment	**BS Degree with MA units**	**13**	**40.63**
	Master's Degree	7	21.88
	DA/PhD/EdD	5	15.63
	with Doctoral units	4	12.50
	Baccalaureate Degree	3	9.38
Years of Experience as a Coach	**6-15 years**	**15**	**46.88**
	5 years and below	12	37.50
	26 years and above	3	9.38
	16-25 years	2	6.25
Number of Memberships to Professional Organizations	**1-3**	**16**	**50.00**
	None	10	31.25
	4-6	5	15.63
	7 and above	1	3.13
Number of Related In-Service Trainings, Conferences and Seminars	**7 and above**	**14**	**43.75**
	1-3	12	37.50
	4-6	4	12.50
	None	2	6.25

The data imply that though educational attainment is given a premium at the tertiary level in the country, which counts as an excellent percentage for promotion, this is not, however, reflective of the study's results. Hence, the desire to complete the Master's degree accounts because most respondents are on their way to embark on their post-graduate studies.

Y**ears of Experience as a Coach.** It is reflected from the table that coaches have been serving 6-15 years, as indicated by the frequency of 15 or 46.88%. Twelve (12) or 37.50% have been making a coaching career for five years and below, and three (3) of them or 9.38% served as a coach for 26 years and above. However, only 2 or 6.25% have exemplified a coaching service for 16-25 years. This indicates that the experience is grounded on the age of respondents; it is tantamount to saying that their service as a coach goes with it. "The younger the age, the lesser the number of years of coaching," imparts.

Number of Memberships to Professional Organizations. It is seen on the table that most of the respondents have 1-3 memberships to professional organizations, as evidenced by the frequency of 16 or 50.00%. There has been one (1) or 3.13% and five (5) or 15.63% with seven and above and 4-6 memberships. Moreover, ten (10) or 31.25% have no affiliations. This indicates that the coaches are active in joining sports and related organizations because these are avenues for great opportunities for peer collaboration and support aside from acquiring new knowledge from the experts.

A number of Related In-Service Trainings, Conferences, and Seminars. Data shows that most respondents had attended seven and above seminars, as indicated by the frequency of 14 or 43.75%, while only 2 or 6.25% never attended meetings related to sports coaching. The data implies that participation in professional development training and enhancement programs is necessary because their participation in activity indicates its significance to their field of endeavour.

Because sports need teamwork and the development of social skills, participation in training aids in developing good character. Being in training gives one the chance to establish meaningful self-awareness. Now is the moment to observe reactions and develop suitable responses to challenging circumstances. Instead of just broadening the selection of sports facilities that are available to students, universities need to focus on training investments to meet sports development demands (Harrow, 2016)

Profile of the Coaches as to the Awards Received and Sports/Events Handled

Awards Received. It is shown on the table that four (4) or 12.50% of the coaches have received gold medals in the national competitions. Eight (8) or 25.00% have achieved gold medals at the regional level. Likewise, four (4) or 12.50% got gold medals at the local level. The data implies distinction in the field of sports because at least there are achievements acquired by the coaches though minimal. Hence, other coaches are encouraged to step up and desire to have awards that can be conclusive of their merit.

Table 3. Profile of the Coaches as to the Awards Received and Sports/Events Handled

N=32

Profile	Categories	Frequency	Percentage
Awards Received as Coach			
	National **(Gold)**	**4**	**12.50**
	(Silver)	0	0.00
	(Bronze)	0	0.00
	Regional **(Gold)**	**8**	**25.00**
	(Silver)	0	0.00
	(Bronze)	0	0.00
	Local **(Gold)**	**4**	**12.50**
	(Silver)	1	3.16
	(Bronze)	0	0.00
Events/Sports Handled	Badminton	5	15.63
	Basketball	4	12.50
	Volleyball	4	12.50
	Chess	3	9.38
	Taekwondo	3	9.38
	Archery	2	6.25
	Athletics	2	6.25
	Table Tennis	2	6.25
	Karatedo	1	3.13
	Speak Takraw	1	3.13
	Swimming	1	3.13
	Softball	1	3.13
	Arnis	1	3.13
	Beach Volleyball	1	3.13
	Lawn Tennis	1	3.13

Coaches are disreputable for avoiding the spotlight. It is a selfless job that involves assisting others in achieving their goals. For the majority of coaches, seeing them on the podium is enough. Coaches are rewarded in various ways, but one of the most rewarding is seeing athletes mature and move away to maintain a balanced existence in sport. The best instructors are generally those who work in the shadows, pleased to watch an athlete win an Olympic medal and recalling how awkward they were when they first began their sports career (Hooper, 2017)

Events/Sports Handled. The table shows that most coaches handle badminton as indicated by the frequency of 5 or 15.63%. Four (4) coaches or 12.50% each handle basketball and volleyball: three coaches or 9.38% each for chess and Taekwondo. Besides, two coaches, or 6.25%

each for archery, athletics, and table tennis, while an individual coach or 3.13% handles Karatedo, speak takraw, swimming, softball, arnis, beach volleyball, and lawn tennis.

According to Ordoñez (2019), coaching is a growing practice in the Philippines that provides a new path for Filipinos to reach their full potential, personally and professionally. In addition, in the Philippine Sports Commission's National Sports Coaching Certification Course, around 300 coaches got online sports specialized lectures on athletics, badminton, and volleyball to help them improve more.

The Extent of Practice of the Agile Management Skills of Coaches of Higher Education Institutions in the Province of Tarlac

In Terms of Forming

During the forming stage, members avoid disagreement and are more concerned with themselves, their performance, and whether it will be acceptable to the group. At this point, the first leaders will emerge. Individuals will be assessing and forming opinions on the other members of the group, what they may bring to it, and whether they belong within it during the forming stage, which often has a solid social component because it is spent getting to know the rest of the group while forming interpersonal relationships.

The table shows that the coaches highly practice the agreement with the rules and regulations of the team with the players, as evidenced by the mean of 3.48. Rules are deemed to be the factors in establishing rapport between coaches. On this note, this implies that before the selection and recruitment of players in the school, they emphasized the value of compliance and allegiance to the rules and the policies being implemented.

Likewise, developing training techniques and robust sports competition system has been moderately practiced, as supported by the mean of 3.31. It indicates that since coaches are highly spirited and it is their passion to handle athletes within the bounds of their expertise, techniques in coaching are in place. The system of competition is always taken into its most significant consideration.

When coaches build sound training procedures, players gain more control and mindfulness, which leads to them discovering their tone of sports skills and paying attention to them to a more significant level (Moen et al., 2015)

Table 4. The extent of Practice of the Agile Management Skills of Coaches of Higher Education Institutions in the Province of Tarlac in Terms of Forming

N=32

Indicators	**Mean**	**Verbal Description**
Agrees with the rules and regulations of the team.	3.48	Highly Practiced
Develops training techniques and strong sports competition system.	3.31	Moderately Practiced
Shows inexperienced teams how agile practices work and does more listening and asking questions to help the team improves	3.13	Moderately Practiced
Develops an action mechanism to create viable sports team	2.94	Moderately Practiced
Settles unclear policies and mechanisms on practice, team plays and rollouts	2.94	Moderately Practiced
Leads the team through strong recruitment and selection procedure	2.66	Moderately Practiced
Generates sustainable and feasible coaching process	2.63	Moderately Practiced
Weighted Mean	**3.01**	**Moderately Practiced**

Legend:

3.26 – 4.00	Highly Practiced
2.51 – 3.25	Moderately Practiced
1.76 – 2.50	Slightly Practiced
1.00 – 1.75	Least Practiced

The coaches also moderately practice the indicator, show inexperienced teams how agile practices work, and pay more attention and ask questions to help the group develop, as indicated by the mean of 3.13. This means that openness in the group is encouraged. The freedom to express oneself in the team is positively urged. On the same wavelength, the coaches moderately practice the development of an action mechanism to create a viable sports team with a mean of 2.94. This indicates that whatever challenges arise because these are all innate in a sports team, the coaches deal with these with the scheme or a policy so that these conflicts will not become more severe and critical. They have the established flow to follow in effectively dealing with the problems within the team setting.

Teams with high performance are more pull than push. High-performance coaches understand how to instill energy and passion in their teams. Players feel inspired, as though they are working toward a common goal and that what they are doing is critical. A team that was

constantly in dispute and disagreement, a team that was walking on eggshells and scared to speak up or express genuine feelings (Folkman, 2016).

Settling unclear policies and mechanisms on practice, the team plays, and roll-outs have been moderately practiced by the coaches as supported by the mean of 2.94. This is indicative of the pre-judgment skills of the coaches that they know if there are unclear policies in the team like the scheduling of the practice and the game plan during competitions. They manage the players by considering the welfare of the team and the players.

Similarly, leading the team through solid recruitment and selection procedure has been moderately practiced by the coaches as supported by the mean of 2.63. This implies that the coaches are guided by the rules of recruitment and selection procedure for them to have the highest potential athletes capable of bringing honor to the school.

It could be gleaned from the table that generating a sustainable and feasible coaching process has been assessed as moderately practiced, as indicated by the mean of 2.63. This means that the coaches tend to establish a coaching process seen in their individual sports coaching programs. They see that their sports development program in their specific field is quite strong, and all details of the coaching mechanisms are being transpired.

Overall, the coaches moderately practiced their agile management skills in forming, as evidenced by the weighted mean of 3.01.

In Terms of Norming

Norming is observed when the coach begins to be respected by the players/athletes, accepts his authority, shows a more vital dedication to the group's objectives, and performs much better. Here, the team is more assured, and motivation levels rise. As individual conflicts are resolved, and team members can accept one another's skills and qualities, the group starts to come together. Additionally, they become more at ease asking for assistance and giving the other group members and themselves constructive criticism.

Overall, the coaches moderately practiced their agile management skills in norming, supported by the weighted mean of 3.02.

As to the specific indicators, the table shows that understanding the complexities within the team in every prospect social, psychological, and political has been moderately practiced, as supported by the mean of 3.25. This implies that the coaches have the heart to understand that everything that happens in the team is not exactly perfect. All sides are being considered because it is believed that sports are not only the school but it is also multi-dimensional. Coaching is multilayered, structural, dynamic, and socially engaging because it addresses so many aspects of the past and couples them with the intricacies of the present. This is a crucial tool for every coach

to have in their toolkit, as it will enhance everyone's professional, personal, and athletic development. (Smith, 2020)

The table further shows that the coaches have moderately practiced helping the team to shift from command-and-control management practices to collaborative, team-centric environments, as indicated by the mean of 3.22. This means that a democratic way of management is exercised over just the autocratic method.

Likewise, mentoring the potential players on coaching strategies has been moderately practiced by the coaches, as indicated by the mean of 3.13. This means that coaches are giving pieces of advice to the performing players to become coaches in the future.

Monitoring the development of agile teams to ensure goals are effectively achieved and tasks are efficiently accomplished has been moderately practiced by the coaches as supported by the mean of 3.00. The data imply that the coaches monitor the success of the team and that all the set multilevel objectives are fulfilled.

Likely, spending significant time working with sports managers and engaging with obstacles outside the direct control of teams has been moderately practiced by the coaches as supported by the mean of 2.84. Collaboration is the key that this data implies.

Table 5. The extent of Practice of the Agile Management Skills of Coaches Higher Education Institutions in the Province of Tarlac in Terms of Norming

N=32

Indicators	**Mean**	**Verbal Description**
Understands the complexities within the team in every prospect social, psychological, and political	3.25	Moderately Practiced
Helps the team to shift from command-and-control management practices to collaborative, team-centric environments	3.22	Moderately Practiced
Mentors the potential players on coaching strategies	3.13	Moderately Practiced
Monitors the development of agile teams to ensure goals are effectively achieved and tasks are efficiently accomplished.	3.00	Moderately Practiced
Spends significant time working with sports managers and engages with obstacles outside the direct control of teams	2.84	Moderately Practiced
Is non-intrusive and should try to steer the team in the right direction without getting in their way.	2.84	Moderately Practiced
Brings specific skills to organizations and helps train corporate teams on the agile methodology	2.84	Moderately Practiced
Weighted Mean	**3.02**	**Moderately Practiced**

Legend:

3.26 – 4.00	Highly Practiced
2.51 – 3.25	Moderately Practiced
1.76 – 2.50	Slightly Practiced
1.00 – 1.75	Least Practiced

The coaches have moderately practiced being non-intrusive and should try to steer the team in the right direction without getting in their way, as indicated by the mean of 2.84. This suggests that the coaches consider the welfare of the group.

Similarly, the coaches have moderately practiced bringing specific skills to organizations and helping corporate train teams on the agile methodology, as indicated by the mean of 2.84.

In Terms of Performing

Performing happens when the coach and the team reach their full potential, and as team members become more accustomed to responsibility, structure, and hard work, their roles on the

team become more fluid. Athletes who have reached the performing stage of group growth are more understanding of the shortcomings of their colleagues. They accept them with an all-encompassing attitude. As a result, the team is making the most progress possible, and everyone is giving their best effort.

The table shows that the coaches have moderately practiced the agile management skills of coaches of Higher Education Institutions in the Province of Tarlac in terms of performance, as indicated by the weighted mean of 3.11.

Moreover, it could be gleaned from the table that the coaches assessed executing "experienced" ability with agile processes and techniques as moderately practiced, as indicated by the mean of 3.19. This implies that the outstanding power of coaches is seen in their execution. That is why the coach who will be designated is the one who has profound knowledge, aptitude, and expertise in the sports being handled.

Likewise, the coaches have assessed that practicing the team through rough patches until they can find their way as moderate as indicated by the mean of 3.19.

Other indicators which are evaluated as moderately practiced are the following entries that are arranged in descending means: demonstrates non-usual yet alternative variations of skills (3.06), shows exceptional sports coaching proficiency (3.06), and addresses limitations of players (3.19).

It could be seen further on the table that the coach has moderately practiced achieving excellent results for the players to emulate, as evidenced by the mean of 3.13.

This means that the coaches intend to make themselves role models to which the players should look up.

Table 6. Extent of Practice of the Agile Management Skills of Coaches of Higher Education Institutions in the Province of Tarlac in Terms of Performing

N=32

Indicators	**Mean**	**Verbal Description**
Executes "experienced" ability with agile process and techniques.	3.19	Moderately Practiced
Practices the team through rough patches until they can find their own way	3.19	Moderately Practiced
Demonstrates non-usual yet alternative variations of skills.	3.06	Moderately Practiced
Shows exceptional sports coaching proficiency.	3.06	Moderately Practiced
Addresses limitations of players.	3.19	Moderately Practiced
Achieves excellent results for the players to emulate	3.13	Moderately Practiced
Drives results by focusing on developing the capabilities of players over optimizing for immediate, short-term results	2.97	Moderately Practiced
Weighted Mean	**3.11**	**Moderately Practiced**

Legend:
3.26 – 4.00 Highly Practiced
2.51 – 3.25 Moderately Practiced
1.76 – 2.50 Slightly Practiced
1.00 – 1.75 Least Practiced

The coaches have also moderately practiced driving results by focusing on developing players' capabilities by over-optimizing for immediate, short-term results, as indicated by the mean of 2.97.

Significant Relationship Between the Extent of Practice of the Agile Management Skills of Coaches Across Their Profile Variables

Spearman's Rho results revealed that age has correlated with the agile management skills of coaches along with forming (0.34, sig=0.03) and norming (0.36, sig=0.04). This means that the older the coaches, the higher their skills to handle and put in place all procedures that govern sports coaching. This may be attributed to their experience as manifested in the very crucial age in managing players at all costs and means.

Table 8. Significant Relationship Between the Extent of Practice of the Agile Management Skills of Coaches Across Their Identified Variables

N=32

Variables		Forming	Norming	Performing
Age	Spearman's Rho	**0.34**	**0.36**	0.33
	p-value	**0.03**	**0.04**	0.85
Monthly Family Income	Spearman's Rho	0.31	0.19	0.23
	p-value	0.08	0.29	0.21
Highest Educational Attainment	Spearman's Rho	**0.35**	0.16	0.31
	p-value	**0.05**	0.39	0.09
Years of Experience as a Coach	Spearman's Rho	**0.48**	**0.35**	0.24
	p-value	**0.01**	**0.05**	0.18
Number of Memberships to Professional Sports Organizations	Spearman's Rho	0.22	0.23	0.12
	p-value	0.24	0.211	0.53
Number of Relevant Seminars Attended	Spearman's Rho	0.28	**0.49**	0.30
	p-value	0.12	**0.01**	0.09

Similarly, the highest educational attainment of coaches is connected with their skills in forming, as indicated in Spearman's Rho results of 0.35 (sig=0.05). Since the significance value is lower than the significance level, the hypothesis is hereby rejected. The data imply that the more knowledge coaches adopted from their schooling in post-graduate studies, the better their agile management skills contribute to this may be credited to their exposure and learnings acquired from their professors and peers.

In like manner, the years of experience as a coach have also been very significant in their agile management skills, especially in forming and norming, as revealed by Spearman's Rho results of (0.48, sig=0.01) and 0.35 (sig=0.05), respectively. The data is indicative of the experience and the high level of know-how of coaches. Their agile management skills are boosted by the number of years of exposure to the sports they handle.

Lastly, the number of relevant seminars attended has also been very substantial in the agile management skills of coaches, as supported by Spearman's Rho results of 0.49(sig=0.01). This means that seminars are indeed very crucial in the development of coaches. The data also imply that the more conferences attended, the higher the tendency to increase the agile management skills of coaches.

Significant Relationship Between the Extent of Practice of the Agile Management Skills of Coaches Across their Sex, Awards Received and Sports/Events Handled

Cramer's V results revealed that there is no significant relationship between the extent of the practice of the agile management skills of coaches along with forming, norming, and performing and their sex, awards received, and sports/events handled.

Table 9: Significant Relationship Between the Extent of Practice of the Agile Management Skills of Coaches Across Their Identified Variables

N=32

	Forming			**Norming**			**Performing**		
	x^2	*p-value*	*Cramer's V*	*x^2*	*p-value*	*Cramer's V*	*x^2*	*p-value*	*Cramer's V*
Sex	7.48	0.06	0.48	7.19	0.07	0.47	8.56	0.04	0.52
Awards Received as Coach	17.60	0.82	0.43	22.16	0.57	0.48	20.98	0.64	0.47
Sports/Events Handled	**14.60**	**0.05**	**0.03**	**4.16**	**0.00**	**0.00**	**5.98**	**0.04**	**0.00**

It is imperative to conclude that sex has no importance in better equipping coaches in the practice of their agile management skills. The gender is of no consequence as to how they manage the athletes, as indicated in the p-values of 0.06 (sig=0.48), 0.07 (sig=0.47), and 0.04 (sig=0.52) for forming, norming and performing, respectively.

It also means that awards received as a coach would not help coaches to be more creative, flexible, and critical thinkers in catering to the needs and interests of the players and athletes.

However, there is a significant relationship between sports/events handled and the extent of agile management skills in all areas, as revealed by the p values of 0.05 (sig=0.03), 0.00 (sig=0.00), and 0.04 (sig=0.00), respectively which are all lower than the 0.05 level of significance.

Moreover, coaches may also improve coaches' forming, norming, and performing skills. Once enhanced, athletes may also improve creativity, flexibility, and thinking skills, directly benefiting coaches' potential and abilities. Therefore, coaches may also develop players' performance. Specifically, significant and immediate relationships were identified between the awards received and the agile management skills in forming, norming, and performing.

Meanwhile, coaches have a big impact on achievement in both the professional and collegiate ranks, according to uchicago.edu (2017), notably with their experience in the sports they handle and their leadership. Furthermore, rapid technological advancements have had an impact on sports coaches' abilities. To improve their coaching skills, higher education institutions have

steadily integrated modern technologies into the coaching process and curriculum (Carkanji, et al, 2020).

In the same tone, Table 10 also presents the innovative work plan in a tabular form to improve the agile management skills of coaches of Higher Education Institutions in the Province of Tarlac.

Table 10: Innovative Work Plan to Improve the Agile Management Skills of Coaches of Higher Education Institutions in the Province of Tarlac

Area of Concern	Process	Objectives	Strategy	Personnel Involved	Budgetary Requirement	Expected Outcome
Forming						
Leads the team through solid recruitment and selection procedure	**Robust Enlistment Scheme**	• To institutionalize the extensive recruitment and selection process of players that the coaches execute. .	Most comprehensive structure for the dissemination of information on the recruitment process	Sports Directors and Coordinators Sports Coaches	19,000.00	A sturdy scheme of coaches for recruitment and selection has been recognized.
	Selection Structure Management	• To establish a more concrete and viable arrangement of procedures for selection and recruitment	Administration of a potent structure for athlete selection	Sports Directors and Coordinators Sports Coaches	18,000.00	The selection structure is extensively formed.
Generates sustainable and feasible coaching process	**Innovative Coaching Mechanism**	• To put in place a coaching process that is up-to-date and more sustainable.	Innovative and Up-to-date Coaching Strategy	Sports Directors and Coordinators Sports Coaches Athletes	18,000.00	A creative coaching process is crafted.
Norming						
It is non-intrusive and should try to steer the team in the right direction without getting in their way.	**Eclectic way of Athlete Management**	• Construct a style of coaching that motivates the athletes and apply being democratic and autocratic, if needed.	Administer best types of coaching styles depending on the types of athletes being handled	Coaches Athletes	2,000.00	A more systemic administration of athletes is promoted.
It brings specific skills to organizations and helps train corporate	**Skill-Target Development**	• To develop the skills of coaches in training the team	Skills Enhancement Programs	Sports Directors and Coordinators Coaches	10,000.00	The skills of the coaches are improved.

teams on the agile methodology. **Performing**						
Drives results by focusing on developing the capabilities of players over-optimizing for immediate, short-term results	**Achievement Long-Term Goal over Enabling Goal**	• To inculcate to the coaches the value of the long-term rather than the short one and to appreciate the value of the product of time and determination.	Goal Appreciation	Sports Directors and Coordinators Coaches	------	The coaches much appreciate long-term over short-term goal.
TOTAL					**67,000.00**	

IV. Conclusion and Recommendation

The study concluded that most sports coaches are 23-34 years old, male, have a monthly family income of 23,382.00-46,761.00, with MA units, 6-15 years of service, and have 1-3 memberships to professional organizations, received gold medals, and handling badminton sports.

The level of agile management skills of sports coaches is relatively exercised. Age, highest educational attainment, years of service, trainings, and sports they handle are linked with the agile management skills of sports coaches. The proposed work plan is deemed very significant in improving the agile management skills of sports coaches.

In light with the prevailing conclusions, the study recommends that the sports coaches may take the opportunity to embark on their graduate studies and be exposed to training and organization affiliations for further enhancement of knowledge and expertise. Further, the Commission on Higher Education (CHEd) may offer scholarships distinctly intended for the teachers of Physical Education and sports because most of the scholarships are along with core disciplines.

An interest in boosting more of the sports coaches' desires, awareness, and yearnings on managing their skills. Players and athletes should be given much priority. Of course, the welfare of the coaches should also be given such attention by providing them with the necessary coaching knowledge, aptitudes, and mindsets.

A provision of a higher level of experience and training for all the coaches because these are correlated with their agile management skills. Likewise, older coaches have more profound value in coaching. That is why they give them more opportunities for the support they need. The

work plan is ready for implementation to improve sports coaches' agile management skills, especially in the Higher Education Institutions of Tarlac Province.

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When the Tide is not Turning: Exploring the Mental Health of a Select Group of Filipino Youth Living with Human Immunodeficiency Virus (YLHIV)

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ABSTRACT. Literature from worldwide revealed that poor mental health is evident among youths living with HIV (YLHIV). However, literature exploring their mental health in the Philippines is limited despite the alarming increase of HIV cases among young people. Hence, this descriptive phenomenological study purported to characterize the mental health of a select group of Filipino YLHIV. In-depth interviews with ten fully consented male participants aged 18-30 were conducted. Field texts were subjected to Collaizi's (1978) seven-step data analysis method. Interestingly, the *Mental Health Tower of Youth Living with HIV* emerged after thoroughly analyzing the data. This model typifies the mental health of YLHIV, which operates in an environment where both internal and external pressures make them experience (a) disruptive thoughts, (b) depressive mood, and (c) deteriorative behavior. The study has vividly described the instability of the YLHIV's mental health. Therefore, it is vital to develop a mental health program specifically designed for youth living with HIV.

1.0. Introduction

In 2016 the World Health Organization (WHO) reported that the Human Immunodeficiency Virus (HIV) continues to be a major public health concern affecting approximately 36.7 million people worldwide, 1.8 million of whom were newly diagnosed (WHO, 2018). Although the rate of the disease varies between countries and regions, it remains most prevalent in Sub-Saharan Africa, which affects one in every 25 adults, almost two-thirds of the worldwide HIV population. Conversely, other parts of the world, like the United States of America, reported a 10% decline in HIV infection from 2010-2014 due to the HIV prevention efforts of the government. However, those men having sex with men (MSM) was the sole group that did not decline (Centers for Disease Control and Prevention [CDC], 2015). In Europe, despite the public health effort of the government, the significant transmission of the virus continued affecting 29, 444 people in 2016 (European Centre for Disease Control and Prevention, 2017).

Similarly, the Western Pacific region, including the Philippines, reported an increase in HIV infection from 1.25 million in 2010 to 1.48 million in 2016. For several years, the Philippines had a minimal increase in HIV infections. However, in recent years, cases of people with this disease amplified dramatically. The Philippines has been tagged as one of the countries with fast-growing HIV cases worldwide, with a more than 50% increase from approximately 4,300 cases in 2010 to an alarming 10,500 cases in 2016 (WHO, 2018).

Globally, it is estimated that 30% of all new HIV cases occur among young people aged 15-25. Alarmingly, the Philippines is one of the ten countries in Asia and the Pacific with increasing rates of HIV among young people (United Nations International Children's Emergency Fund [UNICEF], 2015). Consistent with the report of the Philippine government thru the Department of Health (DOH), the percentage of HIV cases among Filipino youth aged 15-24 years increased from 25% in 2006-2010 to 29% in 2011-2017. In April 2022 alone, the HIV/AIDS and ART Registry of the Philippines (HARP) recorded 1,198 new HIV cases, 29% of which occurred among youth aged 15-24 years (DOH, 2022).

According to Bekker et al. (2015), the prevalence of HIV among youth is due to many transitions (e.g., social, psychological, structural, and developments) they encounter during this period of their lifespan. Consequently, YLHIV is at high risk of experiencing mental health problems, as supported by several western studies. For example, a meta-analysis of 38 articles, mostly from the United States and Europe, indicated that youth living with HIV experienced behavioral and emotional problems higher than other high-risk groups (Mellins & Malee, 2013). The latter findings support the cross-sectional analysis of 1706 youth living with HIV, revealing that 727 (42.6%) participants reported clinical symptoms. Only 39.7% of 727 reported mental health care and 21.9% are taking medications for emotional concerns (Whitely et al., 2014). In another cross-sectional study conducted in Jamaica, youth with HIV ages 15- 25 years were found to have high rates of anxiety (71%), stress (64%), and depression (63%). The participants also reported smoking cigarettes (16.1%), drinking alcohol (11.4%), and use of marijuana (8%) (Brown & Morgan, 2013). In Rwanda, a cross-sectional analysis of 193 youth living with HIV revealed that 26% had depressive symptoms, and 12% had attempted suicide (Smith Fawzi et al., 2016). Another study in America found that 44% of youth with HIV aged 16-21 years were diagnosed with depression a year after HIV diagnosis (Pao et al., 2000).

Qualitative studies also revealed that people living with HIV are prone to experience mental health problems, like the study of Jena (2014) in South Africa depicting those adolescents with HIV showed sadness, anxiety, fear, and pain in their lived experiences. Particularly, they were anxious regarding death due to their illness and reported fear of rejection, stigma, and discrimination (Jena, 2014; MacQueen, 2017). Similarly, the Aboriginal people living with HIV in Canada reported feelings of depression. They responded to their medical condition with shock, disbelief, and often anger (Cain et al., 2013). Meanwhile, the explorative study of Landry (2014) indicated that youth living with HIV reported experiences of isolation, depression, and thoughts of suicide. This suicidal tendency is triggered by the burden accompanying the long-lasting implication of being HIV positive (Kalichman et al., 2000). Further, stigma, discrimination, low self-esteem, and lack of social support are other factors directly associated with suicidal thoughts and behaviors of people living with HIV (Casale et al., 2019; Wang et al., 2018). The study participants, being youth, may account for their vulnerability to being persuaded by a misconception about the said illness. Also, youths today tend to access information from the internet without checking its veracity. This may result in misleading information about their medical condition. Another phenomenological study in Africa, specifically in Botswana, showed that HIV-AIDS diagnosis resulted in internal (self) and external stigmatization that impacted the lives of people with HIV. The progression of the disease and stigmatization led to emotional disturbance, relationship problems, poverty, dependence, and concerns about their family members (Setlhare et al., 2015).

The foregoing discussions of foreign literature established that mental health problems are evident among youth living with HIV. This aspect of YLHIV's health is critical and often neglected (Vreeman et al., 2017) and calls for more empirical investigation adopting various theoretical and methodological lenses. Despite the alarming increase of HIV cases among Filipino youth in the Philippines, there is a lack of literature exploring their mental health status. Hence, this phenomenological study was conducted to characterize the mental health of a select group of Filipino YLHIV.

2.0. Methodology

Research design. In recent years, there has been a growing interest in the use of phenomenology to explore the experiences of people who have HIV and mental health issues (Sharma & Babu, 2017; Zhou, 2010; Cain et al., 2013; Jena, 2014; Landry, 2014; McLeish, 2015). The current study utilized the aforementioned design, particularly the descriptive phenomenology, which is appropriate for understanding the subjective experience of a select group of Filipino YLHIV. According to Lopez and Willis (2004), descriptive phenomenology aims to describe the universal essence of an experience as it is lived by the participants. Hence, it represents the true nature of the phenomenon being studied.

Participants. The ten male participants of the current study were recruited via Pinoy Plus Advocacy Pilipinas, Inc., a pioneer support group dedicated to the welfare of PLHIV in the Philippines. They were identified from the pool of potential participants of the study. The participants were purposively chosen based on the following inclusion criteria: (a) diagnosed with HIV; (b) 18- 30 years old, and (c) Filipino citizen.

The participants were diagnosed with HIV from the year 2007-2018. Regarding their employment, four (4) of them are currently studying, three were employed, and the other three (3) were

unemployed. One of those unemployed participants has a pending case against his employer for forcing him to resign because of his HIV status. Of the 10 participants, two worked as sex workers and were strongly convinced that they had acquired the virus from their customers. All of them signed informed consent and were given meal and transportation allowance for voluntarily participating in the study.

Data Collection procedure. Prior to the conduct of this qualitative inquiry, ethical clearance from the local ethics committee was secured. Permission from the study site- Pinoy Plus Advocacy Pilipinas, Inc., was also sought. Preliminarily, informed consent was secured from the participants, including the consent to record the interviews; participation was stressed to be voluntary without remuneration and that they are free to discontinue during the interviews without any bias.

The researchers adopted the interview protocol of Seidman (2006), which consists of three phases, namely: (a) rapport-building phase, (b) exploratory phase, and (c) clarificatory phase. In the first phase, the researchers established rapport by displaying friendly gestures to the participants. Then, in the exploratory phase, a semi-structured interview guide was employed to uncover the lived experiences of the youth living with HIV in relation to mental health. Lastly, the researchers raised clarifications during the clarificatory phase to better capture the participants' responses.

Mode of Data Analysis. The recorded interviews were individually transcribed and converted into field texts. Since the language used by the participants is Filipino, their responses were properly translated into English and interpreted to preserve their original meaning. The translation was accomplished with the help of an English Editor with a Ph.D. degree in Language Education and a Master of Arts in Teaching English Language. Then, the researchers used Colaizzi's (1978) seven-step method to analyze the data. The process included: (1) familiarizing with the field texts by reading and reading them; (2) pulling out significant statements from the field texts; (3) formulating meaning units from the significant statement; (4) categorizing the meaning units into clusters of themes; (5) developing a full and inclusive description of the phenomenon by incorporating all the themes produced at step 4; (6) condensing the exhaustive description down to a short, dense statement that captures just those aspects deemed to be essential to the structure of the phenomenon; and (7) returned the fundamental structure statement to all participants.

3.0. Results

This qualitative study yielded interesting findings about the mental health of youth living with HIV. Three themes collectively described the mental health of YLHIV, namely (1) disruptive thoughts, (2) depressive moods, and (3) deteriorative behaviors.

Disruptive thoughts

Learning that they were infected with HIV was very disturbing for the participants. They experience disturbing or troubling thoughts such as irrational, anxious, and suicidal thoughts.

The participants were besieged by several irrational thoughts, such as overestimation of danger and illogical interpretation of their diagnosis. Particularly, they are terrified by the thoughts of dying at a young age and are worried about their future. As verbalized by the participants:

> "That time, I keep on thinking that I was dying, that there is no treatment for HIV. I was worried for my family because I am the breadwinner" (Participant 2, personal communication, January 23, 2020).

> "I thought I was dying. I was thinking what will happen to the dreams I have for my family. Moreover, I was bothered on how to tell my parents about my health condition" (Participant 10, personal communication, January 25, 2020).

Besides entertaining irrational thoughts, the participants were likewise disturbed by anxious thoughts as manifested by their fear of being rejected and discriminated against by family, friends, romantic partners, and the workplace. As expressed by the participants:

> "How can I work if I have this illness? They might trace it through medical examination" (Participant 1, personal communication, January 23, 2020).

> "I am afraid to form romantic relationship because I might transmit the virus, I don't want my partner to get sick because of me" (Participant 3, personal communication, January 23, 2020).

> "My fear was rejection especially if it is from my own family" (Participant 10, personal communication, January 25, 2020).

Cognizant of their present health condition, the participants were also bombarded with suicidal thoughts, as evidenced by their death wish, thinking of killing themselves, and thoughts that other people are better off without them. As expressed, "Sometimes, I was thinking that instead of dying from the opportunistic infections of this virus, I wish I would not wake up the next day" (Participant 1, personal communication, January 23, 2020). Another participant verbalized, "There was a time that I thought of hanging myself to die. After knowing that I am HIV+, I seldom go to work, most of the time I locked myself in the room, I thought of slitting my wrist, and taking all my medicine at the same time" (Participant 4, personal communication, January 24, 2020).

The irrational and anxious thoughts of the participants were entrenched in certain triggers in their environment, particularly the lack of available information about HIV. They articulated that they have limited information about the illness and are mostly misconceptions about its transmission, treatment, and prevention. As expressed,

"During that time, I thought HIV and AIDS are the same. So for me, it is like a death sentence" (Participant 3, personal communication, January 23, 2020). This misconception elicited disturbing thoughts of dying at a young age and worrying about their future. Another participant verbalized, "I thought I cannot find a job anymore. I was so hesitant to apply for a job because they might discover that I am HIV+" (Participant 2, personal communication, January 23, 2020).

> This wrong notion triggered the fear of being rejected and discriminated against in the workplace. Meanwhile, the suicidal thoughts of the participants are primarily triggered by an internal factor, internalized stigma- the internalization of negative beliefs, feelings, and attitudes about PLHIV. As verbalized,
>
> "I would rather die than to be associated with this illness for a long time. I felt so gross/dirty. I am a mess" (Participant 10, personal communication, January 25, 2020).

Summarily, the mental health condition of a select group of Filipino youth living with HIV is typified by certain disruptions which make them entertain irrational, anxious, and suicidal thoughts. These thoughts are provoked by internal and external factors, particularly internalized stigma and lack of available information.

Depressive mood

Accommodating the fact that they were infected by HIV was not easy for the participants. They were blasted with the emotional turmoil intruding on their daily activities at home, school, and even the workplace. Particularly, the participants articulated their experiences of emotional distress, persistent feelings of sadness, and hopelessness.

The participants' emotional distress is manifested in their experience of a deep state of agony and disbelief. As verbalized by the participants:

> "I felt like I was going crazy that time, some of my friends told me not to stress myself, but it stresses me a lot. From time to time, it sinks in. I really don't know what to do" (Participant 1, personal communication, January 23, 2020).
>
> "It seems that I was blown away when they told me about the result. I even tried to ask for a second opinion, I can't believe it. Gosh, I was extremely terrified that time. I'm sure, my parents will kill me, I uttered" (Participant 2, personal communication, January 23, 2020).

Other participants experienced a persistent feeling of sadness. They verbalized feelings of aloneness and loneliness, especially the undisclosed YLHIV. Generally, they feel miserable and unhappy instigated by their health condition. The following are some of the verbalizations of the participants:

> "After knowing the HIV test result, I kept it for a long time. When I was with my parent, I pretend to be OK, but in reality, I felt so sad most of the time" (Participant 7, personal communication, January 24, 2020).

> "I felt lonely; I have difficulty coping up with loneliness. I can't do the things that I previously enjoyed; I lose my interest in almost everything" (Participant 6, personal communication, January 24, 2020).

Likewise, the participants conveyed their experience of hopelessness as they struggled with HIV. They are uncertain about their future and express pessimism about life in general. As expressed,

> "It is like, I lose hope in life, there was a time that I applied for a job and got hired, but I backed out because of the medical exam" (Participant 1, personal communication, January 23, 2020).

The indices of depressive mood such as emotional distress, persistent feeling of sadness, and hopelessness were prompted by discrimination. One participant shared that he experienced discrimination from his own family. He said,

> "After telling my parents about it, I felt they avoided using the utensils we used to share like drinking glass, spoon, and fork. I even shared a room with my brother before, but now they asked my brother to move out of my room. I felt very sad, but I have to accept it. Maybe that is the consequence of having this illness" (Participant 1, personal communication, January 23, 2020).

Aside from their family members, the participants also received discrimination from their friends and workplace. As articulated:

> "After I disclosed my status with my immediate supervisor, I noticed that most of my workmates kept distance from me. Almost every day, I was crying discreetly in the office because of the way they treated me" (Participant 2, personal communication, January 23, 2020).

Another factor that incited the depressive mood of the participants is an internal trigger, non-disclosure. They have difficulty disclosing their health condition due to the stigma associated with the illness. Since they are undisclosed, they feel they are brawl with the illness alone. As shared by one of the participants,

> "Actually, right now my pressing concern is on how to tell my parent about it. Not even one from my family knows my status. I feel so empty and alone. Alone battling with this condition" (Participant 2, personal communication, January 23, 2020).

Another participant uttered,

> "In my case, I am afraid to disclose because my family might throw me away. They will also discover the obscenity I have done in my life. I do not know when to tell them. I am really struggling" (Participant 5, personal communication, January 24, 2020).

Lack of social support is another factor that fueled the emotional struggles of the participants. They mentioned that it is more emotionally painful when they do not get support from their significant others. They badly wanted to feel the comfort of their family, friends, and other people significant to their lives. As verbalized by the participants:

> "I feel so lonely, because until now I am fighting this illness alone. I disappointed my family, so it's very challenging to get support from them" (Participant 5, personal communication, January 24, 2020).

> "It's very painful that I want to hug my friends as I usually do when I have problems. But right now, it seems that gradually they are moving away from me" (Participant 1, personal communication, 2020).

Collectively, the mental health of the selected Filipino YLHIV is characterized by depressive mood, which is manifested by emotional distress, persistent feelings of sadness, and hopelessness. These indices of depressive mood are aggravated by personal and environmental factors such as non-disclosure, discrimination, and lack of social support.

Deteriorative behavior

Besides disruptive thoughts and depressive moods, the participants also struggled with deteriorative behavior that impairs their physical and social well-being, such as loss of interest, self-neglect, and social withdrawal.

After knowing their HIV status, they started to engage in several deteriorative behaviors like losing interest. Particularly, the participants started to lose interest in their work, refused to go to school, and disengaged themselves with activities they previously enjoyed. As uttered by the participants:

> "To the point that almost two months, I did not go to work. I lose my willingness to work" (Participant 5, personal communication, January 24, 2020).

> "Before, I love going to the gym. I usually spend an hour or two for twice or three times a week. But now, I don't go to gym anymore" (Participant 6, personal communication, January 24, 2020).

> "All I want is to stay home, I don't want to go to school. I don't even play with my pets anymore that I used to enjoy doing" (Participant 10, personal communication, January 25, 2020).

Moreover, the participants experienced self-neglect while living their lives with chronic illness. They disregard the regular intake of food and eat on an irregular schedule. As expressed,

> "What happened to me was, I missed some meals in a day. There was a time that I ate for one meal a day. I had difficulty getting up to do the usual" (Participant 9, personal communication, January 25, 2020).

As a form of avoidant coping mechanism, some participants were even engrossed in dangerous vices like substance use. As expressed,

> "The time that I learned my HIV diagnosis, I engaged in inappropriate behavior like smoking cigarettes, drinking alcoholic beverages, I even tried taking marijuana. I felt so devastated that is why I did not care about my health anymore" (Participant 1, personal communication, 2020).

For people living with HIV, doing the usual social interactions was challenging. The participants reported that they experienced withdrawal from other people, such as their friends and workmates. As expressed,

> "Actually, I started to avoid mingling with my friends. I am afraid that every time I was with them, they might discover my health condition" (Participant 10, personal communication, January 25, 2020).

> "I tend to isolate myself from my workmates, although I only disclosed it with my immediate supervisor. I felt that they know about my condition" (Participant 8, personal communication, January 25, 2020).

Some participants also avoid socializing activities like playing sports, as verbalized,

> "I used to play badminton with my neighbors, but I choose to avoid playing with them" (Participant 1, personal communication, 2020).

Notably, personal and environmental factors contribute to the deteriorative behavior of youth living with HIV. For instance, their loss of interest and self-neglect are triggered by internalized stigma, specifically the thought of dying young. This negative notion about the illness fueled their unwillingness to perform their usual task. Meanwhile, engaging in dangerous vices is entrenched in denial of HIV status. They refuse to accept their diagnosis; hence, they engage in avoidant coping mechanisms. As uttered,

> "During that time, it seems that I escaped from reality, after learning my HIV status, my coping was to drink alcohol and smoke cigarettes" (Participant 2, personal communication, 2020).

Further, the participants' experience of discrimination from family and friends provoked social withdrawal. They tend to detach themselves from social activities because they are repudiated by their own family. As stated,

> "I do not want to hang out with my friends anymore. I feel that they will just reject me like what my family did" (Participant 6, personal communication, January 24, 2020).

By and large, deteriorative behavior, a characteristic of the mental health of a select group of Filipino youth living with HIV, is exhibited by loss of interest, self- neglect, and social withdrawal, which were shaped by internal and external factors such as internalized stigma, denial, and discrimination.

4.0. Discussion

After carefully analyzing the themes, this study allowed the emergence of an interesting model that typifies the mental health condition of the participants. Labeled as the *Mental Health Tower of Youth Living with HIV* (Fig.1), this model conceptualizes instability that permeates the thinking, feeling, and doing aspects of a young person suffering from this dreaded condition. Similar to a tower, YLHIV operates in an environment where both internal and external pressures make them entertain disruptive thoughts, depressive moods, and deteriorative behavior.

Figure 1. Mental Health Tower of Youth Living with HIV

The findings of the study indicated that YLHIV was bombarded with disruptive thoughts, depressive mood, and deteriorative behavior. This finding converged with the study of Jena (2014) in one wellness clinic in South Africa, indicating that adolescents living with HIV showed anxiety. They were anxious regarding death due to their illness and reported fear of rejection, stigma, and discrimination. Moreover, youth living with HIV were also bothered with suicidal thoughts such as thoughts of killing themselves and death wishes. According to Badiee et al. (2012), suicidal thoughts are common among people with HIV compared to the general population. Alarmingly, suicide rates have been reported at elevated levels in this population (Carrico, 2010).

The present study also revealed that young people infected by HIV faced emotional turmoil manifested by depressive symptoms such as distress, sadness, and hopelessness. This finds concurrence with the Joint United Nations Programme on HIV/AIDS (UNAIDS) (2018) that people with HIV have a higher risk of developing mental health conditions like depressive symptoms. The report supports the claim that medical conditions like HIV could be a major source of stress that negatively affects a person's mental health (US Department of Health and Human Services, 2020). Similarly, the study by Twesigye (2011) found that psychosocial issues experienced by PLHIV in Denmark include stress, frustration for those who could not live a normal life, and long-term sadness.

Besides disruptive thoughts and depressive mood, our study found that YLHIV displayed deteriorative behaviors that impair their physical and social well-being. This deteriorative behavior includes loss of interest, self-neglect, and social withdrawal. The loss of interest of people living with HIV is manifested by their unwillingness to perform usual activities (Andersen et al., 2015), like going to work and performing school tasks. YLHIV also display behaviors that harm their physical health, such as smoking cigarettes, drinking alcohol, and using marijuana (Brown & Morgan, 2013). These behaviors are coping mechanisms of YLHIV in dealing with psychological distress brought by their illness (Duko et al., 2019). Moreover, the deteriorative behaviors of the participants are manifestations of avoidance coping mechanisms. This mechanism is employed by people who are in denial of their medical conditions, that instead of facing reality, they choose to deny it by engaging in various inappropriate behaviors.

5.0. Conclusion

Using the phenomenological design, this study attempted to describe the mental health of a select group of Filipino youth living with HIV. Notably, the study afforded the development of a model identified as the Mental Health Tower of Youth Living with HIV, which typifies the mental health struggles of this group. Characteristically, their mental health is defined by disruptive thoughts, depressive moods, and depressive behaviors, which operate on the thinking, feeling, and doing levels, respectively. Such conditions of the participants were triggered by personal (denial, internalized stigma, and non-disclosure) and environmental factors (lack of available information, discrimination, and lack of social support).

This study advances the current literature about the mental health struggles of Filipino YLHIV by crafting a model which can serve as an interesting platform for understanding the lived experiences of youth living with HIV. The model shows the mental health conditions of YLHIV that need to be addressed. Further, it invites the need to consider personal and environmental factors in developing a mental health program specifically designed for this population. As illustrated, the model has vividly described the instability of the YLHIV's mental health and the factors affecting such conditions, which can serve as valuable inputs for policy-making bodies, government and non-government organizations, and support groups to consider mental health in their respective programs for YLHIV. Particularly, early assessment of the mental health status of YLHIV should be considered alongside the development and provision of a mental health program for this group.

6.0. Declaration of Conflicting Interest

The authors have no conflict of interest to declare.

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Regulation of the immune system by administering lactic acid bacteria to suppress the effects of aflatoxin B1 in mice (*Mus musculus*)

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ABSTRACT

Aflatoxin B1 (AFB1), which is a toxic compound produced by the filamentous fungus *Aspergillus* sp., is highly carcinogenic, damages vital organs, and may cause death. Prevention of aflatoxin poisoning through proper food storage and physical treatment is an added cost, thus there is a need to identify alternative methods including treatment with probiotic bacteria. We evaluated the effect of *Lactobacillus bulgaricus* on activating immune cells in mice exposed to Aflatoxin B1. The study used a post-test control design consisting of five treatment groups including a negative control, positive control, and T1, T2, and T3 groups treated with lactic acid bacteria at doses of 105 colony forming unit (CFU)/ml, 107 CFU/ml, and 109 CFU/ml, respectively, administered on days 7–28 and AFB1 at a dose of 0.2 mg/kg bw orally on days 15–28. The relative number of lymphocytes consisting of CD11c+transforming growth factor-beta (TGF-β)+, CD4+CD8+, and B220+IgG+, was measured using flow cytometry. The data were analyzed using a one-way analysis of variance test. The results indicated that *L. bulgaricus* bacteria increased the relative number of CD11c+TGF-β+, B220+IgG+, and CD4+CD8+ cells in mice exposed to the mycotoxin. *Lactobacillus bulgaricus* may function as an immunostimulator against mycotoxins by inducing the humoral and cellular immune response.

INTRODUCTION

Mycotoxins, such as aflatoxin B1 (AFB1), are secondary metabolites produced from filamentous fungi that are toxic, carcinogenic, and immunosuppressive to animals and humans. The estimated world production of feed contaminated with fungus is approximately 5%–10% (Oswald *et al*., 2005; Qian *et al*., 2012; Tomkova *et al*., 2001). Aflatoxin reportedly caused up to 100,000 turkey deaths following the consumption of contaminated peanut mushrooms. Broiler feed contaminated with a mycotoxin mixture (3.5 mg/kg diet as 79% of AFB1, 16% AFG1, 4% AFG2, and 1% AFB2) can lead to weight loss and inflammation of the liver and kidneys. Aflatoxin B1 is a potent agent that causes immunosuppression in pigs at a dose of 140–280 µg/kg of feed by inhibiting DNA synthesis and immune cells, such as lymphocytes, but does not affect the humoral immune response (Perczak *et al*., 2018; Pierron *et al*., 2016).

Aflatoxin B1 suppresses the cellular immune system, in particular T lymphocytes, because of decreased complement production by the liver, phagocytosis by macrophages, and neutrophil activity (Perdigon *et al*., 2001). T lymphocytes affected by the toxin, as well as other lymphoid cells, such as cytotoxic T cells and natural killer cells, can promote tumor cell function directly or indirectly. The cellular components of the immune system produce cytokines for protection against tumor progression; however, cytokines may also play a role in the inflammation mechanism that causes damage to various organs (Ibrahim, 2013).

Methods such as heating, chemical treatment, or radiation can destroy and eliminate mycotoxin (Zain, 2011);

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however, the cost is prohibitive and it may impair the nutritional value of the feed. The decline in fungal growth may increase during feed production or storage (Munoz *et al*., 2010). Lactic acid bacteria (LAB) may exhibit antifungal activity, especially *Lactobacillus* sp (Sadiq *et al*., 2019). *Lactobacillus pentosus* and *Lactobacillus brevis* bacterial strains at a concentration of 3.5 × 108 colony forming unit (CFU)/ml can bind and release aflatoxin B1 by 17.4% and 34.7%, respectively, in liquid media as measured by ELISA (Hamidi *et al*., 2013).

Microorganisms, such as *Saccharomyces cerevisiae* and LAB, may be used as biopreservatives in feeds, so it is possible to extend the shelf-life and increase food safety with microflora supplementation. Antimicrobial products of microorganisms also have potential as probiotics and may improve health (Tran *et al*., 2020); however, there is limited data demonstrating the immunostimulating effects of LAB. Therefore, we evaluated the effect of LAB on the cellular and humoral immunity profile of mice.

MATERIAL AND METHODS

Preparation of animal

Male mice (*Mus musculus*), strain Balb/c aged 8–12 weeks (n = 25), with a body weight of 25–30 g, were obtained from Brawijaya University's bioscience laboratory and divided into five groups (five mice/group). The treatment groups consisted of a positive control (mice induced with AFB1 0.2 mg/kg bw on days 15–28), negative control (healthy mice), and treatment groups, T1, T2, and T3 in which mice were administered 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

Bacterial suspension preparation

Lactobacillus bulgaricus (LAB) was obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya, and confirmed by biochemical tests and Gram staining. The bacteria were grown on de Man, Rogosa, and Sharpe (MRS) agar media at 37°C for 24 hours. A bacterial suspension was prepared using MRS broth media and the bacterial concentrations were measured using a spectrophotometer. The bacteria were diluted with phosphate buffer saline (PBS) for the experiments.

Aflatoxin B1 preparation

Aflatoxin B1 (Sigma Company catalog: A6636®) is potent with respect to acute toxicity, mutagenicity, and carcinogenicity, and one vial of AFB1 contained 5 mg of powder. AFB1 (0.2 mg/kg BW) was diluted in 1 ml PBS, pH 7.2 (Qian *et al*., 2012).

Flow cytometry

The mice were sacrificed on day 29 by cervical dislocation. Spleens were harvested, placed into a petri dish containing sterile PBS, and crushed with the base of a syringe. 10 ml homogenates of the sample were prepared in a volume of 10 ml, centrifuged, and the pellets were resuspended in 1 ml PBS by pipetting (Ardiana and Rifa'i, 2015).

Then, 100 µl of the suspension was placed into a microtube; 500 µl of PBS was added; and the mixture was centrifuged at 2,500 rpm for 5 minutes at 4°C. Then, 50 µl of PE/Cy5 conjugated rat anti-mouse Cd11c, PECy5 conjugated rat anti-mouse transforming growth factor-beta (TGF-β), fluorescein isothiocyanate (FITC) conjugated rat anti-mouse CD4, PE conjugated rat anti-mouse CD8, FITC conjugated rat anti-mouse B220, and PECy5 conjugated rat anti-mouse IgG (Biolegend®, San Diego) were added to the cells and incubated for 20 minutes in 4°C (8). Afterward, 50 µl of cytofix (BD Biosciences Pharmingen) was added and incubated for 20 minutes in 4°C; wash perm solution (BioLegend®, USA) was added; and the mixture was centrifuged at 2,500 rpm at 10°C for 10 minutes. The cells were analyzed by flow cytometry (BD FACSCalibur, USA) using BD Cellquest ProTM software.

Statistical analysis

The data are presented as the relative number of immune cells (CD11c+TGFβ+, CD4+CD8+, and B220+IgG+). Data were analyzed statistically using one-way analysis of variance (ANOVA) with an error level of $\alpha = 0.05$, followed by Tukey's test.

RESULTS

$CD11c^+TGF\text{-}\beta^+$ cells

The results indicated that supplementation of the mice with LAB induced by AFB1 increased the relative number of CD11c+ cells that produce TGFβ+ (Fig. 1). In the positive control group, the relative number of CD11c+ expressing molecule TGF-β+ (1.75%) was different, but no significance was observed when compared with the negative control (0.91%). All treatment groups (T1, T2, and T3) administered LAB at a 105–109 CFU/ml concentration showed an increase in the relative number of CD11c+TGF-β+ cells by 2.05% 3.14%, and 3.06%, respectively, when compared to the negative and positive controls, as shown in Figure 1.

$CD4^+CD8^+$ cells

The results showed that the supplementation with LAB in mice induced with AFB1 increased the relative number of CD4+CD8+ cells in all treatment groups, but the amount did not significantly differ and was similar to that of the negative control, as shown in Figure 2. The negative control was higher when compared with the positive control.

$B220^+IgG^+$ cells

The results showed significant differences in the relative number of B220+-expressing IgG+ cells in all the treatment groups following supplementation with LAB after induction with AFB1. The negative control group (24.56%) was significantly different from the positive control group (5.76%), which showed a relatively higher number of B220+IgG+ cells compared with the positive control. There was an increase in the relative number in all treatment groups (7.44%, 10.26%, and 7.67% for T1, T2, and T3, respectively), as shown in Figure 3.

DISCUSSION

Effect of LAB on the relative number of $CD11c^+TGF\text{-}\beta^+$ cells

The results indicated that the relative number of CD11c+ cells that expressed TGF-β was different, but there was no significant between the treatment groups; however, a 107 CFU/ml concentration of LAB increased the average of CD11c+TGF-β+

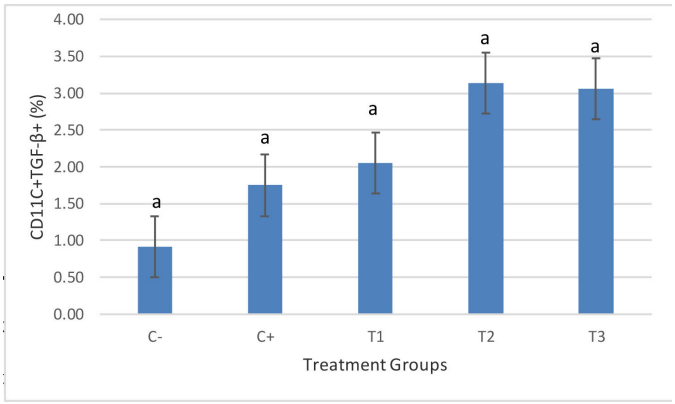

Figure 1. Flow cytometry analysis shows that giving LAB showed an increase in the relative number of CD11c+TGF-β+ cells ($p < 0.05$), but was not significantly different between treatments. There was an increase in the number when compared with the positive control. The highest average increase was seen in the T2 treatment. The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

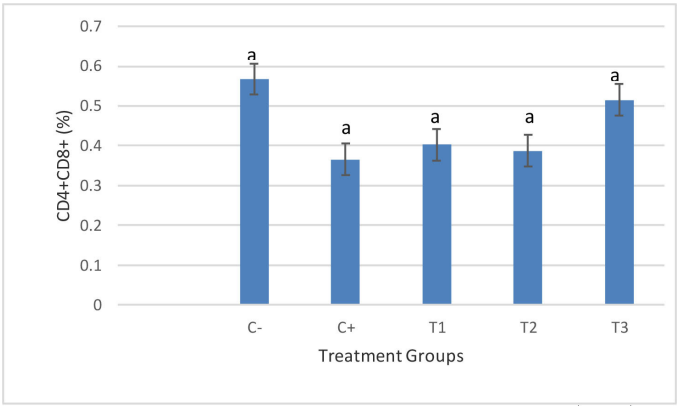

Figure 2. Treatment with LAB showed an increase in the relative number of CD4+CD8+ cells for all treatments, which did not differ significantly ($p < 0.05$). The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

cells in all treatments. Mycotoxin exposure decreased the relative amount of CD11c+TGF-β+ cells. Mycotoxin AF1 altered or decreased anti-inflammatory cytokine synthesis by inhibiting macrophage or T cell activation. In fact, mycotoxin inhibits the synthesis and proliferation of T cells, which prevents macrophage cells from producing anti-inflammatory cytokines. Mycotoxin is cytotoxic to lymphocytes by interfering with lymphocyte receptors or lymphocyte function (Tran *et al*., 2020). A study by Murugesan *et al*. (2015) revealed that mycotoxin was not immunogenic. It could not induce an immune response to pathogens, but interfered with mitogen-activated protein kinase signaling to modulate cell growth, apoptosis, or the immune response. This could expose an individual to a high risk of infection.

Dendritic cells (DC) and CD11c markers are antigen-presenting cells (APCs) that are regulated specifically and nonspecifically by immune cells found in the lamina propria of the small intestine and gut-associated lymphoid tissues, such as

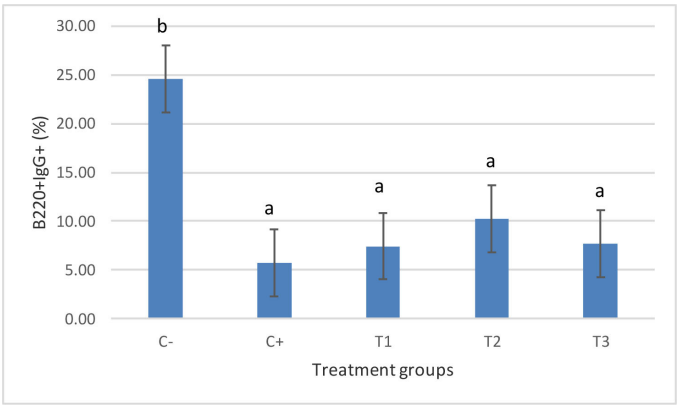

Figure 3. Treatment with LAB showed an increase in the relative number of CD4+CD8+ cells for all treatments, which did not differ significantly ($p < 0.05$). The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

the Peyer's patches. Most dendritic cells (DCs) are present in an immature condition and are less immunogenic because of the low expression of MHC costimulators. Contact with pathogen-associated molecular patterns (PAMPs) or other signals induces the pattern recognition receptor (PRR) signal and activates the NF-κB pathway, resulting in maturation and activation of DC cells (Wells, 2011). Mature DCs may then express high MHC levels, costimulatory molecules, and cytokines which attenuate APC activation and differentiation of T cells to cause inflammation (Mohamadzadeh *et al*., 2005). DCs exposed to AFB1 cannot respond correctly to any invading microbes and fails to initiate antigen presentation to activated T cells that are susceptible to pathogenic agents (Mohammadi *et al*., 2014).

The anti-inflammatory cytokine, TGF-β, inhibits the proliferation of fibroblast epithelial cells, dendritic cells, and macrophages to produce inflammatory cytokines and controls cell growth through adhesion and extracellular matrix formation (Hussain *et al*., 2018). Exposure to mycotoxin could stimulate CD11c+ cells to activate inflammatory pathways that TGF-β may have suppressed. Oral exposure to LAB concentrations of 105 CFU/ml could activate inflammatory cytokines, such as TGF-β, in DCs, which may inhibit CD11c+ cell activity. This was evident in the T1 and T2 group, prior to an observed decrease in the T3 group. According to Vindirelo and Alberto (2015), the higher the concentration of cell bacteria, the greater the binding capacity of AFB1 in liquid media *in vitro*. The concentration of bacteria that can bind AFB1 was 1010 CFU/ml for *L. rhamnosus* GG, *L. casei Shirota*, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *Escherichia coli*. Probiotics have an immunomodulatory effect on the release of cytokines, interleukins, tumor necrosis factor, transforming growth factor, and chemokines from immune cells that play a role in the innate and adaptive immune systems. LAB may interact with enterocytes and DCs, Th1/Th2 cytokines, or T reg cells in the intestine to stimulate the adaptive immune response into a proinflammatory or anti-inflammatory action (Azad *et al*., 2018; Mohamadzadeh *et al*., 2005).

Mycotoxin AFB1 exerts toxicity because it is readily absorbed by the intestine and rapidly binds to serum protein. AFB1 is

genotoxic and immunogenic in animals (Zimmermann *et al*., 2014). Mycotoxin can activate the microbial intestine, and mycotoxin adsorption–desorption is highly dependent on the intestinal environment and digestive enzymes. The LAB, *L. rhamnosus* RC007, stimulates pH, salts, enzymes, and peristalsis at each stage of AFB1 absorption in the digestive tract. Saliva secretion results in low adsorption and high AFB1 reabsorption. Gastric fluids and intestinal fluids do not decrease the AFB1 adsorption of LAB, rather they stimulate higher AFB1 adsorption (Sadiq *et al*., 2019).

Metabolic LAB products inhibit aflatoxin biosynthesis. Heterofermented LAB, such as *L. bulgaricus*, produce a high level of acetic acid and propionic acid at acidic pH (Vinderola and Ritieni, 2015). The mechanism of action of LAB is to inactivate the fungal membrane and inhibit the absorption of amino acids and inactivated products from fungi, such as acetic acid (Perczak *et al*., 2018). Bacteria and yeasts may neutralize mycotoxins in the body by reshuffling, transforming, and breaking them down into nontoxic metabolic products or inactive forms (Murugesan *et al*., 2015). LAB binds mycotoxins to prevent further absorption by the intestine, which are then secreted with feces (Adilah *et al*., 2018). LAB walls contain peptidoglycans that could interact with mutagenic compounds, including mycotoxins, through binding to reduce stability and bioavailability, and stimulate the secretion of anti-inflammatory cytokines by macrophages (Niderkorn *et al*., 2009; Tabari *et al*., 2018). LAB's capacity to bind mycotoxins would be optimal when the bacteria cells die due to a change in the cell surface. Live LABs require a long time to release mycotoxin from the body (Perczak *et al*., 2018). Cell wall protein denaturation may function by creating a broader area to absorb mycotoxins (Tabari *et al*., 2018). The proteins in the ribosomes, nucleus, chromosomes, cytosol, and cellular cytoskeleton components support the forming of the cell wall of bacteria in the exponential growth phase.

On the contrary, binding between the cell wall of LAB with mycotoxin takes place at the beginning of the end of the bacterial growth cycle (Moller *et al*., 2021). AFB1 could bind to the cell wall β-d-glucan through hydrogen or van der Waals bonds. Absorption of AFB1 toxin depends on the availability of the number of binding sites on the surface of microbes, and the equilibrium constant [K (eq)], which could change as a result of genetic, physical, or chemical alterations (Sadiq *et al*., 2019).

LAB may act as an anti-inflammatory agent, resulting in the reduction of oxidative stress from AFB1 exposure (Abbes *et al*., 2016). Probiotics could also stimulate T cell subsets, humoral immune cells, epithelial-associated dendritic cells, and macrophages to increase anti-inflammatory cytokine products Braat *et al*., 2004. The entry of LABs into the body may increase the capacity and phagocytic receptors of leukocyte cells, especially complement receptor 3 (CR3), for bluffing respiratory bursts (Bravo *et al*., 2019).

Effect of LAB on the relative number of $CD4^+/CD8^+$ cells

Our results showed that the administration of LAB (*L. bulgaricus*) had an effect on the relative number of CD4+/CD8+ immunocompetent cells in mice induced with aflatoxin B1 in the treatment groups ($p > 0.05$) based on a Kruskal–Wallis test. The negative control group had a higher relative number of CD4+/CD8+ T cells than the positive group. This result is consistent with that of Qian *et al*. (2012), in which the administration of AFB1 to mice orally for 24 hours reduced CD3 T cells in the intestinal mucosa. AFB1 caused a decrease in the cellular immune response to the specific dose and duration treatments (Zimmermann *et al*., 2014). A low dose of mycotoxin could induce an inflammatory response if activated by enzymes, such as inflammatory inhibitors (Hussain *et al*., 2018). Mycotoxin AFB1 may undergo systemic hydrolysis and further activate metabolism. Phase metabolism includes conjugation with glucuronic acid and sulfate by the whole-cell biotransformation system during immune cell communication (Tran *et al*., 2020).

High LAB concentrations could increase naive T lymphocyte activation and proliferation as well as memory T cells (Wells *et al*., 2011). Upon entering the body, an antigen is presented by DCs in the digestive tract mucosa (CD11c). Histocompatibility complex class II molecules combined with the expression of costimulatory molecules and cytokines (Gaudinoand Kumar, 2019) and activation of T lymphocytes. As a cellular defense, T cells may differentiate into a subset of T1 that activates macrophages. Macrophages and APCs induce T cells to secrete inflammatory cytokines that cause tissue damage (Mohamadzadeh *et al*., 2005). The T1, T2, and T3 groups had a higher relative amount of CD4+/CD8+ T cells compared with the positive control group is shown in Figure 2.

LAB may act as an antifungal agent because it contains metabolites, such as organic acids, carboxylic acids, phenolic acids, cyclic dipeptides, hydrogen peroxide, and compounds that inhibit sporulation, which may decrease mycotoxin production (Sadiq *et al*., 2019). LAB at concentrations of 105 CFU/ml could activate T lymphocyte cells in all groups compared with the positive control group. These results were consistent with that reported by Tsai *et al*. (2012) in which LAB activates the cellular adaptive immune response.

THE EFFECT OF LAB ON THE RELATIVE NUMBER OF $B220^+IGG^+$ CELLS

Our results showed that $B220^+$ cells expressing IgG in the negative control group was higher compared with the positive group, whereas it was not significantly different among all of the treatment groups. The average of B220 cells expressing IgG increased after LAB administration. Mycotoxin can reduce immunoglobulin production in chicken feed supplemented with AFB1 (Nazarizadeh and Pourreza, 2019). LAB stimulates the humoral immune response by increased circulating antibodies and levels of plaque-forming cells in the host when exposed to mycotoxin (Abbes *et al*., 2015). LAB can absorb and eliminate mycotoxin to prevent intestinal absorption and reduce liver damage, which is a target of mycotoxin. Upon entering the host orally, mycotoxin stimulates the secretion of immunoglobulin A (IgA) in the digestive mucosa and antibody IgG in the circulation. IgG represents a secondary response to protect the body against foreign antigens (Chen and Tsai, 2011).

In the normal intestinal epithelium, microflora bacteria act as a barrier antigen. However, when epithelial cells are degraded by infectious and noninfectious substances, alterations in intestinal permeability and inflammation of the intestinal mucosa occur. The inflammatory response that occurs in the intestine activates IgG antibodies and causes translocation of the microflora bacteria (Paludan *et al*., 2020).

LAB plays a role in the body to activate the immune response by inducing the formation of Secretory IgA (SIgA) and producing vitamins (Wold, 2001). Antibody SIgA is dominant in the

mucous membrane, which is the first defense immune system against a dangerous environment. SIgA antibodies play a role in neutralizing toxins, viruses, salivary exotoxins, and eliminating pathogenic microbes (Hayati *et al*., 2018). *Lactobacillus* bacteria and other probiotics are commensal microorganisms that interact with the mucosa or the immune cells. LAB stimulates specific functions of the mucosal immune system and produces secretory IgA. The presence of receptors, such as Toll-like receptors, nucleotide oligomerization domain-like receptors, and C-type lectin receptors may stimulate *Lactobacillus*. *Lactobacillus* associates with microbe-associated molecular patterns to activate APC and modulate their function through the expression of surface receptors, secretion of cytokines and chemokines, and other nonspecific immune effector cells (Mohamadzadeh *et al*., 2005). Our results indicate that there was an increase in the relative number of IgG+ cells resulting from LAB induction in mice exposed to mycotoxin. This is consistent with the results obtained by Tran *et al*. (2020) in which IgG levels in the serum of Balb/c mice increased when *Lactobacillus* was administered for 7 days (infected with *Salmonella typhimurium* bacteria). DCs play a role in the adaptive immune response. LAB stimulates DC cells to activate specific immune responses in the intestinal mucosa to maintain homeostasis, protect against pathogenic microbes, and maintain intestinal permeability (Mohamadzadeh *et al*., 2005). LAB increases the cellular and nonspecific humoral immune response in mice exposed to mycotoxin AFB1.

CONCLUSION

L. bulgaricus bacteria exhibited a potent effect as an immunostimulator resulting from exposure to mycotoxin AFB1.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee (certified no. 012-KEP-UB-2020) of Institut BioSains.

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COMPETING INTERESTS

The authors declare that there are no financial and nonfinancial conflicts of interest.

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DATA AVAILABILITY

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AUTHORS' CONTRIBUTIONS

DQS obtained the funding; designed the study, analysis and interpretation of data; and was a major contributor in writing the manuscript. DQS, SM, and IAA analyzed the flow cytometer data, treated the animals, and collected the data. All authors read and approved the final manuscript.

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YOGURT FORTIFIED WITH PURPLE ROSELLE EXTRACT AS PREVENTION OF DIOXIN INTOXICATION BASED ON MALONDIALDEHYDE LEVELS AND HISTOPATHOLOGY OF RATS (*RATTUS NORVEGICUS*) KIDNEY

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ABSTRACT

Dioxin is one of the chemical compounds produced through the chemical industry's combustion process, can pollute the environment,and harms the health of living things. Exposure to dioxin that accumulates in the body can disrupt normal organ function, including the kidneys. This study aimed to determine the preventive effect of giving yogurt fortified with purple roselle extract on the conditions of dioxin intoxication based on the parameters of Malondialdehyde MDA levels and kidney histopathology. This study used 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has the highest toxic potential among other dioxins congeners. TCDD was diluted in corn oil. This study used white male rats (*Rattus norvegicus*), 30 animals were divided into 5 treatment groups (1) negative control without treatment, (2) positive control was given TCDD dose of 200 ng/kg b.wt./day, (3) P1 given TCDD 200 ng/kg b.wt./day and roselle yogurt concentration of 0.5% 1 ml, (4) P2 given TCDD 200 ng/kg b.wt./day and roselle yogurt 1% concentration 1 ml, (5) P3 were given TCDD 200 ng/kg b.wt./day and roselle yogurt with a concentration of 1.5% 1 ml. The treatment was given for 12 days, orally. The parameters gathered were MDA levels and kidney histopathological changes. MDA levels were analyzed quantitatively using ANOVA continued with the Tukey test for comparison of treatment (α = 5%). Histopathological changes were analyzed descriptively. This study concludes that yogurt fortification with purple rosella extract with a concentration of 0.5%, 1.0%, and 1.5% as prevention on dioxin intoxication has not been able to decrease MDA levels of rat kidney and to prevent the histopathological damage of kidney rats.

Key words: Dioxin. MDA, kidney, purple roselle extract, yogurt

Introduction

Many activities caused environmental pollution. One of them is the combustion process of industrial waste and burning products containing chlorine. The combustion of waste has a negative effect because the compound created has a prolonged degradation rate, both in air, soil, and water. These waste compounds has long-term and short-term impacts on human, animal, and ecological health. According to Fiedler (2003) these dangerous industrial wastes are known as dioxins.

The World Health Organization data in 2012 states that dioxin levels are mostly found in eggs, poultry, milk, and fish. When dioxins enter the human or animal body, it will cause various disorders, including immune, nervous, endocrine, reproductive, and liver function changes. According to Indraningsih and Sani (2014) Trichloro dibenzo-p-dioxins (TCDDs) residues were found at a high level on beef meat in some areas in Indonesia. According to Susanti (2004), TCDD will accumulate in the tissues and have toxic effects that only appear after several years, starting with the binding of dioxins-AhR (Aryl Hydrocarbons Receptor), the chaperones dissociate, resulting in AhR translocating into the nucleus and dimerizing with ARNT (AhR nuclear translocator), leading to changes in gene transcription. Then inducing the expression of cytochrome enzymes P450 CYP-A1 and CYP-B1. These enzymes contribute to cellular reactive oxygen species (ROS). ROS formation contributes to cell damage and disease development. Exposure to dioxins shows an increase of ROS indicator in the organs of animal models, including the kidney (Sakin *et al*., 2011). To prevent the overproduction of ROS, the body requires antioxidants.

Yogurt is a fermented milk product with high antioxidant content, involving microorganisms, namely, *Lactobacillus bulgaricus* and *Streptococcus thermopillus* bacteria. Antioxidants are available from natural ingredients, especially in spices, fruits, and vegetables. A study conducted by Suharto *et al*. (2016) found that the antioxidant activity of natural ingredients is found in plant extraction. One of the plants known to have high antioxidant activity is the purple roselle. Purple roselle (*Hibiscus sabdariffa L*) has the main component of anthocyanin pigments that form flavonoids as antioxidants (Nugroho *et al*., 2018). According to Arviani *et al.* (2018). The levels of vitamin C in purple rosella were 241 mg/100 g higher than oranges (49 mg/100 g), star fruit (35 mg/100) and papaya (78 mg/100 g).

Fortification of purple rosella extract in yogurt has the organoleptic preference due to color and taste (Noviatri *et al*., 2020), and is expected to have a preventive effect of reducing the number of free radicals due to exposure to dioxins. Hence, this study was conducted to determine whether fortified yogurt with purple roselle extract could increase antioxidant activity against dioxin exposure in terms of MDA levels and kidney histopathology.

Materials and Methods

Chemicals

Dioxin compound used was TCDD (Supelco 45899), cow's milk yogurt, purple roselle extract, yogurt starter

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(Yogourmet® LYO-SAN.INC) contains *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus*, 10% phosphate buffered saline (PBS), paraffin block, corn oil, physiological NaCl, 2-Thiobarbituric acid (TBA), formaldehyde 10%, aquadest, and hematoxylin and eosin (HE) stain.

Yogurt fortification purple roselle extract

Dried roselle petals were ground until smooth until they became flour, then sieved using a sieve. The roselle powder was dissolved in warm water (ratio 20 g: 100 ml water). After that, the pasteurization was carried out at 63ºC - 65ºC for 30 minutes. After 30 minutes, the liquid and sediment were separated (Suharto *et al.,* 2016).

Yogurt starter was made by pasteurized 100 ml of cow's milk at a temperature of 72ºC for 15 minutes. Then, the cow's milk was cooled down to a temperature of 45ºC. After that, the yogurt starter was inoculated and homogenized. Then, it was incubated at 45ºC for 4 hours until it reached a pH of 4.4-4.5 (Mahdi *et al.,* 2016). The starter was inoculated as much as 3% of the volume of cow's milk to make cow's milk yogurt (F1) (Mahdi *et al.,* 2016). Plain yogurt homogenized using a blender and divided into 3 parts. Then the purple roselle extract was added with a concentration of 0.5%, 1%, and 1.5%. Analysis of nutrition value of yogurt fortified with purple roselle extract was done using proximate assay and antioxidant activity analyzed by DPPH modified Pinela *et al.* (2012) method.

Animals and experimental design

The study was approved by the University Institutional Animal Care and Use Committee. The animals used were rats (*Rattus norvegicus*) divided into five groups, with six (6) rats per group. Feed, and drinking water was given *ad libitum* for 7 days. Rats were housed under standard laboratory conditions (room temperature 25°C - 26°C, 12 h light-dark cycle). 2,3,7,8-Tetrachlorinedibenzo-p-dioxin (TCDD) diluted with corn oil, gave orally using a gavage every day for 12 days.

Experimental groups were: Negative control group (NC) received normal food and water; Positive control group (PC) given a dose of 200 ng/kg b.wt./day TCDD; Yogurt of cow's milk with purple rosella extract with each concentration (0.5%, 1%, 1.5%) was given to each treatment (P1, P2, and P3). Group P1 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 0.5% roselle yogurt; Group P2 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 1% roselle yogurt; and Group P3 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 1.5% of roselle yogurt.

Sample collection and analyses

The experimental rats (*Rattus norvegicus*) were euthanized at the end of the experiment. The abdominal cavity was incised, and kidneys were extracted immediately. The left kidney was immersed in a 10% formaldehyde solution to be used for making histopathological preparations. Then, the right kidney was wrapped in aluminum foil and stored at -20° for biochemical analyses.

Determination of (*malondealdehyde*) MDA levels using the Thiobarbituric acid (TBA) method. The kidney sample was weighed as much as 0.5 grams, crushed with a mortar, then added 200 µl of physiological NaCl. The homogeneous sample was put into a polypropylene tube and added with distilled water. Then added 100 µl of homogenized TBA, added 250 µl of 1N HCl, and homogenized again. The homogeneous mixture was added with Na-Thio 1% as much as 100 µL and centrifuged at 500 rpm for about 10 minutes. The supernatant was taken then heated in a water bath of 100°C for 20 minutes. The supernatant was then cooled at room temperature, and the absorbance value of the sample was determined using a UV-Vis spectrophotometer at its maximum wavelength (Widyaningsih *et al*., 2015).

Kidney samples were immersed into xylol concentration levels 1-3 each for 5 minutes. The dehydration was carried out by inserting the sample into stratified ethanol starting from 1-3 absolute ethanol, 95%, 90%, 80%, and 70% ethanol for 5 minutes each. The preparations were then immersed in distilled water for 5 minutes. After that, put in a hematoxylin dye for 10 minutes. The preparations were washed in running water and soaked in distilled water again to remove excess eosin. After that, the dehydration process, the preparations were put into the ethanol series, graded from 80%, 90%, and 95% to absolute ethanol 1-3. The last process, namely clearing by inserting the preparations into xylol 1 and 2, then drying. The mounting process was carried out using Etellan®. After the mounting process, the prepared slides were observed and examined under the light microscope equipped with a digital camera and the images were processed using a software and each sample was observed in five fields view. Observations were made to determine epithelial necrosis, glomerular and tubular damage.

Data analysis

Statistical analyses using One Way Analysis of Variance (ANOVA) continued with Honest Real Difference (HSD) Tukey's test (α = 5%). The results of the observation of the histopathological preparations of the kidney were analyzed descriptively.

Results and Discussion

Table 1 shows the result on yogurt fortified with purple roselle extract nutritional and antioxidant activity analysis. Antioxidant activity assay for yogurt fortified with purple roselle extract IC_{50} result was increasing as the concentration of the purple roselle extract increase.

Table 1:Analysis of yogurt fortified with different concentration of purple roselle extract

Sample	Test	0.5%	1.0%	1.5%
Yogurt fortified with purple roselle extract	Protein (%)	2.26	1.98	1.99
	Fat (%)	3.32	3.92	3.75
	Water (%)	89.21	89.17	89.25
	Ash (%)	0.75	0.74	0.74
	Carbohydrate (%)	4.46	4.19	4.27
	Antioxidant IC_{50} (mg/mL)	181.10	140.68	137.70

Table 2 shows the MDA level assay of all treatment groups. MDA level on all treatment groups showed a significant increase compared to negative control group.

Fig. 1 shows the kidney histopathological changes on each group with HE staining under light microscope with 400x magnification. The histopathology of rat kidney showed differences in each treatment group according to the changes in glomerulus, Bowman's space, and kidney tubular.

Table 2: MDA level of rat's kidney of all groups in the study

Groups	Kidney MDA level (ng/mL) Mean±SD
NC	180.46±245.91[a]
PC	414.22±13.46[b]
P1	425.50±32.60[b]
P2	444.11±2.35[b]
P3	418,02±10.91[b]

Differences in superscript notation indicate a significant difference between groups at P<0.05

Qualitative analyses of damaged kidney cells were carried out by determining a score based on the percentage of damage, and the number of cell damage, according to Windahartono *et al.* (2013). Table 3 shows the histo-pathological observations of rat kidney.

Table 3: Histopathological findings observed in the kidney tissues of the rats according to the experimental groups

Group	Necrosis	Inflammatory cells	Haemorrhage
NC (A)	-	++	-
PC (B)	+++	+++	+++
P1 (C)	+++	++	++
P2 (D)	+++	++	++
P3 (E)	++	+++	+++

(-) none, (+)1%-2.99% ,(++)3%-4.99%, (+++)5%-6.99%

According to Khairan (2010), biochemical processes in the body, such as inhalation of oxygen, aerobic metabolic processes, and excessive food processing will produce low amounts of free radicals, one of the products of free radicals produced, called malondildehyde. MDA

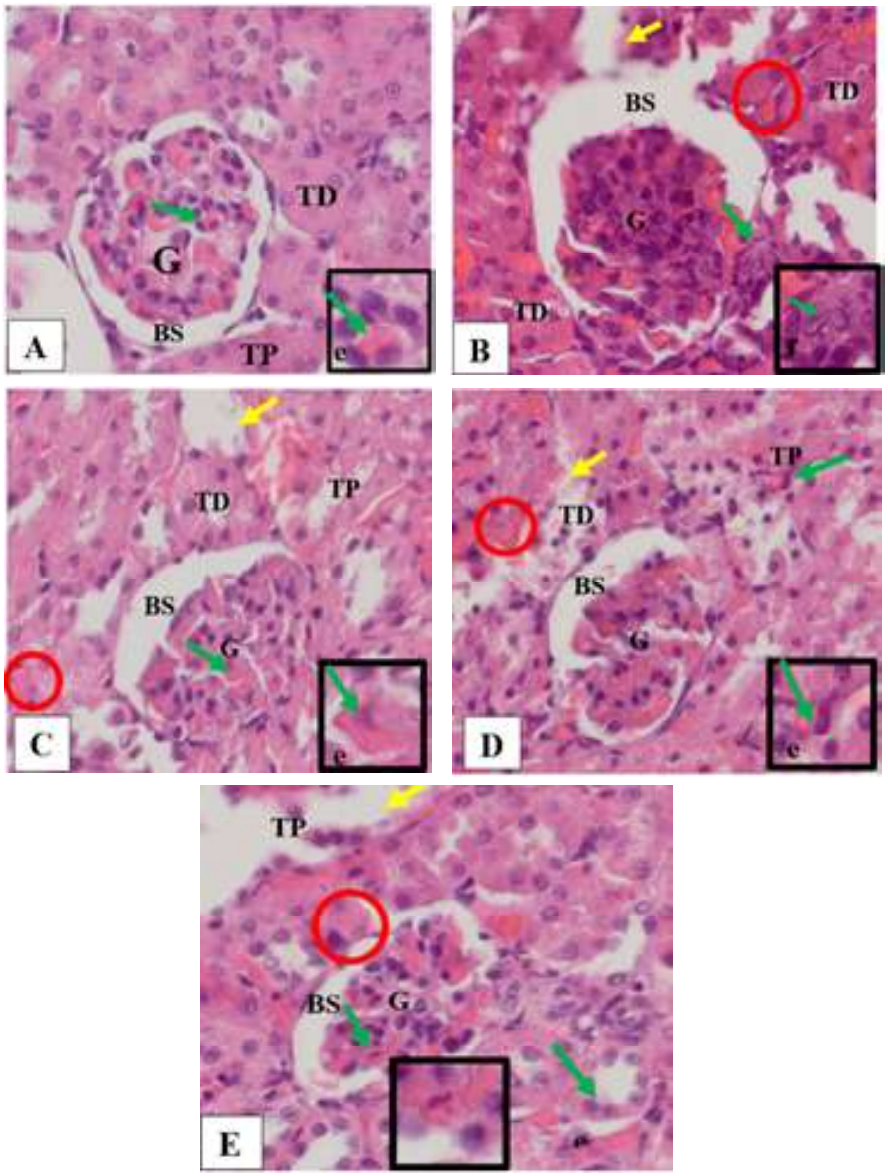

Fig. 1: Rat's kidney histopatological appearance. Group NC (A), Group PC (B), Group P1 (C), Group P2 (D), Group P3 (E): HE staining; Magnification 400x and 1000x (e).G (glomerulus); BS (bowman's space); TD (distal tubule); TP (Proximal tubule); (green arrow) = erythrocytes; (yellow arrow) = necrotic cells, e : Neutrophils, f : lymphocytes; red circle = haemorhage.

levels in the PC group exposed to TCDD at a dose of 200 ng/ kg b.wt., showed a significant increase ($P<0.05$) compared with the NC group. This proves that TCDD exposure has an effect on the kidneys, which can cause oxidative stress. TCDD has lipophilic properties; when it enters the body, it easily accumulates in the adipose tissue, making it difficult to remove. The elimination time of TCDD in mice lasts 12-31 days and in humans lasts for years, so that with these properties, TCDD will continue to be in the kidney adipose tissue for a long time and will continue to initiate oxidative stress (Rozewicz, 2016).

Based on the average MDA level, there was no preventive effect of fortified yogurt with purple roselle extract on the damage caused by dioxin intoxication in rat's kidney.These results can be caused by several reasons, including the concentration factor of purple roselle extract fortification, which at the given concentration did not have an effect on preventing the increase in MDA levels of the kidney due to dioxin intoxication. The analyses on the antioxidant activity test of yogurt fortified a concentration of 0.5%, 1.0%, and 1.5% purple roselle extract had a relatively low value of antioxidant levels (Table 1). Based on the antioxidant activity value of IC_{50} according to Alfian and Susanti (2012), the IC_{50} value is said to be very strong if it is less than 0.01 mg/mL, strong 0.01-0.05 mg/mL, moderate at 0.05-0.1 mg/mL, and weak if the value is more than 0.01 mg/ mL. For this reason, a higher concentration of purple roselle extract is needed in order to have an efficient effect on preventing increased levels of MDA in rat kidneys.

Pathological changes in the kidney tissues of the positive control group (PC) given with TCDD at a dose of 200 ng/kg b.wt., had a visible difference compared with the negative control (NC). The PC group showed necrosis that was present in almost all tubules, there are several inflammatory cells (neutrophils and lymphocytes), the size of the glomerulus is reduced, and the bowman space looks very wide. Reyner *et al*. (2016) explained that the blood flow to the kidneys is very large, and the kidneys also function in the excretion of metabolic products and foreign chemicals, so it is very risky to carry toxic materials and will cause damage to kidney tissue, especially in structural changes, kidney structure, and function.

Glomerular atrophy caused by TCDD deposited in the adipose tissue will indirectly cause the kidney to experience a gradual injury over a long period of time, then haemodynamic changes will occur and will trigger atrophy in the glomerulus, the space between the glomerulus and bowman's capsule will be filled with a lot of sediment causing the glomerulus is compressed and shrinks so that Bowman's space is widened. In the kidneys, haemorrhage is also found. Haemorrhage occurs due to TCDD, which is a toxic substance that damages blood vessels, so that blood and blood components go out into the tissue, characterized by finding red spots on the tissue. When there is hemorrhage in the tissue, the cells do not get blood intake. The cells will experience swelling, after that there is denaturation of proteins by the cells, and the cells experience death, characterized by loss of cell nuclei and chromatin image, the nucleus looks denser, becomes crooked, more color dark (pycnosis), divided into torn fragments (karyorrhexis), and pale (karyolysis). This event will increase the load when there is the filtration of the glomerulus, and inflammation occurs. The inflammation that occurs will trigger the release of inflammatory cells. According to Ulilalbab *et al*. (2018), giving antioxidants, especially orally for 36 days, can play a role in neutralizing or protecting the effects of compounds due to free radicals. Meanwhile, regenerating the renal epithelium, especially the tubules, needs approximately 1 month or 28 days to repair themselves (Yokote *et al*., 2012).

Recent study concludes that, based on the MDA level and the histopathological features of kidney tissues in rats TCDD exposure can increase the MDA level and histopathological changes in all groups exposed to TCDD. The prevention using yogurt fortified with purple roselle extract concentrates at 0.5%, 1.0%, and 1.5% has no effect on preventing kidney damage due to dioxin intoxication. These results suggest that an increase in concentration on purple roselle extract might increase the antioxidant properties and the protective effect.

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