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PREFACE

In this edition, Volume 29 No 4, we proudly present articles from animal and veterinary sciences including genetic, reproduction; animal physiology; and veterinary from scientist all over the world. The articles published in this edition are:
"The Follicular Distribution during the Estrus Phase and the Enhanced Estrus Behavior of Crossed Ongole Heifers Stimulated with a Non-superovulation Dose of PMSG"; "Characterization of Protein Degradation in Tropical Dairy Feedstuff Using the *In Sacco* Method"; "Effect of Total Mixed Ration Feeding System on Dry Matter Intake, Nutrient Intake, and Onset of Estrus in Growing Dairy Cattle"; "Emerging Challenges: Methicillin and Vancomycin Resistance in *Staphylococcus aureus* from Urinary Tract Infections in Ewes of Diyala Governorate, Iraq"; "Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]"; "Growth Response and Carcass Yield of Male Japanese Quail Fed Diets Contained Fermented Rubber (*Hevea brasiliensis*) Seed Meal"; "Supplementation of Selenium-enriched Black Soldier Fly (*Hermetia illucens*) Larvae Meal on Growth Performance, Blood Parameters, and Immune Function in Broiler Ducks"; and "Qualitative Traits of Local Bambu Apus Rabbits".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, December 2024

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LIST OF CONTENT

The Follicular Distribution during the Estrus Phase and the Enhanced Estrus Behavior of Crossed Ongole Heifers Stimulated with a Non-superovulation Dose of PMSG

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ABSTRAK

Putro KB, Amrozi, Winarto A, Boediono A, Manalu W. 2024. Distribusi folikel pada fase estrus dan peningkatan performa estrus sapi PO dara yang distimulasi dengan PMSG dosis non-superovulasi. JITV 29(4):172-180. DOI:http://dx.doi.org/10.14334 /jitv.v29.i4.3160.

Penelitian ini dilakukan untuk mempelajari sebaran folikel pada fase estrus dan kaitannya dengan performa estrus dan perubahan biometrik organ reproduksi sapi Peranakan Ongole (PO) dara yang diinjeksi *Pregnant Mare Serum Gonadotrophin* (PMSG) dengan dosis non-superovulasi (NSO). Sembilan ekor sapi PO dara dibagi ke dalam tiga kelompok secara acak berdasarkan dosis PMSG 0 (plasebo), 0.5, dan 1.0 IU/Kg BB yang diinjeksi pada awal gelombang folikel kedua. Dosis PMSG 0.5 dan 1.0 IU/Kg BB terbukti mampu meningkatkan rerata diameter folikel besar (> 0.8 cm) sebesar 7.08 % ($P>0.05$) and 15.04 % ($P<0.05$), akan tetapi total jumlah folikel besar pada dosis 1.0 IU/Kg BB masih tinggi (5.33±2.52, $P<0.05$) sehingga berpotesi menginduksi *multiple calving*. Skor performa estrus terbukti meningkat seiring dengan peningkatan dosis PMSG yang diikuti dengan perubahan biometrik organ reproduksi, khususnya ovarium, uterus, dan serviks ($P<0.05$). Hasil penelitian menunjukkan bahwa pemberian PMSG dengan dosis NSO mampu meningkatkan pertumbuhan dan perkembangan folikel bersamaan dengan peningkatan kualitas estrus tanpa meningkatkan risiko *multiple calving*.

Kata Kunci: Distribusi Folikel, Sapi PO Dara, Ovarium, PMSG, Biometrik Traktus Reproduksi

ABSTRACT

Putro KB, Amrozi, Winarto A, Boediono A, Manalu W. 2024. The follicular distribution during the estrus phase and the enhanced estrus behavior of crossed Ongole heifers stimulated with a non-superovulation dose of PMSG. JITV 29(4):172-180. DOI:http://dx.doi.org/10.14334/jitv.v29.i4.3160.

The experiment's objective was to study the follicle distribution during the estrus phase and the estrus performance of the Ongole-crossed heifer by injecting a non-superovulation (NSO) dose of PMSG. Nine PO heifers were randomly assigned to one of three treatment groups, each receiving a different dose of PMSG injection, i.e., 0, 0.5, and 1.0 IU/kg BW, respectively; this was done at the early second follicular phase. The evaluation was conducted during the estrus phase to assess follicle distribution and estrus performance and to collect the reproductive tract for biometrics evaluation. The results demonstrated that PMSG injection at doses of 0.5 and 1.0 IU resulted in an increase in mean follicle diameter size above 0.8 cm by 7.08 % ($P>0.05$) and 15.04 % ($P<0.05$), respectively. However, the total number of follicles above 0.8 cm at 1.0 IU/kg BW dose remains high (5.33±2.52, $P<0.05$), indicating a continued propensity for multiple calving. The results demonstrated that the estrus performance score was enhanced with the administration of increasing doses of PMSG, which was accompanied by an increase in the biometrics of the ovaries ($P<0.05$) and the diameter of the uterine body, uterine horn, and cervix ($P<0.05$). It was concluded that the NSO dose of PMSG could facilitate follicle development and enhance estrus quality without increasing the risk of multiple calving.

Key Words: Follicle Distribution, Ongole-crossed Heifers, Ovary, PMSG, Reproductive-tract Biometrics

INTRODUCTION

The Ongole-crossed cattle is an endemic breed distributed throughout Indonesia, particularly prevalent in the Central Java and East Java provinces. Ongole-crossed cattle represent the results of a grading-up breeding process between Java and Sumba Ongole (SO) cattle (Astuti 2004). Although the Ongole-crossed cattle results from a grading-up breeding program, this breed frequently encounters difficulties in terms of

reproductive performance. Some reproductive performances categorized as low in this breed of cattle are anestrus, especially in heifers (Wahyunani 2014), calving rate, birth weight, weaning weight, and post-weaning growth rate of calves (Astuti 2004). These reproductive performances are markedly inferior to those observed in Bali cattle (Saili et al. 2011). The estrus cycle of heifers is often disrupted. Most heifers exhibit signs of anestrus during their initial and subsequent natural estrus cycles, with or without estrus synchronization. Ma'ruf et al. (2017) reported that more than 60% of Ongole-crossed heifers treated with medroxyprogesterone acetate (MPA) experienced anestrus. This problem frequently causes Ongole-crossed heifers to give birth at older ages, i.e., above 24 months (Astuti 2004).

The growth and development of follicles were stimulated by administering Pregnant Mare Serum Gonadotropin (PMSG) to enhance the livestock reproductive performance. Several studies have demonstrated that PMSG injection increases the endogenous secretion of estradiol in thin-tailed sheep (Sugiyatno et al. 2001) and has been shown to enhance the estrus performance of Boer goat (Salleh et al. 2021). In Chinese Holstein cows, estrus signs manifested in 70-80% of animals within 74-98 hours after administering a low dose of PMSG (Fu et al. 2013). Furthermore, gilts exhibited enhanced follicle development and estrus performance following PMSG injection during the luteal phase (Zeng et al. 2019), a phenomenon also observed in does (Kavitha et al. 2018).

The implementation of this methodology has the potential to enhance the reproductive and productive performance of mammalian animals markedly. Furthermore, offspring born to the mother stimulated by PMSG injection prior to mating exhibited elevated birth weights (Manalu et al. 1997; Manalu et al. 2000). The administration of PMSG to ewes prior to mating has been demonstrated to result in an average increase in weaning weights productivity in the small-scale farm (Manalu 2012). Furthermore, the efficacy of PMSG in augmenting endogenous secretion of pregnant hormones has been demonstrated in the sow (Rayer et al. 2015). This method also has been demonstrated to enhance lamb resilience to *H. contortus* (Arif et al. 2018).

The majority of experiments conducted on the enhancement of reproductive performance in Ongole-crossed cattle in Indonesia have been limited in their utilization of luteolytic agents and improved nutrition and have demonstrated a lack of comprehensive follicle distribution analysis during the estrus phase of monotocous ruminants. The administration of an NSO dose of PMSG at the second follicular wave in Ongole-crossed heifers represents a potential alternative method for enhancing the reproductive performance of Ongole-crossed cattle and mitigating the risk of multiple calving in monotocous animals. This experiment's objective was to study the follicle distribution during the estrus phase and the subsequent estrus performances, as well as biometric changes in the reproductive tracts of Ongole-crossed cattle in response to an NSO dose of PMSG.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, and reproductive tracts were collected at the local slaughterhouse. The Animal Ethics Committee, School of Veterinary Medicine and Biomedical Science, IPB University, approved all experiments (approval number 090a/KEH/SKE/XI/2017).

Preparation of heifers and experimental design

Nine Ongole-crossed heifers, aged between two and a half years and with a body condition score of 2.5 to 3.0, were selected based on the aforementioned criteria. They were observed to have regular estrus cycles based on the observation using real-time ultrasonography (USG, Aloka model SSD 500, linear probe 7.5 MHz, Aloka Co. LTD, Tokyo, Japan). They were deemed to have satisfactory reproductive tract health. Over one month, the heifers were acclimatized to the experimental conditions by being fed a similar ration twice daily.

The experimental heifers were allocated to a completely randomized design comprising three treatments of dose of PMSG injection, namely 0.0, 0.5, and 1.0 IU/kg BW, each with three replications. The heifers were categorized into three groups, designated as D0.0, D0.5, and D1.0, respectively. Following the acclimatization period, heifers were subjected to an estrus synchronization procedure, whereby they were administered an injection of $PGF_{2\alpha}$ (Lutalyse, Zoetis, Dublin, Ireland) at a dosage of 25 mg/heifer in two separate intramuscular injections with an interval of 12 days between each injection. A PMSG injection (Folligon, MSD, Intervet BV, The Netherlands) was administered intramuscularly at the onset of the second follicular wave. The emergence of the second follicular wave was monitored via real-time ultrasound. The emergence of a cohort of small antral follicles (0.3-0.5 cm) was employed to indicate the advent of the follicular wave. Subsequently, the heifers were administered an injection of $PGF_{2\alpha}$ 48 hours later, which resulted in lysis of the existing corpus luteum (CL). This procedure ensured that the dynamics of the ovary continued uninterrupted until the estrus phase. The ultrasound examination continued until a dominant follicle and estrus mucus confirmed the appearance of estrus signs. All heifers that exhibited signs of estrus were humanely euthanized by slaughtering at a local abattoir, with the reproductive tract and the ovary collected for further analysis.

Follicle distribution

On the day that estrus was detected, an ultrasound scan (USG) was performed to evaluate the distribution of follicles in size across both ovaries. The number of follicles was counted and divided into three categories based on sizes: 0.20-0.59 cm (small), 0.60-0.80 cm (medium), and above 0.80 cm (large). Moreover, the mean diameter of the large follicles (above 0.80 cm) was determined to acquire information regarding ovarian dimensions and correlation with the propensity for an increased number of large follicle formations.

Observations of estrus's performances

The occurrence of estrus was determined through the observation and scoring of the estrus signs, employing the methodology described by Saili et al. (2011) and Abidin et al. (2012). The appearance of estrus was indicated by an increase in vaginal temperature, alterations in the vaginal coloration, and swelling of the vulva, which were classified as physical changes. Additionally, behavioral changes were observed, including mounting other cattle or remaining standing when mounted by the other cattle. The scoring was divided into three categories: score 1 was assigned when the physical change was incomplete, whether or not estrus mucus; score 2 was assigned when the physical change was complete, and estrus mucus was present; and score 3 was assigned when the physical change was complete, and estrus mucus was present, along with behavioral changes. Additionally, vaginal temperature was monitored as a further indicator, given its importance as a sign of estrus (Higaki et al. 2019). Vaginal temperatures of heifers were recorded by using a digital thermometer (GEA®) on the same day as the injection of PGF2α and monitored until the appearance of estrus.

Collection of the ovary and uterus

The reproductive tracts collected were of the following types: vulva-vagina, cervix, uterine body, uterine horn, and ovary. Macro-anatomical parameters were measured, including length, diameter, and thickness of the wall of the uterine horn, uterine body, and cervix. The ovary was measured for length, width, and thickness. The number of follicles on the surface of the ovary was counted.

The length of the uterine horn was determined by utilizing a stainless-steel pipe strap, measuring from the bifurcation's base to the uterine horn's extremity. Moreover, the length of the uterine horn was determined by measuring from the base of bifurcation up to the orifice of the cervix, which is in contact with the uterine horn, using a caliper. The length of the cervix was measured from the orifice of the cervix, which is the point of junction with the vagina, to the point of junction with the uterine horn. The whole diameter was also measured. The ovary was separated from the oviduct, and then the dimension of the ovary was measured.

Data analysis

The estrus performances and vaginal temperature were presented descriptively. The data about biometric observation of reproductive tracts and the ovary were subjected to the variance (ANOVA) test, with the results processed using the SPSS 16.0 software. The Duncan test was employed for further analysis when a statistically significant effect was observed.

RESULTS AND DISCUSSION

Follicle distribution

The experiment's results demonstrated a statistically significant difference in the distribution of follicles and the mean diameter of large follicles (above 0.8 cm), as illustrated in Table 1. The highest number of large follicles was found in D1.0 ($P<0.05$), while other groups showed the same results (2 ± 0, $P>0.05$). These findings demonstrate that the highest PMSG dose in this experiment still had a propensity for multiple dominant follicle formation, thus increasing the risk of multiple calving. Moreover, the mean diameter of follicles exceeding 0.8 cm exhibited a notable elevation at the D1.0 dosage, whereas the D0.5 dosage did not reach statistical significance ($P>0.05$) despite an observed diameter increase up to 7.08%.

The experiment results demonstrated a correlation between the elevated NSO doses of PMSG administrations, the augmented prevalence of large follicles, and the dimensions of the ovaries. Pregnant Mare Serum Gonadotropin (PMSG) functions similarly to follicle-stimulating hormone (FSH), which promotes the growth of small follicles, and luteinizing hormone (LH), which induces ovulation in the dominant follicle, particularly in pre-ovulatory follicles (Fu et al. 2013; Depison et al. 2011). The injection of heifers with PMSG has been observed to increase the number of small follicles selected to become dominant, even at the lower dose (Fu et al. 2013). This increased the number and diameter of the pre-ovulatory dominant follicles. Moreover, the increase in the number and diameter of the pre-ovulatory dominant follicle resulted in an ovary enlargement during the estrus phase. Several studies have demonstrated that PMSG treatment in conjunction with estrus synchronization protocols has a considerable effect on the growth and size of the pre-ovulatory follicle

Table 1. Number of follicles based on the size at the estrus phase in experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Sizes of the follicles (cm)	Doses of PMSG injection (IU/kg BW)		
	0	0.5	1.0
0.20-0.59	35.00±15.58[a]	62.33±2.63[b]	34.33±6.65[a]
0.60 – 0.80	0±0[a]	0.33±0.47[a]	0.67±0.94[b]
Above 0.80	2±0[a]	2±0[a]	5.33±2.52[b]
The mean diameter of the follicle above 0.8 cm	1.13±0.20[a]	1.21±0.20[a]	1.30±0.48[b]

Different superscripts in the same row mean a significant difference ($P<0.05$)

in various mammals, including buffaloes (Jerome et al. 2016; Terzano et al. 2013), cows (Elmetwally 2021), and goats (Kavitha et al. 2018; Hameed et al. 2020). The results of the present experiment demonstrate that the presence of follicles, particularly large follicles, significantly influences the dimension of the ovary. The highest number of large follicles was observed in D1.0 heifers, significantly impacting the ovary's weight, length, width, and thickness.

Furthermore, the most notable alterations in ovarian dimensions, particularly in weight, were observed in the right ovary. This finding indicates that most follicle growth and development in the present experiment occurs in the right ovary. It has been demonstrated in several studies that the right ovary is more active than the left ovary in both heifers and cows (Pierson et al. 1987; Lopez-Gatius 1997). In heifers, the ovulation of the dominant follicle in the right ovary was observed to occur in 54.1% of cases during the first estrus and 59.6% during the second estrus. Heifers' ovulation in the right ovary is 56.5% of heifer cases (Geres et al. 2011). These findings were evaluated in the recent report through a retrospective study in calves, which demonstrated that the right ovary has a higher propensity for a more significant number of antral follicles, larger follicle diameter, greater intrafollicular fluid volume, and a larger follicle surface (Dangdubiyyan & Ginther 2019). The present study demonstrated that the dominant follicles are predominantly formed in the right ovary, as evidenced by a significantly higher weight in the heifers treated with 0.5 and 1.0 IU of PMSG/kg BW.

Estrus performances

The heifers in the control group exhibited the lowest score, indicating incomplete physical changes. The swelling of the vagina was not detected even though there was a change in vaginal color and an increase in vaginal temperature that was associated with the secretion of estrus mucus (Table 2). The D0.5 heifers showed a higher score compared to the control heifers. All the D1.0 heifers exhibited complete physical changes accompanied by behavioral alterations. The vaginal temperatures were comparable at the outset of the treatment period, with a mean of approximately 38.4°C. A notable increase was observed during the estrus phase, reaching a peak (Figure 1). The control heifers reached the peak vaginal temperature (38.67°C) on the third day after the $PGF_{2\alpha}$ injection or the fifth day following the administration of the PMSG injection. In contrast, the D0.5 and D1.0 heifers reached the peak vaginal temperatures on the sixth day after PMSG injection with an average of 38.73°C in the D0.5 heifers and 38.98°C in D1.0 heifers. The D0.5 and D1.0 heifers exhibited estrus signs 24 hours later than the control heifers.

The remaining outcomes of the present study corroborate the hypothesis that heifers administered a higher dose of PMSG exhibited more robust estrus-related behavior and has been demonstrated to have a positive correlation with plasma estradiol concentration (Lyimo 2000), which in turn increases with the stimulation provided by an increasing dose of PMSG. A previous study reported a significantly higher estrus response and duration following estrus synchronization with a combination of progesterone, prostaglandin, and PMSG (Kavitha et al. 2018). Tirpan et al. (2019) additionally observed that Angora goats exhibited heightened levels of estrus behavior following estrus synchronization with a PMSG combination protocol. These increasing estrus behaviors are highly correlated with the increasing diameter of the pre-ovulatory follicle diameter (Jitjumnong et al. 2019; Putro et al. 2020), followed by a higher level of plasma estradiol (Pandey et al. 2018; Mohamed et al. 2021; Hunter & Lopez-Gatius 2020). The aforementioned experiments demonstrate that when the plasma estradiol reaches its peak, estrus behavior manifests with considerable intensity. Therefore, stimulation of heifers with PMSG during the follicle wave 2 in this study will improve the observed estrus signs, thus increasing the probability of successful mating.

Furthermore, the vaginal temperatures observed in the present experiment demonstrated a pattern of increase and reached the peak temperature during the estrus phase. An elevated vaginal temperature has been demonstrated to be closely associated with hormonal regulation during the estrus phase in a range of species, including the Bligon goat (Widiyono et al. 2011), the

Table 2. Scoring of estrus performances of Ongole-crossed heifers injected with low doses of Pregnant Mare Serum Gonadotropin (PMSG)

Doses of PMSG injection IU/kg BW	Number of heifers	Estrus score*		
		1	2	3
0	3	2 (66.67%)	1 (33.33%)	-
0.5	3	-	2 (66.67%)	1 (33.33%)
1.0	3	-	-	3 (100%)

*Based on the number of heifers that showed the same score.

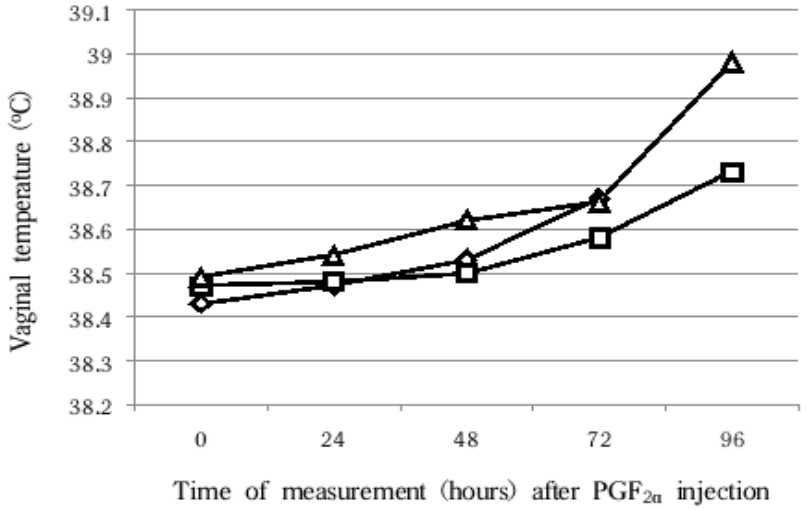

Figure 1. Vaginal temperatures of experimental Ongole-crossed heifers injected with PMSG at doses of 0 (—◇—), 0.5 (—□—), and 1.0 (—△—) IU/kg BW. Vaginal temperatures were measured from the injection of $PGF_{2\alpha}$ up to the appearance of estrus

dairy cow (Wang et al. 2020), camel (Mohamed et al. 2021), and other mammals (Lopez-Gatius 1997). The highest vaginal temperature was observed when the plasma estradiol reached its peak concentration, coinciding with the most complete and robust estrus signs exhibited by the heifers (score 3).

The other finding of the present experiment is the delayed onset of estrus signs following $PGF_{2\alpha}$ injection, with a mean of 4 days in D1.0 heifers versus 3 days in control heifers. The control heifers exhibited the typical time frame for the appearance of estrus signs following the administration of $PGF_{2\alpha}$ injection. It can, therefore, be concluded that the administration of PMSG has the effect of prolonging the duration of estrus appearance by approximately 24 hours. The delayed appearance of estrus in heifers injected with PMSG is likely attributable to the effect of PMSG on the corpus luteum regression. The mechanism of CL regression is postulated to have a relationship with a decreased level of nitric oxide (NO) and vascular endothelial growth factor (VEGF) (Ferrara et al. 1998; Jaroszewski & Hansel 2000; Shirasuna et al. 2012), as previously discussed in other research which indicates that VEGF plays an important role in CL formation by enhancing luteal angiogenesis (Chou & Chen 2018; Mara et al. 2020). Moreover, gonadotropin has been demonstrated to induce the expression of vascular endothelial growth factor (VEGF) in a dose- and time-dependent manner along with endothelin-2 (EDN2) mRNA, which is induced by luteinizing hormone (LH) (Shreshta et al. 2019). It is hypothesized that the injection of PMSG, acting as an agent of follicle stimulation, enhances the activity of VEGF, NO, EDN2, and other signaling agents, which in turn causes the late regression of the corpus luteum. A longer-lasting corpus luteum will impede the development of selected follicles into dominant follicles due to the elevated level of progesterone. This condition results in a delay in the formation of the dominant follicle, which in turn delays the attainment of peak estradiol concentrations is also delayed.

Biometrics of the ovary

Further observation of the ovary revealed significant differences in the weight and thickness of the ovary (Table 3). The weight and thickness of the ovary were found to increase in proportion to the doses of PMSG

injection administered. The weight of the right ovary in heifers administered D0.5 and D1.0 was significantly higher than in those administered D0.0 ($P<0.05$). Nevertheless, no statistically significant difference was observed in the ovary weights between the D0.5 and D1.0 heifers. The weight pattern of the left ovary differed from that of the right ovary. The weights of the left ovary in D0.0 and D0.5 heifers were similar ($P>0.05$), and D0.5 and D1.0 heifers also had similar weights of the left ovary ($P>0.05$). However, the weights of the left ovary in D1.0 heifers were higher ($P<0.05$) than in D0.0 heifers.

No significant differences ($P>0.05$) were observed in the other parameters, namely the length and width of the ovary, among the doses of PMSG injection. The length of the right ovary in all groups of heifers showed values above 3 cm, while the average length of the left ovary was shorter than the length of the right ovary across all doses of PMSG injection. The width of the right and left ovaries had almost similar values, with the lowest value observed in the left ovary of control heifers and the highest value of the left ovary of D1.0 heifers. Although no significant difference was observed, the longer right ovary compared to the left ovary indicated a tendency for the right ovary to be more active and responsive to the stimulation of PMSG at a lower dose.

The highest mean values for ovarian thickness were observed in D1.0 heifers ($P<0.05$) (Table 3). However, in the right ovary, a significant difference in the thickness of the ovary between the D0.0 and D0.5 heifers and between the D0.5 and D1.0 heifers was not found ($P>0.05$). The D1.0 heifers exhibited a significantly greater ovarian thickness on the right side than the control. No significant difference was observed in the thickness of the left ovary between the D0.5 and D1.0 heifers ($P>0.05$). Nevertheless, the ovary's thickness was significantly higher in the D0.5 and D1.0 heifers than the control heifers ($P<0.05$).

The enlargement of the reproductive tract in heifers was associated with the increased doses of PMSG injection. The largest is observed in D1.0 heifers, followed by D0.5 and D0.0 heifers. In general, stimulation with PMSG has been observed to elicit effects in increased numbers and diameters of dominant follicles. An increase in the diameter of the dominant follicle has been observed to result in an elevation of plasma estradiol concentration during the estrus phase (Lopes et al., 2007). Plasma estradiol during estrus plays a direct role in inducing the activation of physiological secretion processes of the endometrium and intensifying protein-lipid metabolism (Bondranenko et al. 2019). This mechanism results in the secretion of estrus mucus, which affects the size of the reproductive tract (Laksi & Trilaksana 2020) and increases the probability of conception (Damarany 2020).

Biometrics of the reproductive tract

Most reproductive tract parameters exhibited no statistically significant differences ($P>0.05$) across the doses of PMSG injection, as illustrated in Table 4. No significant differences were observed in the length of the right uterine horn between D1.0 and D0.0 heifers or between D0.5 and D1.0 heifers ($P>0.05$). However, heifers administered the lowest dose of PMSG (D0.5) exhibited a greater length of the right uterine horn than those administered the highest dose (D1.0). The diameters of the left and right uterine horns, as well as the diameters of the uterine body and cervix, showed similar patterns, i.e., there was a significant increase in the diameter with the increased dose of PMSG injection (D0.5 and D1.0) compared to control heifers. However, the increased doses of PMSG injection from 0.5 to 1.0 IU/kg BW did not significantly increase the diameters of the right and left uterine horns, as well as the diameters of the uterine body and cervix ($P>0.05$). The diameter of

Table 3. Morphological characteristics of the experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Ovary	Parameters	Doses of PMSG injection (IU/kg BW)		
		0	0.5	1.0
Right	Weight (g)	6.57±0.80^{a}	7.43±0.13^{b}	7.78±0.44^{b}
	Length (cm)	3.05±0.22^{a}	3.22±0.06^{a}	3.01±0.43^{a}
	Width (cm)	2.12±0.05^{a}	2.61±0.18^{a}	2.43±0.37^{a}
	Thickness (cm)	1.73±0.07^{a}	1.98±0.16^{ab}	2.44±0.20^{b}
Left	Weight (g)	4.69±0.90^{a}	6.69±0.18^{ab}	7.01±0.08^{b}
	Length (cm)	2.63±0.22^{a}	2.82±0.89^{a}	2.93±0.22^{a}
	Width (cm)	2.03±0.22^{a}	2.22±0.23^{a}	2.64±0.40^{a}
	Thickness (cm)	1.78±0.15^{a}	2.14±0.04^{b}	2.24±0.06^{b}

Different superscripts in the same row mean significant difference ($P<0.05$)

Table 4. Morphological characteristics of the reproductive tracts of the experimental heifers injected with different doses of Pregnant Mare Serum Gonadotropin (PMSG)

Uterus		Parameters (cm)	Doses of PMSG injection (IU/kg BW)		
			0	0.5	1.0
Horn	Right	Length	14.93±0.32[b]	18.72±0.67[a]	17.35±2.30[ab]
		Diameter	1.43±0.22[a]	2.99±0.21[b]	3.58±0.16[b]
		Thickness	0.65±0.11[a]	0.64±0.03[a]	0.75±0.02[a]
	Left	Length	14.92±0.61[a]	17.06±0.39[a]	16.44±1.52[a]
		Diameter	1.98±0.21[a]	2.45±0.18[b]	3.49±0.15[b]
		Thickness	0.66±0.13[a]	0.68±0.05[a]	0.69±0.02[a]
Body		Length	7.22±1.67[a]	7.91±0.79[a]	6.99±0.83[a]
		Diameter	4.06±0.24[a]	5.58±0.71[b]	5.75±0.20[b]
		Thickness	0.74±0.07[a]	0.71±0.04[a]	0.79±0.02[a]
Cervix		Length	5.38±0.69[a]	6.16±0.33[a]	6.39±0.33[a]
		Diameter	4.14±0.45[a]	5.22±0.61[b]	5.82±0.08[b]
		Thickness	0.95±0.06[a]	1.09±0.15[a]	1.08±0.10[a]
Vagina		Length	18.56±2.66[a]	23.47±0.44[a]	22.37±0.64[a]
		Diameter	4.44±0.20[a]	4.59±0.22[a]	5.10±0.56[a]
		Thickness	0.83±0.07[a]	0.67±0.02[a]	0.83±0.22[a]

Different superscripts in the same row mean significant difference (P<0.05)

the uterine horn in the D0.0 heifers was below 2 cm. In contrast, in the D0.5 heifers, the diameter of the uterine horn ranged from 2 to 3 cm, and in the D1.0 heifers showed an average diameter above 3 cm.

The diameter of the uterine body was found to be significantly (P<0.05) smaller in control heifers compared to both D0.5 and D1.0 heifers, which exhibited the largest diameter of the uterine body. The D1.0 heifers had the highest diameter of the uterine body among the groups of doses of PMSG injection. The diameter of the cervix in heifers administered with PMSG demonstrated a positive correlation with the dosage of PMSG administered. The highest diameter of the cervix was shown by D1.0 heifers, which D0.5 heifers followed. The heifers in the D0.0 group exhibited the most minor diameter of the cervix among the group receiving PMSG injections.

The wall thickness of all heifer groups administered different doses of PMSG was similar (P>0.05). However, a significant increase (P<0.05) in diameter was observed with elevated doses of PMSG injection. Thus, the observed increase in diameter is a consequence of the increased volume of secreted estrus mucus. An increase in plasma estradiol concentrations during estrus can result in heightened tension of the endometrium, enhanced mucus secretion of the uterus, and alterations in the size, color, and temperature of the reproductive tract, particularly in the vulva and vagina (Forde et al. 2011). The observed phenomena indicate that a lower dose of PMSG can enhance the quality of estrus, ultimately increasing the probability of mating and conception success.

CONCLUSION

The results of the experiment and the subsequent analyses indicate that administration of lower doses of PMSG can enhance the quality and performance of estrus, manifested as an improvement in estrus behavior of the crossed Ongole heifers; this, in turn, facilitates the detection and confirmation of estrus through discernible changes in the dimensions of the reproductive tract. The present study's findings suggest that the observed phenomena enhance the success of mating and conception. This approach could be an alternative method to improve reproductive efficiency in monotocous animals, such as local buffalo and cattle.

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Characterization of Protein Degradation in Tropical Dairy Feedstuff Using the *In Sacco* Method

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ABSTRAK

Permana IG, Rosmalia A, Rahmat SFI, Despal, Zahera R. 2024. Karakterisasi degradasi protein pada bahan pakan untuk ternak perah tropis dengan metode *in sacco*. JITV 29(4):181-192. DOI:http://dx.doi.org/jitv.v29i4.3382.

Penelitian ini bertujuan untuk menentukan karakteristik degradasi protein pada 27 bahan pakan tropis untuk ransum ternak perah dengan dua puluh tujuh jenis bahan pakan. Dua puluh dua bahan pakan tropis dikelompokkan menjadi A1 (pakan lokal rendah serat rendah protein: jagung, dedak padi, gaplek, onggok, gandum, dan pollard), A2 (pakan lokal rendah serat tinggi protein: bungkil inti sawit, ampas tahu, dan ampas tempe), dan A3 (pakan lokal tinggi serat: akasia, alfalfa, narra, gliricidia, indigofera, kaliandra, bauhinia, leucaena, albizia, agati, piper, kelor, dan daun jack), dibandingkan dengan A4 (pakan impor rendah serat tinggi protein: kedelai, kedelai sangrai, DDGS, CGM, dan CGF) menggunakan metode in sacco. Hasil penelitian menunjukkan bahwa A1, A2, dan A3 memiliki kandungan protein lebih rendah namun tinggi serat dibandingkan A4. Fraksi kelarutan protein (a) lebih tinggi pada A1 dan A2, sedangkan fraksi yang berpotensi terdegradasi (b) lebih tinggi pada pada A2 dan A3. A1 dan A2 memiliki fraksi RDP lebih tinggi daripada A3 dan A4. Pakan tinggi RDP meliputi pollard, gandum, kedelai, CGF, ampas tempe, alfalfa, gamal, indigofera, agati, dan kelor, sedangkan pakan tinggi RUP meliputi jagung, bungkil kelapa sawit, angsana, kaliandra, lamtoro, sengon, daun asam jawa, sirih hutan, daun nangka, kedelai sangrai, bungkil kedelai, CGM. Pakan tropis menunjukkan karakteristik degradasi protein yang beragam sehingga bermanfaat dalam memformulasikan ransum yang tepat pada sapi perah.

Kata Kunci: Hijauan, Konsentrat, Rumen Degradable Protein, Rumen Undegradable Protein, Sapi Perah

ABSTRACT

Permana IG, Rosmalia A, Rahmat SFI, Despal, Zahera R. 2024. Characterization of protein degradation in tropical dairy feedstuff using the *in sacco* method. JITV 29(4):181-192. DOI:http://dx.doi.org/jitv.v29i4.3382..

A study was conducted to determine the protein degradation characteristics of 27 tropical feedstuffs for dairy rations. Twenty-two tropical feedstuffs were grouped into A1 (local low fiber and low protein sources: corn, rice bran, cassava, cassava waste, wheat, pollard), A2 (local low fiber and low protein sources: palm kernel meal, tofu waste, tempe waste), and A3 (local high fiber sources: acacia, alfalfa, narra, gliricidia, indigofera, calliandra, bauhinia, leucaena, albizia, agati, piper, moringa, jack leaves), and compared to A4 (imported low fiber high protein sources: soybean, roasted soybean, DDGS, CGM, CGF) using the *in sacco* method. The study revealed that A1, A2, and A3 had lower protein content but higher crude fiber than A4. Protein solubility (*a*) was higher in A1 and A2, while the potentially degraded fraction (*b*) was higher in A2 and A3. A1 and A2 had higher RDP fractions than A3 and A4. High RDP feedstuffs include pollard, wheat, soybean, CGF, tempe waste, alfalfa, gliricidia, indigofera, agati, and moringa. In contrast, high RUP feedstuffs include corn, palm kernel meal, narra, calliandra, leucaena, albizia, tamarind, piper, jack leaves, roasted soybean, soybean meal, and CGM. Tropical feedstuffs exhibit diverse protein degradation characteristics, making them valuable for strategic ration formulation in dairy cattle.

Key Words: Concentrate, Dairy Cattle, Forages, Rumen Degradable Protein, Rumen Undegradable Protein

INTRODUCTION

The tropical regions have a high biodiversity potential and natural resources. The characteristics of tropical ecosystems are species richness in various taxa and complex biotic interactions among component species (Orians 2000). High species diversity allows for exploring plants, including grains and forages, that have the potential as livestock feed. Lee (2018) indicated that tropical forage plants have a lower nutritional value compared to those in temperate regions, with a 2% reduction in crude protein (CP), a 12% decrease in dry matter digestibility (DMD), and 49% organic matter digestibility (OMD). In contrast, tropical forages have higher fiber content, including 19% more neutral detergent fiber (NDF), 11% more acid detergent fiber (ADF), and 3% more acid detergent lignin (ADL). Hence, tropical feedstuffs have various qualities that fluctuate between the dry and rainy seasons (Despal et al., 2014). For instance, Napier grass (*Pennisetum purpureum*) has lower CP and DMD in the dry season (12.4% CP and 59.1% DMD) compared to the rainy season (13.7% CP and 61.8% DMD) (Evitayani et al. 2004). Conversely, *Leucaena leucocephala* shows

higher CP content during the dry season (24.19%) compared to the rainy season (21.75%) (Tahuk et al. 2018). Moyo and Nsahlai (2021) reported that feed degradability was highest in the cold (715 g/kg) and temperate regions (745 g/kg), compared to lower values in tropical (664 g/kg) and arid regions (621 g/kg).

Dairy cattle feed should provide mainly energy, protein, minerals, and vitamins. In the ruminant, the protein quality of feed is expressed by amino acid content, degradability, and digestibility (Patton et al. 2014). Rumen degradable protein (RDP) and rumen undegradable protein (RUP) are also used to determine the quality of protein for dairy cattle (NRC 2001). RDP expresses the amount of protein degraded by rumen microbes into ammonia (NH_3), which then is used to synthesize microbial protein (Hristov et al. 2019) and is essential to maintain the balance of the microbial population (Uddin et al., 2015). RUP is feed protein that by-pass from the rumen. The RDP content of the feed is correlated with crude protein content but uncorrelated with crude fiber (Rosmalia et al. 2021). The Dairy National Research Council (NRC) standard (2001) recommends that the minimum RDP and RUP requirements be 60% and 40%, respectively.

The common feed used for dairy ration in Indonesia, namely concentrates and forages (Rosmalia et al. 2022; Sahroni et al. 2021), still adopts the dairy NRC standard for the formulation without considering RDP and RUP due to the lack of information on local feed. Concentrate feed supplies protein and energy (Woods et al. 2003), including agro-industrial by-products such as soybean meal, corn gluten meal, tofu waste, and palm kernel meal. By-product feeds have undergone mechanical or physical processing, especially heating, which can reduce soluble protein and RDP (Doiron et al. 2009). Cereal grains provide energy in the rumen and are a source of carbon skeletons for microbial protein synthesis when RDP is sufficient (Ferraretto et al., 2013; Rastgoo et al., 2020). Cereal grains provide protein and support the formulation of cost-effective dairy rations due to their relatively low prices, such as wheat pollard at 210 USD per metric ton compared to soybean meal at 500 USD per metric ton. Legume has a high protein content and is an alternative feedstuff in the tropical dairy ration to substitute protein content in feed concentrate (Castro-Montoya et al. 2019). A previous study reported that feeding dairy cattle a legume-based ration led to higher dry matter intake (DMI) by 1.3 kg/day and increased milk production by 1.6 kg/day compared to a grass-based ration (Johansen et al. 2018). The use of legumes in dairy cattle rations is limited by the low degradation in the rumen caused by crude fiber content and the presence of antinutrients (Jouan et al., 2020; Piluzza et al., 2014).

It is important to know the degraded and non-degraded fractions in the rumen related to the utilization of protein for rumen microbes and the host. Efforts to increase the productivity of dairy cows through improved feed with a fulfillment approach based on protein adequacy and a balance of RDP and RUP will provide an accurate measurement of the estimated dairy production so that feed protein efficiency increases and costs incurred for reduced feed. Dairy ration based on RDP and RUP balance at the optimum level can also reduce nitrogen emission to the environment by improving nitrogen efficiency (Martins et al. 2019). The objective of this study was to compare degradation characteristics between tropical and imported (temperate origin) feed and to identify the RDP and RUP content in tropical feedstuff, including concentrate and forage protein, as an inventory of information on the nutritional content of the feed to further serve as a reference in preparing feed formulations and meeting nutrient needs for dairy cattle in Indonesia.

MATERIALS AND METHODS

Tropical feedstuff preparation

A total of 29 kinds of feedstuff, including concentrate and forage protein, were used in this study. Feedstuffs were grouped into A1 (local low fiber low protein sources), A2 (local low fiber high protein sources), A3 (local high fiber sources), and A4 (imported low fiber high protein sources). Local energy sources comprised corn, rice bran, cassava, cassava waste, wheat, and pollard. Protein sources include palm kernel meal, tofu, and tempe waste). Forage sources consisted of acacia (*Acacia mangium*), alfalfa (*Medicago sativa*), narra (*Pterocarpus indicus*), gliricidia (*Gliricidia sepium*), indigofera (*Indigofera zollingeriana*), calliandra (*Calliandra calothyrsus*), bauhinia (*Bauhinia purpurea*), leucaena (*Leucaena leucocephala*), albizia (*Albizia chinensis*), agati (*Sesbania grandiflora*), piper (*Piper aduncum* L.), moringa (*Moringa oleifera*), and jack leaves (*Artocarpus heterophyllus*). In contrast, imported sources consisted of soybean, roasted soybean, DDGS, CGM, and CGF.

The concentrate samples were ground through a 2-mm screen for concentrate feedstuff and dried in an oven at 60 ℃ for 48 h. The leaves and stems commonly eaten by dairy cattle were taken as samples for forage sources. The forage samples were chopped and dried in the room for three days, then dried in an oven at 60 ℃ for 48 h before ground to a 2-mm size. All feedstuff was analyzed for nutrient composition, such as dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE) using AOAC (2005) method, and gross energy (GE) was estimated according to Weiss and Tebbe (2019).

In sacco degradability measurement

This research was conducted using the *in sacco* method according to NRC (2001). The samples were

weighed 5 g and put in a nylon bag (ANKOM, porosity ±50 µm), 5 x 10 cm for concentrate feed and 10 x 20 cm for forage protein feed (Despal et al. 2022; NRC 2001; Van Emon et al. 2015). Each sample consists of 3 bags. The nylon bag is tied with a rope and inserted into the fistulated dairy cattle rumen. The nylon bags were inserted before morning feeding. This study used two fistulated Friesian Holstein bulls (BW±510 kg). Dairy cattle were fed 2% of BW twice daily at 7.00 am and 3.00 pm. Diets contained 60% Napier grass (*Pennisetum purpureum*) and 40% concentrate mixture (%DM basis). The diets contained 10.25% CP, 20.70% CF, and 60% TDN.

The nylon bags were incubated in the rumen for 0, 3, 6, 9, 12, 15, 24, and 48 hours for concentrate feed, then until 72 h incubation for forage protein feed. For 0 h incubation, the nylon bags were only rinsed under tap water. The nylon bag contains a sample of feed at a predetermined time. The nylon bag was washed and dried at 60°C for 48 hours in an oven. The nylon bag was weighed, and the residual sample was separated for protein analysis using the Kjeldahl method (AOAC 2005). Crude protein disappearance (CPD) (%) was estimated as follows:

$$CPD = \big((BW + S1) - (BW + RW)\big)x\ 100\ (S1xCP)$$

where BW is bag weight, RW is residue weight, S1 is sample weight, and CP is the crude protein of the original sample.

The degradation of protein or kinetic parameters was calculated based on an exponential equation according to the (Ørskov & Mcdonald 1979):

$$y = a + b(1 - e^{(-ct)}) \quad (1)$$

Where *y* is protein disappearance in the rumen (%), *a* is a soluble fraction (%), *b* is an insoluble but potentially degradable fraction (%), *c* is degradation rate constant of the b fraction (%/h), *t* is degradation time (0, 3, 6, 9, 12, 15, 24, 48 and 72 h), *e* is base for natural logarithm. Potential degradation was calculated with the formula:

$$PD = a + b \quad (2)$$

The RDP and RUP were calculated, which refer to NRC (2001) with the following equation:

$$RDP = a + b\ [{}^{c}/_{(c+k)}] \quad (3)$$

$$RUP = 100 - RDP \quad (4)$$

where *a*, *b*, and *c* are the same as in equation (1), *k* is the rumen outflow rate, assumed to be 6%/h.

Statistical analysis

The nutrient composition and ruminal degradability of protein were analyzed descriptively. A One-Way Analysis of Variance (ANOVA) was performed using the SAS program (SAS Institute Inc., Cary, NC, USA) to evaluate the kinetic parameters and estimate RDP and RUP. The animals were treated as a block. Duncan's Multiple Range Test further tested the differences ($P<0.05$).

RESULTS AND DISCUSSION

Nutrient composition

In this study, there were four groups of tropical dairy feedstuff consisting of local low-fiber and low-protein sources (A1), local low-fiber and high-protein sources (A2), local high-fiber sources (A3), and imported low-fiber and high protein sources (A4). The nutrient composition of the tropical dairy feedstuff varied depending on the feed sources presented in Table 1. The crude protein content (CP) of A4 was highest compared to other group feedstuff, with an average of 38.30%±14.26. The data showed that imported feed sources had higher protein content than local feed sources in both concentrate (low fiber) and forage (high fiber) categories. Moyo and Nsahlai (2021) reported that the CP content of feed from cold and temperate climates (imported protein sources) was higher than in tropical climates (local protein sources) due to rapid lignification in tropical climates.

In contrast, the A1 group as an energy source had the lowest protein and the highest nitrogen free-extract (NFE), averaging 8.76%±4.74 and 70.00%±19.05, respectively. Storage carbohydrates act as energy sources, so the A1 group is supposed to have low protein content and is easily degradable (Klevenhusen and Zebeli 2021). The gross energy predicted using the Weiss and Tebbe (2019) equation showed that the A4 group had the highest energy density, driven by its high CP and ether extract (EE) content. In contrast, although A1 was rich in carbohydrates (NFE), its overall energy density was moderated by lower protein and fat levels, resulting in the lowest GE among the groups.

The A1 group is a low-fiber and low-protein feed source group primarily used as an energy source in formulating dairy cow rations. Its low protein content also influences the extent of protein degradation in the rumen due to the different carbohydrate components. Pollard has the highest protein content compared to other A1 feed ingredients. Besides being used as an energy source, pollard is also a protein source for dairy cows (Chuzaemi et al. 2020).

The CP content is closely related to effective rumen degradation (Fulkerson et al., 2007). Tofu waste, a feedstuff commonly used by smallholder dairy farmers, had the highest CP content with low crude fiber (CF) and high NFE compared to other A2 group feedstuff. For the A3 group, indigofera had a high CP, followed by gliricidia and leucaena. Meanwhile, tamarind had the lowest CP. However, the crude fiber (CF) content of A3 was lower than A2, 16.30%±5.32 and 25.98%±10.56, respectively. It is due to protein sources from by-product agroindustry and non-conventional feed containing high

Table 1. Nutrient composition of tropical dairy feedstuff

Group	Feedstuff	Nutrient composition						
		DM (%)	Ash (%DM)	CP (%DM)	EE (%DM)	CF (%DM)	NFE (%DM)	GE (Mcal/kg)[1]
A1	Corn	90.59	1.31	7.88	3.74	1.24	85.83	4.45
	Rice bran	92.61	16.21	6.91	3.22	25.60	48.06	3.78
	Cassava	91.17	1.78	5.18	0.43	1.60	91.02	4.22
	Cassava waste	93.35	37.04	3.72	0.52	11.69	47.03	2.72
	Wheat	89.52	1.50	12.78	1.73	3.63	80.36	4.41
	Pollard	91.58	6.80	16.10	1.80	7.59	67.71	4.23
Average±SD		91.47±1.38	10.77±14.08	8.76±4.74	1.91±1.36	8.56±9.25	70.00±19.05	3.97±0.65
A2	Palm kernel meal	94.02	4.77	15.87	11.24	28.46	39.66	4.81
	Tofu waste	92.68	3.47	18.80	4.50	14.40	58.83	4.55
	Tempe waste	91.72	2.62	12.65	2.51	35.07	47.15	4.40
Average±SD		92.81±1.16	3.62±1.08	15.77±3.08	6.08±4.58	25.98±10.56	48.55±9.66	4.59±0.21
A3	Acacia	24.89	4.68	16.63	1.93	19.04	50.04	4.34
	Alfalfa	26.22	9.53	14.47	2.79	28.40	38.18	4.15
	Narra	29.39	6.00	21.63	1.98	21.51	42.34	4.35
	Gliricidia	19.86	9.46	25.18	2.25	14.15	41.34	4.27
	Indigofera	24.16	9.32	26.18	2.37	12.52	40.23	4.30
	Calliandra	31.41	5.78	21.39	1.55	15.84	48.41	4.34
	Bauhinia	37.04	8.94	21.71	3.02	23.07	35.76	4.29
	Leucaena	28.56	7.92	22.68	2.46	16.33	43.26	4.31
	Albizia	34.55	4.61	17.56	2.31	14.02	54.64	4.37
	Tamarind	39.21	7.12	11.60	1.99	18.51	55.30	4.17
	Agati	23.40	8.39	19.69	2.54	10.50	50.82	4.26
	Piper	20.98	18.53	21.69	1.47	10.44	39.54	3.80
	Moringa	18.55	11.41	20.59	3.44	10.38	46.08	4.19
	Jack leaves	29.91	11.12	15.53	0.86	13.42	51.89	4.00
Average±SD		27.72±6.33	8.77±3.54	19.75±4.12	2.21±0.66	16.30±5.32	45.56±6.34	4.22±0.16
A4	Soybean	92.67	6.13	34.78	13.59	11.99	33.50	5.14
	Roasted soybean	94.32	6.23	38.92	15.31	9.55	30.00	5.28
	Soybean meal	92.10	8.47	49.30	1.39	2.26	38.58	4.61
	DDGS	88.77	5.77	29.81	6.15	6.73	51.55	4.69
	CGM	92.94	1.74	58.63	2.51	0.72	36.40	5.08
	CGF	90.42	4.90	18.37	2.49	10.13	64.11	4.38
Average±SD		91.87±1.98	5.54±2.21	38.30±14.26	6.91±6.08	6.90±4.54	42.36±12.95	4.86±0.35

DM= dry matter, CP= crude protein, EE= ether extract, CF= crude fiber, NFE= nitrogen-free extract, GE= gross energy, A1= local low fiber low protein sources, A2= local low fiber high protein sources, A3= local high fiber sources, A4= imported low fiber high protein sources. [1]GE was calculated using the equation proposed by Weiss and Tebbe (2019) with GE = (%CP x 0.056) + (%EE x 0.094) + ((100 – %CP – %EE – %ash) x 0.042)

fiber. Abdeltawab and Khattab (2018) reported that the CF content of palm kernel meal reached 24.90%, with ADF 43.70% and NDF 66.70%. Most feedstuffs in the A4 group had a high protein content except CGF due to the gluten (protein) cut off during the wet-milling process (Li et al. 2011).

The In Sacco protein degradability of tropical dairy feedstuff

The ruminal CP degradation (CPD) of tropical dairy feedstuff at each incubation time is shown in Figure 1. The rise of CPD was followed by increasing incubation time. At 0 h incubation time, protein degradation showed the amount of soluble protein without incubation in the rumen. Protein solubility is the main factor determining proteolytic microbes' activity to access feed protein and degrade it (Bach et al. 2005). The data showed a wide variety of protein degradation at 0 h incubation. The range of protein degradation at 0 h incubation time for concentrate source (A1, A2, and A4) and fiber source (A3) were 0%─78% and 0%─32%, respectively. The highest protein degradation at 0 h incubation time for concentrate and forage sources was found in cassava meal and agati; this indicates that cassava meal and agati contain more soluble protein than other feeds.

In the A1 group, over 50% of CPD rice bran, cassava, cassava waste, wheat, and pollard had been degraded for three hours of incubation. Corn had to be degraded by over 50% CPD for 12 hours. Tempe waste had lower time incubation (3 h) to reach more than 50% CPD compared to tofu waste (9 h) and palm kernel meal (12 h). In the A4 group, CGF had more than 50% CPD with lower time incubation (3 h) rather than soybean and DDGS (9 h), roasted soybean and soybean meal (15 h), and CGM (48 h). According to Figure 1, the level of CPD between soybean and roasted soybean was different due to the heating process obtained by roasted soybean. The heating process in roasted soybeans causes low protein degradation (Petit et al. 2002). Heating treatment of feed reduced RDP by decreasing the soluble fraction (a) and the potential degradation ($a+b$) (Rosmalia et al. 2024). The low CPD in CGM and corn was due to CGM and corn coming from maize protected by a complex endosperm texture, starch structure, and starch granule shape associated with protein and fat, inhibiting rumen microbes from accessing and degrading the protein (Rastgoo et al. 2020). The characteristics of high-starch feed that interact with protein or fat take longer to be degraded (Menezes et al. 2019).

In the forage group, alfalfa, indigofera, agati, and moringa had reached 50% of CPD at 3 h incubation. Meanwhile, gliricidia and bauhinia got more than 50% of CPD at 9 h, piper at 12 h; jack leaves at 15 h, then narra and Leucaena at 48 h. In contrast, acacia and albizia have not reached 50% degradation after 72 h incubation. Protein degradation of forage protein depends on the part of the plant, fertilization rates, maturities, and antinutrients, which have different characteristics among forages (Elizalde et al. 1999). Acacia and albizia contained high tannin compared to agati and gliricidia (Alam et al. 2007; Yusiati et al. 2018).

Kinetic of protein degradation on tropical dairy feedstuff

The kinetic degradation of protein in tropical dairy feedstuff is presented in Table 2. The values of a, b, and PD were significantly different among types of feed ($p<0.05$), while the coefficient c was not significant ($p>0.05$). The average soluble fraction (a) on A1 (32.06%) and A2 (30.96%) was higher than A3 (16.24%) and A4 (12.40%). In the A1 group, cassava was the highest value, followed by cassava waste, pollard, corn, wheat, and rice bran. The high soluble fraction of cassava is due to high nonstructural carbohydrate concentration (Daza et al., 2019). The most significant value in the A2 group was tempe waste compared to tofu waste and palm kernel meal. The high percentage of total carbohydrates in tempe waste can affect soluble fractions. Lee et al. (2017) revealed that the soluble fraction positively correlates with carbohydrates, especially non-fiber carbohydrate (NFC) content. The highest and lowest values in the A3 group were agati and acacia, respectively. The low value can be caused by the inability of rumen microbes and their enzymes to degrade the substrate at a certain level. The presence of associated nutrients (cross-linking bonds) that are difficult to degrade is also a limiting factor (Dijkstra et al. 2005). In the A4 group, CGF had the highest value, while soybean meal and roasted soybean had a lower value than other A4 feedstuff. The negative value in soybean meal and roasted soybean can be related to the loss of finer particles from the bags in this treatment instead of a higher solubility (Belachew et al. 2013).

The value of b was highest in A4 (90.03%) compared to A2 65.00%, A1 55.64%, and A3 59.10%; this is in line with Moyo and Nsahlai (2021) that the b value was higher for concentrate feed compared to roughages. Wheat and corn had a high b value, followed by pollard, rice bran, cassava waste, and cassava. Tofu waste had the highest b value compared with palm kernel meal and tempe waste. In the forage group, the b value was highest for piper, followed by moringa, jack leaves, indigofera, gliricidia, agati, leucaena, bauhinia, alfalfa, narra, tamarind, acacia, calliandra, and albizia. In the A4 group, soybean meal, roasted soybean, and soybean had a high b value. In contrast, DDGS, CGM, and CGF had the same level of b coefficient ranging from 35%─60%. The b values of soybean meal and roasted soybean were overestimated by more than 100%. Referring to Figure 1, this might be due to the low and slow degradation rate

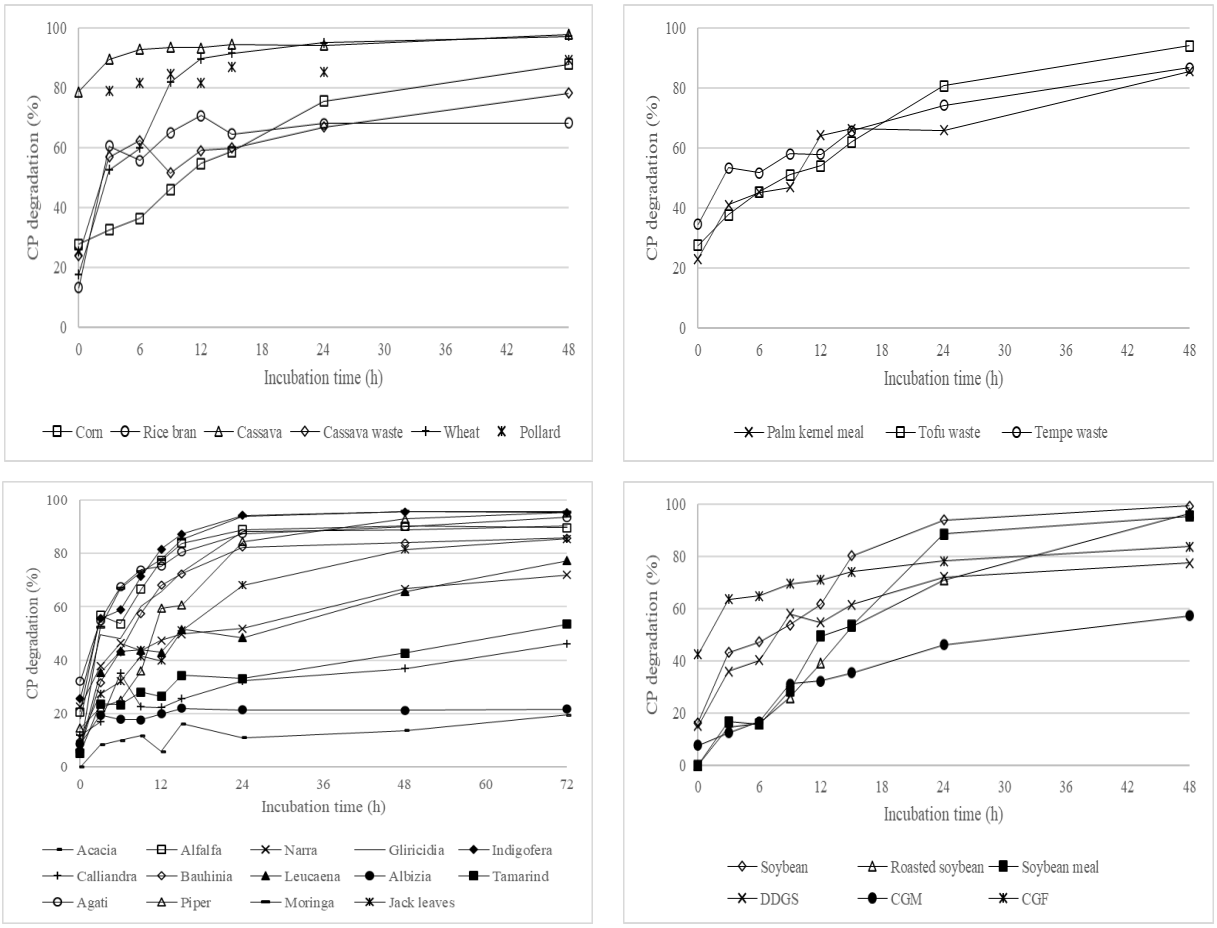

Figure 1. Ruminal crude protein degradation (CPD) of tropical feedstuff in the rumen

at the beginning of incubation for up to 15 hours of incubation time. After 24 hours of incubation, more than 70%─80% of the protein had been degraded in the rumen.

The PD value describes potential degradation. Imported protein sources had the highest PD value, followed by local protein, energy, and forage protein sources. Corn, wheat, and cassava had a higher potential degradation, followed by pollard, cassava waste, and rice bran. There is no difference in PD values among feedstuff in A4 (P>0.05). The potential degradation for tofu waste was highest compared to other A4 feeds. Piper, moringa, and agati had a high PD value, while albizia had the lowest.

The rate of degradation (*c* value) was not significantly different among types of feed, including the feedstuff in the group (P>0.05). The *c* value ranged from 0.04─0.63 h^{-1}. However, corn's degradation rate tends to be low compared to other A1 feedstuff. According to Herrera-Saldana et al. (1990), corn contains high starch and has a low rate of starch degradation. Table 2 shows that the *c* value decreased for soybeans heated by roasting. The heating process is one way to protect feed protein from rumen degradation (Micek et al. 2020). Petit et al. (2002) reported that the rate of protein degradation decreased, and the concentration of RUP increased with heating temperature due to the Maillard reaction.

Estimation of rumen degradable protein and rumen undegradable protein

Table 3 shows the estimation of RDP and RUP on tropical dairy feedstuff, including concentrate and forage protein. The data indicate that RDP and RUP values differed for all types of feed (P<0.05). A1 had the highest RDP value, followed by A2, A4, and A3. In contrast, A3 had the highest RUP values in comparison to other types. Almost 70% of the protein of A1 had degraded in the rumen used to serve the energy and the carbon skeleton for microbial protein synthesis (Rastgoo et al. 2020). Cassava had the highest RDP value compared to other A1 feedstuff. The high RDP is related to the degradability of dry matter in feedstuff. Wanapat and Kang (2015) reported that dry matter degradability for cassava, cassava waste, rice bran, and corn were 92.5%, 63.6%, 63.0%, and 59.3%, respectively. A1 feeds contain carbohydrates, including sugar, starch, fructan, and pectin (Villalba et al., 2021). It was reported that cassava had a high pectin substance

Table 2. Kinetic degradation of protein

Group	Feedstuff	Coefficient of kinetic parameters[1]			
		a (%)	*b* (%)	PD (%)	*c* (h^{-1})
A1	Corn	24.59^{bc}	75.94^{a}	100.54^{a}	0.04
	Rice bran	13.84^{c}	52.74^{bc}	66.58^{c}	0.63
	Cassava	78.83^{a}	16.29^{d}	95.12^{a}	0.40
	Cassava waste	31.40^{b}	42.54^{c}	73.95^{c}	0.11
	Wheat	17.96^{bc}	79.89^{a}	97.85^{a}	0.16
	Pollard	25.42^{bc}	59.86^{b}	85.29^{b}	0.84
Average±SD		32.06±24.15^{a}	55.64±23.37^{b}	87.70±13.38^{ab}	0.39±0.36
A2	Palm kernel meal	26.00^{b}	60.44^{b}	86.44^{b}	0.06
	Tofu waste	27.53^{b}	79.69^{a}	107.22^{a}	0.04
	Tempe waste	39.36^{a}	54.87^{b}	94.23^{ab}	0.04
Average±SD		30.96±6.66^{a}	65.00±11.79^{ab}	95.96±9.72^{ab}	0.05±0.02
A3	Acacia	6.25^{e}	30.88^{f}	37.13^{gh}	0.29
	Alfalfa	17.23^{bcd}	50.37^{e}	67.60^{d}	0.12
	Narra	15.75^{bcd}	43.94^{e}	59.68^{ef}	0.06
	Gliricidia	22.26^{ab}	54.37^{cde}	76.63^{bc}	0.11
	Indigofera	21.04^{bc}	61.99^{bcd}	83.03^{ab}	0.13
	Calliandra	15.37^{bcd}	27.91^{f}	43.28^{g}	0.05
	Bauhinia	14.26^{bcde}	50.99^{de}	65.25^{de}	0.11
	Leucaena	17.15^{bcd}	51.27^{de}	68.42^{d}	0.07
	Albizia	12.44^{cde}	22.22^{f}	34.65^{h}	0.39
	Tamarind	21.95^{ab}	32.94^{f}	54.89^{f}	0.10
	Agati	29.69^{a}	55.00^{cde}	84.69^{a}	0.12
	Piper	9.18^{de}	79.90^{a}	89.09^{a}	0.06
	Moringa	20.60^{bc}	66.24^{b}	86.84^{a}	0.16
	Jack leaves	12.21^{cde}	63.51^{bc}	75.72^{c}	0.05
Average±SD		16.24±9.91^{b}	59.10±24.64^{b}	75.35±27.60^{b}	0.28±0.97
A4	Soybean	18.78^{b}	86.01^{bc}	104.80	0.07
	Roasted soybean	-5.52^{d}	143.81^{ab}	138.30	0.04
	Soybean meal	-6.57^{d}	157.61^{a}	151.05	0.03
	DDGS	16.62^{b}	60.51^{c}	77.13	0.10
	CGM	5.81^{c}	56.94^{c}	62.75	0.05
	CGF	45.30^{a}	35.28^{c}	80.58	0.15
Average±SD		12.40±18.82^{b}	90.03±50.53^{a}	102.43±38.44^{a}	0.07±0.05

[1]Means in the same row with different superscripts differ significantly ($P<0.05$). a= soluble fraction, b= insoluble but potentially degradable fraction, PD= potential degradation, c= degradation rate constant of the b fraction, A1= local low fiber low protein sources, A2= local low fiber high protein sources, A3= local high fiber sources, A4= imported low fiber high protein sources.

Table 3. Estimation of RDP and RUP on tropical dairy feedstuff

Group	Feedstuff	CP (%DM)	RDP (%CP)	RUP (%CP)
A1	Corn	7.88	55.00^{c}	45.00^{a}
	Rice bran	6.91	61.42^{c}	38.58^{a}
	Cassava	5.18	92.61^{a}	7.39^{c}
	Cassava waste	3.72	59.19^{c}	40.81^{a}
	Wheat	12.78	76.33^{b}	23.67^{b}
	Pollard	16.10	80.77^{b}	19.23^{b}
Average±SD		8.76±4.74	71.95±14.32^{a}	28.05±14.32^{b}
A2	Palm kernel meal	15.87	57.04^{b}	42.96^{a}
	Tofu waste	18.80	59.19^{ab}	40.81^{ab}
	Tempe waste	12.65	62.08^{a}	37.92^{b}
Average±SD		15.77±3.08	59.43±3.05^{ab}	40.57±3.05^{ab}
A3	Acacia	16.63	10.46^{g}	89.54^{a}
	Alfalfa	14.47	69.79^{a}	30.21^{g}
	Narra	21.63	47.49^{d}	52.51^{d}
	Gliricidia	25.18	64.08^{ab}	35.92^{fg}
	Indigofera	26.18	73.88^{a}	26.12^{g}
	Calliandra	21.39	26.79^{ef}	73.21^{bc}
	Bauhinia	21.71	58.89^{bc}	41.11^{ef}
	Leucaena	22.68	46.15^{d}	53.85^{d}
	Albizia	17.56	19.38^{fg}	80.62^{ab}
	Tamarind	11.60	28.90^{e}	71.10^{c}
	Agati	19.69	72.64^{a}	27.36^{g}
	Piper	21.69	52.59^{cd}	47.41^{de}
	Moringa	20.59	72.41^{a}	27.59^{g}
	Jack leaves	15.53	46.45^{d}	53.55^{d}
Average±SD		19.75±4.12	49.28±21.07^{b}	50.72±21.07^{a}
A4	Soybean	34.78	65.31^{ab}	34.69^{cd}
	Roasted soybean	38.92	42.92^{cd}	57.08^{ab}
	Soybean meal	49.30	50.72^{bc}	49.28^{bc}
	DDGS	29.81	54.27^{bc}	45.73^{bc}
	CGM	58.63	31.95^{d}	68.05^{a}
	CGF	18.37	69.85^{a}	30.15^{d}
Average±SD		38.30±14.26	52.50±15.08^{b}	47.50±15.08^{a}

CP= crude protein, RDP= rumen degradable protein, RUP= rumen undegradable protein, A1= local low fiber low protein sources, A2= local low fiber high protein sources, A3= local high fiber sources, A4= imported low fiber high protein sources

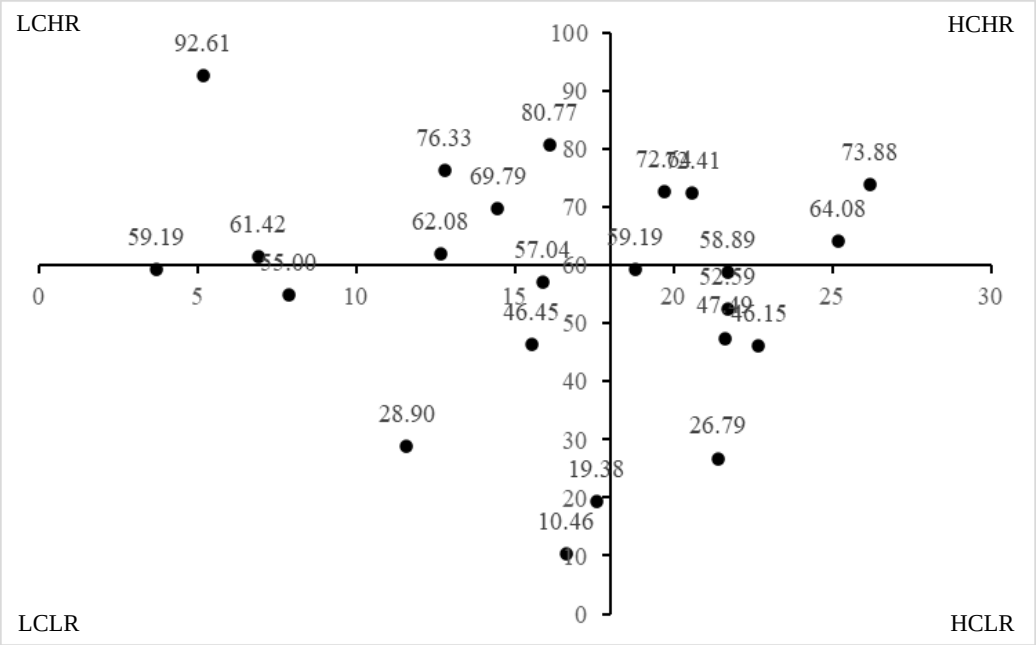

Figure 2. Tropical dairy feedstuff groups based on CP and RDP content

(Staack et al. 2019), and corn was rich in starch, approximately 70% (Hall et al. 2010). Zhao et al. (2015) revealed that the degradation rate for feedstuffs rich in pectin was higher than for feedstuffs rich in starch. This is in line with this study's finding that the RDP value of cassava was higher than corn.

Indigofera, agati, moringa, alfalfa, and gliricidia had high RDP values, followed by bauhinia, piper, narra, jack leaves, leucaena, tamarind, calliandra, albizia, and acacia. Putri et al. (2019) also showed that the RDP value of indigofera (74.72%) was high compared to gliricidia and leucaena. It indicates that indigofera can be a forage protein source with a high RDP content. The RDP value of alfalfa in this study (69.79%) was lower than that reported by Elizalde et al. (1999), ranging from 72.9%─81.1% at different stages of maturity (mid-vegetative, early bud, early flowering, and late flowering). The high RUP of acacia was due to its high fiber content. Abdulrazak et al. (2000) reported that acacia had a high proportion of ADF fraction, which means high cellulose and lignin content. Also, the CP is negatively correlated with ADF and the polyphenolic compound.

For A4, CGF had the highest RDP value compared to other imported feedstuff. Maskaľová et al. (2014) reported that the RDP content of soybean meal, CGM, and CGF were 71.0%, 20.3%, and 74.8%, respectively. The data shows that the roasted soybean's RUP value was higher than the soybean's due to the heating process. A previous study revealed that soybeans under heat treatment had a higher RUP value than unheated soybeans (Petit et al. 2002). Tempe waste had the highest RDP value in A2, while palm kernel meal had the lowest. The low RDP value of palm kernel meal is due to its high crude fiber content (Abdeltawab and Khattab 2018). Crude fiber is negatively correlated to the RDP value of feed (Rosmalia et al. 2021).

The quality of feed protein sources is distinguished by their CP content and a balanced RDP and RUP. Feed protein sources contained a minimum protein of 18%─20%, and the minimum RDP and RUP contents were 60% and 40% (NRC 2001). Based on CP and RDP content, tropical dairy feedstuff can be divided into 4 groups, which are shown in Figure 2. The four groups are LPLR (low CP and low RDP), HCLR (high CP and low RDP), LCHR (low CP and high RDP), and HCHR (high CP and high RDP). For concentrate feed, the LCPLR group is corn, cassava waste, and palm kernel meal; LCHR includes cassava, rice bran, wheat, pollard, and tempe waste; HCLR is a soybean meal, roasted soybean, DDGS, CGM, and tofu waste; HCHR is soybean and CGF. For forage feed, LCLR includes acacia, albizia, tamarind, and jack leaves; LCHR is alfalfa; HCLR is narra, calliandra, bauhinia, leucaena, and piper; HCPHR includes gliricidia, indigofera, moringa, and agati. Some factors that influence RDP level are the types of protein, the proportion of NPN, the physical and chemical properties of the protein, the presence of disulfide bonds and cross-linking, antinutrients, retention time, rumen pH, and proteolytic microbial activity (Bach et al. 2005; Broderick et al. 1991; Doiron et al. 2009).

CONCLUSION

The degradability and the RDP and RUP content of tropical dairy feedstuff vary and differ among types of feed. Most energy feed sources are highly degraded, so

they should be combined with high RDP from the protein feed sources. Forage protein could be added to dairy ration as a source of RUP. Furthermore, this information can be used in dairy formulation by considering the protein degradation of feedstuff.

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Effect of Total Mixed Ration Feeding System on Dry Matter Intake, Nutrient Intake, and Onset of Estrus in Growing Dairy Cattle

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ABSTRAK

Barros A, Guadayo GF, Sevilla CC, Bautista JAN, Dizon JT, Loresco MM, Narag RAB, Angeles AA. 2024. Pengaruh sistem rasio pakan lengkap terhadap konsumsi bahan kering dan nutrisi serta awal estrus pada sapi perah yang sedang tumbuh. JITV 29(4):193-200. DOI:http://dx.doi.org/10.14334/jitv.v29i4.3354.

Studi ini dilakukan untuk membandingkan keuntungan pemberian pakan lengkap atau *total mixed ration* (TMR) dibandingkan dengan pemberian pakan komponen (COMP) terhadap konsumsi bahan kering (KBK), dan konsumsi nutrisi dalam hal protein kasar (PK), serat deterjen netral (NDF), dan konsumsi serat deterjen asam (ADF), serta timbulnya berahi. Sepuluh ekor sapi dara dan jantan muda persilangan Friesian Holstein dan Jersey di Dairy Training dan Research Institute digunakan untuk percobaan pakan selama 5 bulan. Penelitian menggunakan Rancangan Acak Kelompok Lengkap (RAKL) dengan 5 blok di bawah 2 kelompok perlakuan. Pakan TMR dan COMP menggunakan bahan pakan yang sama yaitu silase jagung, rumput gajah, konsentrat mash, campuran kacang-kacangan, molase, dan garam beryodium dengan jumlah dan kebutuhan nutrisi yang sama. TMR dicampur terlebih dahulu sebelum diberikan sementara bahan pakan COMP diberikan secara terpisah pada ternak dengan mengikuti jadwal pemberian makan.Data dikumpulkan dari konsumsi pakan harian dari ransum yang diberikan. Sisa pakan ditimbang setiap hari sebelum pemberian pakan dimulai pada pagi hari. Pertambahan berat badan (PBB) ditimbang setiap dua minggu sekali. Sampel dari pakan yang diberikan dan sisa pakan dikumpulkan seminggu sekali untuk dilakukan analisis kandungan nutrisi. Data dianalisis dengan menggunakan prosedur ANOVA dari Perangkat Lunak Statistik SAS. Hasil penelitian menunjukkan bahwa konsumsi BK, PK, NDF, dan ADF lebih tinggi pada kelompok TMR dibandingkan dengan kelompok COMP. Perbedaan rata-rata untuk PBB, rasio konversi pakan, dan timbulnya berahi tidak berbeda nyata. Secara keseluruhan, TMR diharapkan dapat bermanfaat dalam beternak sapi perah.

Kata Kunci: Komponen, Sistem Pemberian Pakan, Budidaya Sapi Perah, Pakan Lengkap

ABSTRACT

Barros A, Guadayo GF, Sevilla CC, Bautista JAN, Dizon JT, Loresco MM, Narag RAB, Angeles AA. 2024. Effect of total mixed ration feeding system on dry matter intake, nutrient intake, and onset of estrus in growing dairy cattle. JITV 29(4): 193-200. DOI:http://dx.doi.org/10.14334/jitv.v29i4.3354.

The objective of the study was to compare the advantages of total mixed ration (TMR) feeding versus component (COMP) feeding concerning dry matter intake (DMI) and nutrient intake in terms of crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) intake, and the onset of estrus. A total of ten growing Holstein Frisian (HF) x Jersey breeds were randomly assigned to participate in five months of feeding trials at the Dairy Training and Research Institute. The design structure employed a randomized complete block design (RCBD) comprising five blocking factors under two treatment groups. The TMR and COMP were composed of the same feed ingredients, including corn silage, Napier grass, concentrate mash, mixed legumes, molasses, and iodized salt, and they had the same quantity and nutrient requirements. The TMR was prepared and mixed in advance, prior to the commencement of the feeding trial. In contrast, the components of the COMP were offered separately to the cattle following the specified feeding schedule. The data were collected from the daily intake of the rations offered. The feed refusal was weighed daily before the morning feeding periods, while the body weight gain (BWG) was recorded every two weeks. While samples of the offered and refused feeds were collected once a week for nutritional analysis, the data were subjected to the ANOVA procedure of SAS Statistical Software. The results demonstrated that DM, CP, NDF, and ADF intake was higher in the TMR than in the COMP group. However, the differences in means for BWG, feed conversion ratio, and the onset of estrus were not significantly different. In conclusion, TMR is expected to be beneficial in raising growing dairy cattle.

Key Words: Component Feed, Feeding System, Growing Dairy Cattle, Total Mixed Ration

INTRODUCTION

Dairy farming represents one of several livestock production systems encompassing a range of farm sizes, from small subsistence farms to large-scale commercial operations. However, dairy farms have encountered numerous constraints due to the unavailability of quality and quantity of feed resources and the impact of these limitations on the growth, health, and reproduction of animals and their subsequent ability to produce milk.

The utilization of feed represents the most significant production expense for dairy farms and, therefore, necessitates efficient management. The expenditure on animal feed can represent as much as 60 to 70% of total production costs, given that it constitutes the most significant single production cost for livestock operations. A variety of feed ingredients can be used in animal rations, none of which are particularly noteworthy. It is of paramount importance to ensure that the nutrients are accessible in order to meet the nutritional requirements of the cattle. Various by-product feed alternatives are now available, offering an inexpensive means of providing additional nutrition.

Nevertheless, the most commonly utilized feed ingredients for animal feed are forage crops including maize (*Zea mays*), sorghum (*Sorghum bicolor*), Napier grass *(Pennicetum purpureum*), and legumes such as Moringa *(Moringa oleifera),* Kakawate *(Gliricidia sepium),* Ipil-ipil *(Leucaena leucocephala),* Indigo *(Indigofera tinctoria),* and Colopo *(Calopogonium mucunoides)* are fed on a fresh or dry matter basis. It is anticipated that the utilization of feed in an effective and efficient feeding system will increase dairy cattle's production traits, thereby reducing feed costs. TMR is one of the feeding systems that can reduce feed costs and is currently the most widely applied in dairy farms worldwide. This strategy allows for the effective and efficient utilization of available feed resources, thereby overcoming the feed shortage of dairy cows (Karunanayaka et al. 2021). A TMR feeding strategy for cattle involves combining feeds with varying nutrient content into a single feed mix. A TMR is provided to dairy cows, which comprises all of the necessary feeds and nutrients. It constitutes a complete and balanced diet, providing the nutritional requirements for optimal cow health. The regular management of TMR can ensure that the cows obtain the nutrients they require for healthy growth, production, and reproduction; this is due to the significant effect that TMR has on the feed intake, feed efficiency, body weight gain, and the attainment of early sexual maturity of growing dairy cattle. The feeding of TMR according to animal requirements is more efficient than the traditional feeding system.

It was hypothesized that the feeding trial of COMP and TMR on growing dairy cattle (HF x Jersey) would demonstrate an advantage of a TMR-based system, including the minimization of selective consumption, the predetermination of daily nutrient intake, and the incorporation of unpalatable or low-quality feeds in the TMR. Notwithstanding the aforementioned advantages, TMR feeding in dairy cattle production remains infrequent at the farm level.

The objective of the experiment was to obtain reliable data that would enable a comparison of the efficiency and effectiveness of TMR over the COMP feeding technique. The feed cost represents a significant proportion of the overall expense incurred by dairy farms. Therefore, ensuring that this component is utilized as efficiently as possible is essential. It represents the most significant financial outlay associated with heifer production. It is anticipated that enhancing feed efficiency through reducing feed costs will facilitate the implementation of an appropriate feeding strategy, thereby optimizing the utilization of feed and enhancing the production traits of dairy cattle.

The TMR feeding system used in this feeding trial was formulated from the following ingredients: Napier grass, corn silage, a mixture comprising Moringa, Kawate, and Ipil-ipil concentrate mash, molasses, and iodized salt; this is the same composition as that of the COMP feeding, with the exception that the COMP feeding was administered separately. The COMP and TMR feeds were formulated to have the exact nutrient requirements and were administered to the cattle at 3% of their body weight per day on a dry matter basis. The TMR feeding system is anticipated to confer an advantage in terms of growth and reproductive performance in replacement heifers.

In addition to genetics and body weight factors, nutritional intake can influence the onset of puberty. Nutritional factors such as protein, energy, minerals, and vitamins have been demonstrated to influence reproductive function. Heifers fed an appropriate diet typically reach puberty between nine and 15 months of age (Senger 2012). Following the onset of puberty, a heifer will continue to exhibit regular estrous cycles, occurring every 21 days (the normal range is every 18 to 24 days). Several hormones and organs regulate the estrous cycle in cattle. The monitoring and reading of the estrus are frequently conducted based on the common estrus signs, which include the cow standing to be mounted by other cows and moving forward with the weight of the mounting cow; this is the most common and accurate symptom of estrus, Mounting other cows is an indicator that the cow is in or approaching the estrous phase. The mucus discharge is an unintended consequence of elevated estrogen levels during estrous. Mucus may be observed on the tail, thighs, flanks, or perineal region of the cow or in the form of long, viscous, clear elastic strands hanging from the vulva. The vulva enlarges in the heat, becoming

moist and red on the internal surface, exhibiting restlessness and a tendency to trail.

MATERIALS AND METHODS

Feeding trials

The experimental units were 10 growing HF x Jersey with an initial average body weight of 195±21.9 kg, assigned in feeding trials. The design structure applied a Randomized Complete Block Design (RCBD) with five blocking factors based on initial body weight and sex in two farms where animals were treated under two treatments. The animals in Treatment 1 (COMP feeding) were provided with corn silage, fresh Napier grass or hay, concentrate mash (26% CP, 2530 kcal/kg ME), and air-dried legume mixture (Moringa, Kakawate, Ipil-ipil), molasses, and iodized salt. The animals in Treatment 2 were fed a TMR comprising the same ingredients. The Napier grass was chopped using a machine chopper with a 2 to 5-cm particle size, the same size as the corn silage. The corn silage was obtained from the silage bunker of the Dairy Training and Research Institute, Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños.

In the initial feeding trial, the treatment groups were fed COMP and TMR formulated with the following proportion: 41.26% corn silage, 13.74% Napier grass, 2% Ipil-ipil and Kakawate, 1% Moringa, 35.76% concentrate mash, and iodized salt. These formulations were created using the Best Mixed software. At the same time, the COMP and TMR were formulated with approximately 15.9% crude protein and 2470.0 kcal/kg metabolizable energy to be provided daily to growing HF x Jersey dairy cattle.

The TMR feed was physically combined by hand mixing shortly after the requisite ingredients were weighed and placed on the floor. This process was repeated until homogeneous mixing was achieved, thereby preventing the cattle from dividing the feed into sections. The TMR was manually combined once a day in the morning, with the quantity required for the cattle being calculated on a daily basis. The cattle were fed for a period of five months, with 14 days to the adjustment period to allow for acclimation to the feed, pen, and environment. Immediately following the adaptation period, the animals were weighed to determine their initial weight. The feed was administered twice daily at 08:30 and 13:30 for the component feed and three times daily for the TMR at 08:30, 13:30, and 16:00, respectively. The amount offered was 3% dry matter per animal's body weight per day.

Data were collected daily through observation of the daily feed intake (in kilograms per day) of the rations offered. The daily feed refusal was weighed before the morning feeding commenced, while the body weight gain was recorded and weighed at two-week intervals. The samples of feed offered and feed refusals were collected once a week for the purpose of nutrient analysis to determine the nutrient intakes of the animals.

Onset of estrus

Six (6) HF x Jersey heifers with average initial weights of 195.08±17.99 kg and average ages of 361±16.70 days were identified as exhibiting signs of estrus through visual observation. The detection of estrous behavior was initiated at the commencement of the feeding trial when the cattle were approximately 12 months of age. The observation was conducted four times daily, at 7 a.m., 9 a.m., and 6 p.m. and 8 p.m. The signs of estrous that were observed included mounting to other cows, bellowing, mucus discharge from the vagina, swelling, and reddening of the vulva, as well as other indications. If a cow was identified as being in estrus for the first time, this was confirmed and verified at the subsequent estrus cycle (21 days) to ascertain a definite estrus day. The onset of estrus indicated that the growing female was reaching sexual maturity.

Data analysis

The data obtained from the daily feed intake (kg/d), nutrient intake, body weight gain (kg), average daily gain (kg/d), and feed conversion ratio were subjected to the analysis of variance (ANOVA) using the SAS Statistical Software. The correlation between age, body weight, and feed intake with the age at first estrus and the number of days of heat occurrence after feeding was measured using an Excel spreadsheet.

RESULTS AND DISCUSSION

Feed and nutrient intake

Table 1 presents the data on feed intake and nutrient intake from the COMP and TMR feeding systems on the growing HF x Jersey, divided into two treatment groups. The total dry matter and daily dry matter intakes of growing dairy cattle fed a diet COMP, and TMR was found to be higher in the TMR group than in the COMP group, with a significant difference at the $P<0.01$ level. A significant difference ($P<0.01$) was observed in crude protein intake between the two treatment groups. The NDF intake exhibited a highly significant difference ($P<0.001$) between the two dietary treatment groups. The result was observed in

Table 1. Feed intake and nutrient intake of the cattle in two different treatment groups

Parameters	Treatments		SEM	P-Value
	COMP	TMR		
Feed Intake, kg:				
Total DMI, kg	953.13[a]	1146.89[b]	44,86	0.0024
DDMI, kg/d	6.14[a]	7.32[b]	0.34	0.0053
Nutrient Intake, kg/d				
CP	1.18[a]	1.34[b]	0.09	0.0062
NDF	3.23[a]	4.20[b]	0.14	0.0004
ADF	1.52[a]	2.55[b]	0.04	<.0001

COMP= component, TMR= total mixed ration, DMI= dry matter intake, DDMI= daily dry matter intake, CP= crude fiber, NDF= neutral detergent fiber, ADF= acid detergent fiber. a-b means within columns with different superscipts were highly significant different (P<0.01)

the cattle-fed COMP, which exhibited a lower NDF value than the cattle that received TMR feeding. The intake of ADF was found to be the highest (P<0.001) in cattle-fed TMR.

The objective of the feeding trial was to ascertain whether there were any advantages to TMR over the traditional component feeding system on DM intake, CP intake, NDF and ADF intake, and the onset of estrus. The rations were formulated on a dry matter basis. The amounts of each feed were fed on as fed basis. All cattle in the group got the same ration and consumed a mixed amount of forages and concentrates intended for production and health. The intake of TMR and component feed demonstrated a significant difference. Feeding a TMR increased feed intake due to the minimization of selective consumption of the growing dairy cattle.

The notable discrepancy in feed intake observed in this feeding trial is consistent with the findings of a recent study (Lee et al. 2015), which reported that TMR feeding resulted in higher feed intake (P<0.05) during the growing period compared to other treatments. The findings of the present study are also supported by the results reported by Li et al. (2021), who observed that TMR feeding resulted in increased average daily feed intake and daily gain. It may be posited that consumption of TMR by growing dairy cattle is greater than that of other ruminants due to the lack of alternative feed options. Consequently, the nutritional profile of each feedstuff was consistent and easily definable, approximating a nutritionally complete diet (Schingoethe 2017). This finding was consistent with the purported benefits of TMR for cattle, including enhanced feed efficiency, increased feed utilization, and the consumption of the optimal proportion of ingredients for a balanced ration. Compared to the COMP feeding regimen provided in a separate manner, the cattle were allowed to select their own diet.

Furthermore, the forage particle size was considered in the analysis of dry matter intake; this is linked to the rate at which feeds are digested in the rumen, which in turn affects the animal's ability to digest the feed (Yang & Beauchemin 2006, as cited by Melendez & Roy 2016). The TMR diet in this feeding trial had a particle size of less than 5 cm, which resulted in rapid mastication and subsequent passage. The rapid passage rate may result in an increase in DMI. Conversely, large particle size will result in a longer chewing time, which will, in turn, affect the intake. However, this will facilitate the degradation of fiber in the rumen and enhance the homogeneity of the feed (Melendez & Roy 2016).

Several factors exert an influence on the voluntary DMI of animals. Individual animal characteristics: physical condition, body weight, the physical fill of the reticulorumen, and metabolic-feedback elements (Illius & Jessop 1996; Mertens 1994, as cited by Allen 2000), can also influence voluntary DMI. In the case of growing dairy cattle of the HF x Jersey crossbreed, their dry matter intake is largely contingent upon the individual response to the feeds, the feed types, and the feeding management that predicts the voluntary DMI.

The highest CP intake was observed in cattle fed a TMR, while the lowest was in cattle that received component feeding. The disparate CP intake was consistent with the findings of Chander (2011), who reported that all animals in the treatment groups were provided with diets containing an identical percentage of CP. However, the observed increase in CP intake may be attributed to variations in the DM intake of the feeds. These findings align with those of Sarker et al. (2020), who conducted a similar study investigating the comparative advantages of TMR feeding over conventional feeding. The data demonstrate that the DM and CP intake of the TMR group was significantly (P<0.05) superior to that of the control group. Sarker et al. (2020) investigated the impact of paddy straw-based TMR on milk yield, milk composition, and rumen parameters in lactating cows. Their findings revealed that CP intake differed significantly (P<0.05) between

cattle-fed TMR blocks and those receiving component feeding.

The NDF intake exhibited a highly significant difference ($P<0.001$) between the two dietary treatment groups. The result was observed in the cattle-fed COMP, which exhibited a lower NDF value than the cattle that received TMR feeding. This result was similar to the findings of Hundal et al. (2004), who observed a significantly higher ($P<0.05$) NDF digestibility when the diet was fed as TMR as compared to the conventional feeding system. The intake of ADF was found to be the highest ($P<0.001$) in cattle that were fed TMR. These findings were also under those of Sarker et al. (2020) who observed a significant difference ($P<0.05$) in ADF intake between TMR feeding groups.

Moreover, the increased consumption of the TMR diet resulted in elevated levels of CP, NDF, and ADF intake; this was because the amount of CP, ADF, and NDF consumed significantly depended on the amount of DM consumed from the TMR. The value of CP, NDF, and ADF intakes from TMR feeding increases proportionately to the quantity of DM consumed. Allen (2000) reported that forage NDF content was more highly related to the DMI of forage than other chemical measures. Furthermore, Waldo (1986), as Allen (2000) cited, suggested that NDF content is the best single chemical predictor of DMI by ruminants. Mertens (1994), as cited by Allen (2000), employed NDF as the sole feed characteristic to forecast the filling effect and energy content of diets; when energy intake is constrained, a positive correlation was observed between DMI and NDF concentration. Conversely, a negative correlation was noted between DMI and NDF concentration when filling intake is limited. Furthermore, the nutritional impact of NDF is contingent upon a multitude of factors, including the form, digestibility, and chemical composition of the TMR (Allen 2000). The digestibility of forages, co-products, and other fibrous feeds varies considerably, impacting the energy supply.

Body weight gain and feed conversion ratio

Table 2 presents the data regarding weight gain and feed conversion ratio for 10 growing dairy cattle (HF x Jersey) over the course of five months. The data presented in Table 2 indicate that there was no statistically significant difference ($P>0.05$) in BWG, ADG, and FCR between the growing dairy cattle that were fed a COMP and those that were fed a TMR diet. However, the observed values for BWG and ADG were higher in the TMR-fed cattle.

Ten (10) growing dairy cows were included in the feeding trial with an average initial weight of 195.10±28.62 kg for cows fed COMP and 194.90±15.99 kg for cows fed TMR. After 153 days (22 weeks) of the feeding trial, there was no significant difference ($P>0.05$) in BWG and ADG between the two treatment groups; this was in line with Saraye (2014), who reported that after the experimental period, there was no significant difference ($P>0.05$) between the two feeding methods. The result also aligned with Lee et al. (2015), who reported that daily gain was not different during the growing period. Sarker et al. (2020) found that the body weight gain was not significantly different ($P>0.05$) but was slightly higher in TMR feeding groups; however, no significant differences were found between the two treatments. The result was also in agreement with Sarker et al. (2020), who reported no differences in body weight gains for roughage and concentrate ratio in TMR formulation; this was in contrast to the finding of Nissanka et al. (2010) that the TMR feeding system was significantly different ($P<0.05$) from conventional feeding in Friesian heifers.

The growing dairy cattle in the two dietary treatment groups showed that the cattle-fed TMR had practically higher BWG than the cattle-fed COMP. However, there was more significant variability in the initial body weight of cattle receiving COMP versus TMR, especially in block 5, as the feeding trials started at 237.5 kg versus 191 kg; this may have affected the body weight gain and may not have shown the difference between the COMP and TRM groups; this is consistent with the results of the study by Ngadiyono et al. (2019), who reported, that the difference between breed and initial body weight significantly affected the ADG in cattle.

Increases in body weight gains were linear for heifers and steers of all ages in both treatment groups. Average daily gains ranged from 0.61 to 1.09 kg, was in line with NRC recommendations based on nutrient requirements. The use of TMR for young heifers may result in a better balance of nutrient intake by avoiding individual preferences for forage or concentrates, as reported by Borland & Kesler (1979) and cited by Nissanka et al. (2010).

The data presented in FCR shows that the cattle fed TMR exhibited a lower feed conversion ratio (FCR) of 9.5:1 in comparison to the COMP group, which demonstrated an FCR of 8.3:1. The physiological capacity of the growing dairy cattle to convert feed into meat indicated that the cattle fed COMP required 8.3 kg of DM to produce 1 kg of meat compared to the TMR group, which required 9.5 kg of DM to produce 1 kg of meat. This value was comparable to the findings of Heinrichs et al. (2016), who reported that the older heifers fed with poor forage had an FCR of 8:1 or more (with some cases exhibiting an FCR of 15:1). It is possible to achieve an efficiency value of 6:1 to 7:1 through the use of TMR feeding. Yearling heifers fed high-quality feeds and forages can readily achieve a feed-to-gain ratio of 5:1. However, when fed less

Table 2. Body weight gain and feed conversion ratio of the cattle in two different treatment groups

Parameters	Treatments		SEM	P-Value
	COMP	TMR		
Initial BW, kg	195.10	194.90	18.52	0.9872
Final BW, kg	314.70	317.90	30.74	0.8772
BWG, kg	119.60	123.00	12.80	0.6776
ADG, kg/d	0.78	0.80	0.08	0.6285
FCR	8.34	9.52	0.09	0.0560

COMP= component, TMR= total mixed ration, BW= body weight, BWG= body weight gain, ADG= average daily gain, FCR= feed conversion ratio

digestible high-forage diets, this ratio can decline to 8:1 or higher. Shike (2013) defined the feed conversion ratio as the ratio of dry matter intake to live weight gain to quantify feed efficiency. A typical range of feed conversion ratios is 4.5 to 7.5, with a lower number being more desirable as it would indicate that a steer required less feed per pound of gain.

Onset of estrus

Table 2 illustrates the impact of the feeding system on the onset of the estrus of growing dairy cattle HF x Jersey breeds, with the animals being fed either a COMP or a TMR diet. The results show no statistically significant difference (P>0.05) in the feeding of TMR about the onset of estrus. However, heifers fed TMR exhibited an earlier onset of estrus following the implementation of feeding trials.

The initial age (day) of the dairy heifers was not significantly different (P>0.05) at the commencement of the experiment. The age at first estrus was lower in the TMR group, although no substantial difference was noted between the two treatment groups. The number of days during which heat occurred following the commencement of feeding was lower in the TMR feeding groups. Nevertheless, the two dietary treatment groups observed no significant difference (P>0.05).

The diet was formulated to meet the nutrient requirements of the heifers in TMR feeding systems. It was established that the ration had been formulated following the recommendations set forth by the NRC with a metabolizable energy content of 2.47 Mcl/kg and a CP of 15.9%. Karunanayaka et al. (2021) stated that a proper nutritional formulation of the diet is essential to ensure the provision of all nutrients. TMR may prove an efficacious nutritional strategy for improving follicular dynamics concerning reproductive hormones and metabolites. The relationship between nutrient intake (Table 3), the onset of estrus, and the number of days of heat occurrences (NDHA) after feeding was moderately negatively correlated; this indicates that dietary factors were among the influences on the age at first estrus in growing dairy cattle HF x Jersey breeds.

In addition to nutritional factors, body weight gain did not affect the age of puberty (Table 3) despite the close relationship between weight gain and the onset of puberty. The onset of puberty in heifers is contingent upon many factors, including but not limited to breed, age, and body weight. Most dairy breeds reach puberty by 11-12 months of age or even earlier if they are fed per the minimum standards set forth by the National Research Council for energy, protein, minerals, and vitamins (Stevenson & Ahmadzadeh 2011). Breeders frequently employ the weight of 275 kg to 350 kg as a criterion for sexual maturity in cows. The aforementioned data exceeded the results obtained from the feeding trial, wherein the age at first estrus of dairy heifers of HF x Jersey breeds was reached at 220 to 286 kg body weight at 400 to 488 days (approximately 13 to 16 months). This finding aligns with Schillo (2011), who reported that heifers fed an appropriate diet typically reach puberty between 9 and 15 months.

Table 3. Means of initial age, age at first estrus, and number of days of heat occurrence in dairy heifers fed COMP and TMR feeding

Parameters	Treatments		SEM	P-VALUE
	COMP	TMR		
Initial age, d	367	356.2	12.6	0.5383
Age at first estrus, d	438	413.5	26.2	0.6011
NDHA, d	85.7	59.5	19.3	0.4849

COMP= component, TMR= total mixed ration, NDHA= Number of days of heat occurrence after feeding

Table 4. Pearson Correlation Coefficient (r^2) of the BWG, Nutrient Intake with the Onset of Estrus and NDHA in Dairy Heifers During the Feeding Trial

Parameter	AE, d	DMI, kg	CPI, kg	NDHA, d	BWG, kg
AE, d	0				
DMI, kg	-0.57	0			
CPI, kg	-0.67	0.98	0.		
NDHA, d	0.87	-0.57	-0.60	0	
BWG, kg	-0.36	0.80	0.73	-0.46	0

AE= Age at first estrous, DMI= Dry matter intake, CPI= Crude protein intake, BWG= Body weight gain, NDHA= Number of days of heat occurrence after feeding

However, it should be noted that there is considerable variation in the average age at puberty, with figures ranging from approximately 8 to 24 months. For heifers to reach early puberty, it is essential that they receive an adequate nutritional intake, as once this stage is reached, the heifer must be able to maintain a level of nutrition that allows for the continuation of the estrus cycle; this is by Bindari et al. (2013) that nutrition plays a major role in enhancing reproductive efficiency in all animals. Energy and protein are the major nutrients required in the greatest amounts and should be the topmost priority to optimize reproduction efficiency in dairy cattle.

CONCLUSION

According to the study's findings, the TMR feeding system did not affect the growth performance of BWG, ADG, and FCR, except for DMI, CPI, NDFI, and ADFI, which were more significant in the TMR feeding group. The occurrence of the estrus was not affected by the TMR feeding system. However, there was a moderately negative correlation between DMI and CPI with the onset of estrus and the number of heat occurrences after feeding the ration.

Overall, TMR performed a better feeding system. Growing dairy cattle consumed more TMR because they had no choice among the feeds offered. As a result, each feed consumed was uniform, definable, and as close as one can make a nutritionally complete diet. The TMR feeding is expected to be beneficial in raising growing dairy cattle.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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Emerging Challenges: Methicillin and Vancomycin Resistance in *Staphylococcus aureus* from Urinary Tract Infections in Ewes of Diyala Governorate, Iraq

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Abstrak

Bak AIH, Al-Ezzy AIA, Al-Zubaidi RMH. 2024. Tantangan: resistensi methicillin dan vancomycin terhadap *Staphylococcus aureus* akibat infeksi saluran kemih pada domba betina di Provinsi Diyala, Irak. JITV 29(4):201-207. DOI:http://dx.doi.org /10.14334/jitv.v29i4.3407.

S. aureus yang resistan terhadap methicillin merupakan penyebab infeksi saluran kemih (ISK) yang jarang terjadi. Penelitian saat ini bertujuan untuk mengisolasi dan mengidentifikasi S. aureus dari sampel urin domba betina yang mengalami ISK dan menentukan pola sensitivitas antimikroba untuk resistan terhadap methicillin-Vancomycin. Sebanyak 71 sampel urin dikumpulkan. S. aureus diisolasi menggunakan agar garam manitol dan dikonfirmasi oleh sistem VETEK2 dan PCR konvensional, menggunakan primer (Staur 4, 6) dan (mecA). S. aureus diisolasi dari (9,85%). Semua S. aureus yang diisolasi dari domba betina, (100%) memiliki resistensi terhadap Penisilin, sefalosporin dan resistensi methicillin dideteksi oleh uji skrining cefoxitin dan dikonfirmasi oleh deteksi gen MecA. Resistensi s. aureus terhadap antibiotik polipeptida dideteksi pada 100% untuk vancomycin dan (85,72%) untuk Teicoplanin. Resistensi S. aureus terhadap antibiotik makrolida dan Lincosamides terdeteksi pada (14,28%) untuk Azitromisin dan Klindamisin masing-masing. aureus yang diisolasi dari domba betina memiliki sensitivitas absolut terhadap aminoglikosida, kuinolon, makrolida (Eritromisin), oksazolidinon, tetrasiklin, antibiotik nitrofuran, Fusidane, An-samycins dan Sulfonamida. Studi ini menemukan prevalensi MRSA yang signifikan di antara galur S. aureus yang diisolasi dari wanita dengan ISK. Ini menunjukkan tingkat resistensi yang tinggi terhadap antibiotik beta-laktam, yang menimbulkan tantangan untuk pengobatan yang efektif. Studi ini juga mengidentifikasi resistensi vankomisin di antara isolat MRSA. Vankomisin sering dianggap sebagai pertahanan garis terakhir terhadap bakteri yang resistan, sehingga keberadaan S. aureus yang resistan terhadap vankomisin mengkhawatirkan dan membatasi pilihan pengobatan.

Kata Kunci: Domba Betina, *Staphylococcus aureus,* Infeksi Saluran Kemih

Abstract

Bak AIH, Al-Ezzy AIA, Al-Zubaidi RMH. 2024. Emerging challenges: Methicillin and vancomycin resistance in *Staphylococcus aureus* from urinary tract infections in ewes of Diyala Governorate, Iraq. JITV 29(4):201-207. DOI:http://dx.doi.org/ 10.14334/jitv.v29i4.3407.

Methicillin resistant s. aureus is a rare cause of urinary tract infections (UTIs). Current study aims to Isolates and identify *S. aureus* from urine samples of ewes with UTIs and determination of antimicrobial sensitivity pattern for methicillin-Vancomycin resistant. A total of 71 urine samples were collected. S. aureus was isolated using mannitol salt agar and confirmed by VETEK2 system and conventional PCR, using (Staur 4, 6) and (mecA) primers. *S. aureus* was isolated from (9.85%). All *S. aureus* isolated from ewes, (100%) have resistance for Penicillines, cephalosporins and methicillin resistance was detected by cefoxitin screen test and confirmed by detection of MecA gene. Resistance of s. aureus to polypeptides antibiotics was detected in 100% for vancomycin and (85.72%) for Teicoplanin. Resistance of S. aureus to macrolides and Lincosamides antibiotics was detected in (14.28%) for Azithromycin and Clindamycin respectively. *S. aureus* isolated from ewes have absolute sensitivity for aminoglycosides, quinolones, macrolides (Erythromycin), oxazolidinone, tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides. The study found a significant prevalence of MRSA among the isolated strains of S. aureus from ewes with UTIs. This indicates a high level of resistance to beta-lactam antibiotics, posing challenges for effective treatment. The study also identified vancomycin resistance among the MRSA isolates. Vancomycin is often considered a last-line defense against resistant bacteria, so the presence of vancomycin-resistant S. aureus is concerning and limits treatment options.

Key Words: Ewes, *Staphylococcus aureus*, Urinary Tract Infection

INTRODUCTION

The urinary system is one of the most important systems in an animal's body. It removes harmful waste from the body and regulates the components of body fluids, as well as controlling hormone production which promotes bone marrow to red blood cell synthesis (Pugh et al. 2020) The colonization and infection of one or more urinary system sections is referred to as urinary tract infection (UTIs) (Mohammed et al. 2020). Bacteria infiltrate the gastrointestinal system and colonize the external genitalia, as well as invade the bladder and urethra and impede urine flow UTI also causes vascular damage in the urinary bladder, reducing kidney competence and interfering with metabolic end product excretion (El-Deeb & Elmoslemany 2016).

S. aureus is opportunistic and its virulence depends on enzymes and exotoxins, that contribute to causing a wide range of diseases in human (Cheung et al. 2021)and animals (Dai et al. 2019) . Its variable sensitivity and resistance for numerous antibiotics represent the major reason behind the cosmopolitan distribution (Rasmi et al. 2022). Production of β-lactamases was the main trait for *S. aureus* that cause continues failure in treatment with beta lactam antibiotics as penicillin. Methicillin-resistant *S. aureus* (MRSA) possesses reduced affinities for binding to β-lactam antibiotics by producing a specific penicillin-binding protein, PBP2 , resulting in β-lactam antibiotic resistance (Lade & Kim 2021; Bush & Bradford 2020). Methicillin resistance is extended to different antimicrobial agents, including "the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (Cabrera et al. 2020). While (Bitrus et al. 2015) was reported resistant to all cephems, cephalosporins, and other β-lactams such as (amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, ampicillin-sulbactam, carbapenems, and the piperacillin-tazobactam). This study aimed to isolation and characterization of *S.aureus* from urine samples of ewes with UTIs by classical bacteriological methods, Vitek 2 system and conventional PCR using Staur 4, 6 specific primers, Determine antimicrobial sensitivity pattern for methicillin-Vancomycin resistant s.aureus in ewes with urinary tract infections.

MATERIALS AND METHODS

Collection of urine specimens

Urine specimens were collected from 71 ewes with UTIs from 1st October 2022 to the end of May 2023. Samples were delivered to "the postgraduate lab.at the college of veterinary medicine –university of Diyala, Iraq" in a cool box.

Processing of specimens

Samples were cultured on MSA for 18-24 h. A golden yellow colonies were selected for full identification of MRSA through Vitek system 2 and PCR according to (Fajer et al. 2023).

Molecular detection of *S. aureus* specific and Methicillin resistance genes

Conventional PCR was applied to confirm the diagnosis and detection of methicillin resistance gene according to Table 1.

Antimicrobial Susceptibility Test (AMS)

AMS test was achieved by Vitek 2 system as stated by Clinical and Laboratory Standards Institute (Humphries et al. 2018 ; Aljboori et al. 2022; Fajer et al. 2023).

Ethical consideration

This study conducted according to the approval of an ethical review committee of pathology department, college of veterinary medicine, Diyala University, Iraq (Al-Khalidi et al. 2020; Hameed et al 2020; Hassan et al. 2020).

Table 1: Molecular markers used in conventional PCR for Detection of *S. aureus* and MRSA from urine specimens of ewes

Gene	Primers	Base pairs	Sequence (5'- 3')	PCR Protocol			Reference
				Den.	Ann.	Ext.	
S.aureus 23srRNA	Staur4	1250bp	5'-ACGGA GTT AC A AAGGAC GAC-3'	94°C/ 45 sec	64°C/ 60 sec	72°C/ 2min	(Sheela 2017)
	Staur6		5'-AGCTCAGCCT TAAC GAG TAC-3'				
Methicillin Resistant Gene A	mecA-F	162bp	5-TCCAGATTACAACTTCAC CAGG-3	94°C/ 45sec	50°C/ 30sec	72°C/ 30sec	
	mecA-R		3-CCACTTCATATCTTGTAACG-5				

PCR= Polymerase Chain Reaction, MRSA= Methicillin-resistant *Staphylococcus aureus*; Den= denaturation; Ann= annealing, Ext= extention

Statistical analysis

All Calculations by using the Statistical Package of the Social Sciences for windows version 17 (SPSS, Armonk, NY: IBM Corp) (Hameed et al. 2024; Hameed & Al-Ezzy 2024).

RESULTS AND DISCUSSION

As shown in Table 2, S. aureus was isolated from 7/71, (9.85%) urine sample of ewes. All S. aureus isolates have resistant to methicillin by cefoxitin screening test in Vitek 2 system. All S. aureus have resistant for vancomycin. As shown in Table 3 and Figure 1 and Figure 2, all *S. aureus* isolates have Staur 4,6 gene and MecA A gene. Table 3 revealed that all *S. aureus* isolated have resistance for Penicillines and Cephalosporins. All *S. aureus* isolates have methicillin resistance that detected by Cefoxitin Screen test and confirmed early by detection of MecA gene. All *S. aureus* isolates have resistance to vancomycin polypeptides antibiotic and (85.72%) for Teicoplanin. Resistance of *S. aureus* to macrolides, mainly Azithromycin was detected in (14.28%). Resistance to Lincosamides antibiotics, Clindamycin was detected in 1/7, (14.28%) .*S. aureus* isolated from ewes have absolute sensitivity for the following classes Aminoglycosides, Quinolones, Macrolides (Erythromycin), oxazolidinone, Tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides.

S. aureus one of the rare pathogenic bacteria among urinary tract infection. In the current study, *S. aureus* was isolated from 7/71, (9.85%) urine samples of ewes. All *S. aureus* isolates have resistance for Penicillines, cephalosporins and methicillin which confirmed by detection of MecA gene. A total of 77 urine samples collected from ewes cultured on blood agar and mannitol salt agar which is a medium encouraging growth of certain bacteria while inhibiting the growth of other. As stated by Fajer et al. 2023 that as selective and differential medium, MSA contains a NaCL,7% and mannitol sugar1% inhibit most bacteria that makes MSA selective against most gram negative and selective for some gram-positive bacteria that tolerate high salt concentrations. As stated by Abubaker & Alythi (2021) that MSA contain a pH indicator, phenol red for detection of acid produced by mannitol-fermenting staphylococci which turn the *S. aureus* colonies in the medium appear yellowish in color. As stated by Anderson et al. (2013) that in addition to MSA which was used for the selective isolation of presumptive pathogenic Staphylococcus species, Gram stain, Catalase and Coagulase tests, Vitek2 system were used for confirmation. Current study come in line with that reported by Alzolibani et al. (2012) in which seven positive samples referred to conventional PCR and VITEK® 2 System confirm matching 99% . In the current study, the rate of *S. aureus* UTIs in ewes was 7/71, (9.85%). All isolates were MRSA which higher than that reported In north-western Greece by Papadopoulos et al. (2018) who found that 57.8% of specimens were *S. aureus* positive and only 3% MRSA positive. While in China (Zhou et al. 2017).

reported that *S. aureus* was isolated from 43.24% of specimens while in Italy, Giacinti et al. (2017) reported that *S. aureus* was isolated from 53.5% among dairy sheep farms and MRSA rates 7%. While in Norway Mørk et al. (2012) recoded *S. aureus* was (32.6%) in sheep.

In current study *S.aureus* 23srRNA gene sequence specific primers (staur4, 6,) for *S. aureus* was detected in all *S.aureus* isolates by Conventional PCR which come in line with that reported by Sheela & Krupanidhi (2017). All *S. aureus* have methicillin resistant gene, (*mecA*) by conventional PCR which is higher than that reported by Galia et al. (2019)

in Italy, they stated that MecA was detected among 58/65(89%) of *S. aureus* isolates. Acquisition of *mec*A renders β-lactams useless against MRSA and alternative therapies. In Italy, Mascaro et al. (2019) reported that in a dairy sheep farms, *S. aureus* isolate might act as a mec C-MRSA reservoir thus, it is recommended that laboratories must search for the *mecC* gene in all the *mecA*-negative isolate . Less percentage done by Omidi et al. (2020) of 146 *S. aureus* isolates, 24 (16.4%) carried *mec*A genes and identified as MRSA strains. In a study by Lakhundi & Zhang (2018) that counted mecA and its new homologues (mecB, mecC, and mecD) on thirteen types in more than ten Allele.

In a study by Joseph et al. (2018) they stated that most strains of *S. aureus* possess ability to produce beta-lactamases, an enzyme that can open beta-lactam rings in cephalosporin and penicillin. Some acquire resistance genes from the environments and/or from other bacteria and thus may exhibit resistance to antibiotics in other classes produced on plasmid encoded as class A β-lactamase (Nomura et al. 2020). Resistance for beta-lactam antibiotics usually by production of beta-lactamases; or by expression of penicillin-binding protein (PBP 2a), which is not susceptible to inhibition by beta-lactam antibiotics. *S. aureus* have beta-lactamase or PBP 2a-directed resistance (or both) (Lakhundi & Zhang 2018).

In the current study, *S. aureus* 100% resistant to methicillin that confirmed by detection of (mecA) gene which records resistant for Oxacillin. All s.aureus have resistance to vancomycin polypeptides antibiotics and 6/7, (85.72%) have resistance for Teicoplanin. Resistance of *S. aureus* to macrolides antibiotics, mainly Azithromycin and Lincosamides antibiotics, mainly Clindamycin was detected in 1/7, (14.28%). *S. aureus* isolated from ewes have absolute sensitivity for Aminoglycosides, Quinolones, Macrolides (Erythromycin), oxazolidinone, Tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides.

Table 2: Isolation rate of *S. aureus*, MRSA and VRSA from urine of ewes

Samples source	Sample number	A (%)	B (%)	C (%)
Ewes	71	7(9.85%)	7/7,(100%)	7/7 ,(100%)

A= Number of S. aureus isolates based on MSA growth, Vitek 2 system, and PCR; B= Number of MRSA from total positive samples based on Cefoxitin Screen test, conventional PCR using Mec A gene primers, C= number of VRSA from total samples based on Vitek 2 system; PCR= Polymerase Chain Reaction, MRSA= Methicillin-resistant *Staphylococcus aureus*

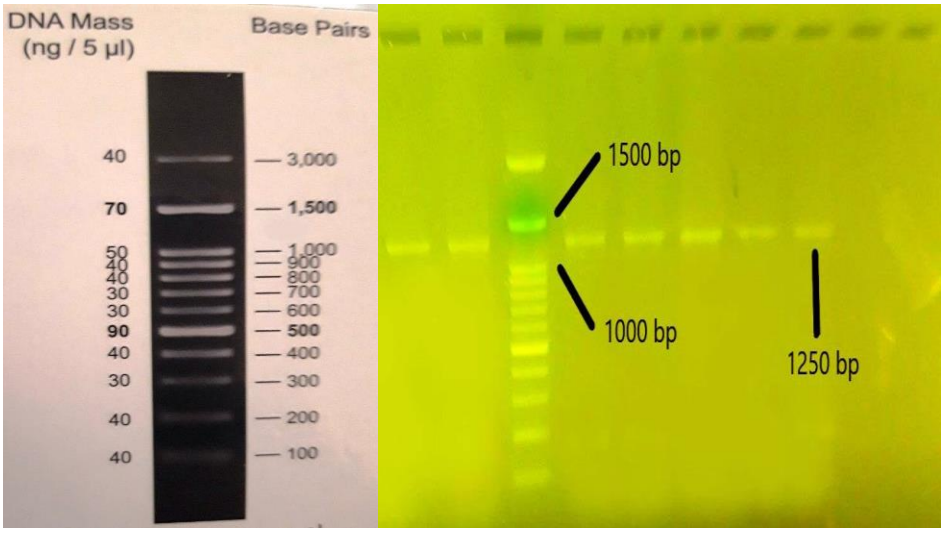

Figure 1. Amplification for staur primers 4&6 (1250bp) by conventional PCR for *S. aureus* isolated from urine of ewes

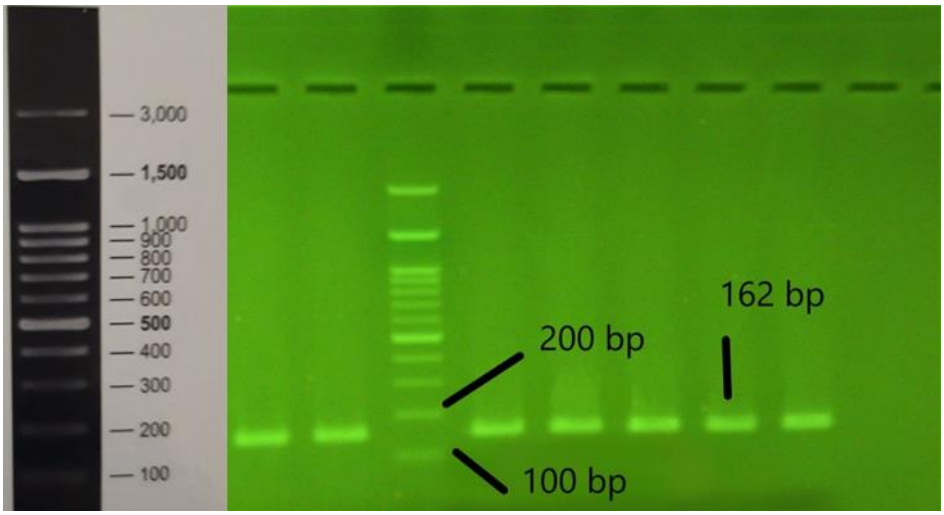

Figure 2. Amplification for methicillin Resistant gene (MecA) primer (1620bp) by Conventional PCR for S. aureus isolated from urine of ewes

In a study of Algammal et al. (2020) they stated that MRSA was determined by PCR and resistance to cefoxitin. Although Sallam et al. (2015) stated that antimicrobial resistance of MRSA detected by penicillin 93.4%, ampicillin 88.9%, and cloxacillin 83.3%, whereas .In Palestine (Azmi et al. 2019) claimed that MRSA isolates identified by cefoxitin disc diffusion and all were vancomycin sensitive and Gentamicin. In Italy, Giacinti et al. (2017) reported that 60.58% of MRSA were susceptible to all the antimicrobials drugs, and 39.42% were resistant to at least one antimicrobial drugs. In particular, 22.12% were resistant to tetracycline, 15.38% to sulfonomides, 13.46% to trimethoprim and sulfa methoxazole, and 8.65% to ampicillin, however only one isolate was resistant to both Fluoroquinolones and aminoglycosides, *S. aureus* isolates displaying resistance to oxacillin, cefoxitin, or both. Resistant to all the β-lactams tested and to erythromycin, streptomycin, kanamycin, and tetracycline.

Table 3. Sensitivity Pattern of S. aureus Isolated from Ewes with UTIs based on Vitek 2 system

Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of s.aureus isolates	Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of s.aureus isolates
Penicillines	Benzylpenicillin	>= 0.5	R	7/7,(100%)	Macrolides	Azithromycin		R	1/7, (14.28%)
	Amoxicillin Clavulanic acid		R	7/7,(100%)		Erythromycin	<= 0.25	S	7/7, (100%)
	Oxacillin	>=4	R	7/7,(100%)	Lincosamides	Clindamycin	<= 0.25	S R	6/7, (85.72%) 1/7, (14.28%)
	Cefoxitin Screen	POS	Methicillin resistance	7/7,(100%)	oxazolidinone	Linezolid	2	S	7/7,(100%)
Cephalosporins	Cefalexin		R	7/7,(100%)	Polypeptides	Teicoplanin	<=0.5	S R	1/7, (14.28%) 6/7, (85.72%)
	Cefazolin		R	7/7,(100%)		Vancomycin	*=32	R	7/7, (100%)
	Cefapime		R	7/7,(100%)					
Aminoglycosides	Gentamicin	<=0.5	S	7/7,(100%)		Doxycycline		S	7/7,(100%)
	Tobramycin	<= 1	S	7/7,(100%)	Tetracycline	Tetracycline	<=l	S	7/7,(100%)
Quinolones Fluoroquinolones	Ciprofloxacin		S	7/7,(100%)		Tigecycline	<= 0.12	S	7/7,(100%)
	Gatifloxacin		S	7/7,(100%)	nitrofuran antibiotic	Nitrofurantoin	<— 16	S	7/7,(100%)
	Levofloxacin	<= 0.12	S	7/7,(100%)	Fusidane	Fusidic Acid	<= 0.5	S	7/7,(100%)
	Moxifloxacin	<=0.25	S	7/7,(100%)	Ansamycins	Rifampicin	<= 0.5	S	7/7,(100%)
	Norfloxacin		S	7/7,(100%)	Sulfonamides	Trimethoprim/ Sulfamethoxazole	<= 10	s	7/7,(100%)

CONCLUSION

The study found a significant prevalence of MRSA among the isolated strains of S. aureus from ewes with UTIs. This indicates a high level of resistance to beta-lactam antibiotics, posing challenges for effective treatment. The study also identified vancomycin resistance among the MRSA isolates. Vancomycin is often considered a last-line defense against resistant bacteria, so the presence of vancomycin-resistant S. aureus is concerning and limits treatment options

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Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]

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ABSTRAK

Saragih HTSSG, Susanto A, Aditya NC, Damayanti SC, Firdaus ABI, Salsabila N, Nuriliani A. 2024. Performa pertumbuhan ayam broiler yang disuplementasi dengan ekstrak buah naga merah [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]. JITV 29(4):208-220. Doi: http://dx.doi.org/10.3443/jitv.v29i4.3445.

Salah satu keunggulan ayam broiler adalah masa panen yang singkat. Faktor yang mempengaruhi pertumbuhan ayam broiler salah satunya adalah kandungan nutrien pakan yang dikonsumsi. Namun, akan sulit untuk mendapatkan pertumbuhan yang optimal apabila hanya mengandalkan pakan konvensional. Oleh karena itu, diperlukan suplemen pertumbuhan yang dapat meningkatkan performa pertumbuhannya. Ekstrak buah naga merah memiliki kandungan flavonoid dan vitamin yang bertindak sebagai antioksidan, sehingga dapat digunakan sebagai suplemen untuk mendorong pertumbuhan pada ayam broiler. Penelitian ini bertujuan untuk mempelajari efek perlakuan ekstrak buah naga merah dalam air minum terhadap pertumbuhan ayam broiler. Penelitian ini menggunakan 300 DOC ayam broiler jantan yang dipelihara sampai umur 21 hari, dilakukan menggunakan Rancangan Acak Lengkap, dengan 5 kelompok dan 5 ulangan, tiap kelompok ulangan terdiri dari 12 ekor DOC. Lima kelompok terdiri dari kelompok kontrol (P0), P1 0,25%, P2 0,50%, P3 1%, dan P4 2%. Parameter yang diamati meliputi struktur histologis usus halus, otot pektoralis, dan lien, serta performa pertumbuhan ayam broiler. Hasil penelitian menunjukkan bahwa perlakuan ekstrak buah naga merah dapat meningkatkan panjang dan luas vili, rasio panjang vili dengan kedalaman kripte, jumlah dan luas sel goblet, luas fasikulus, luas serabut otot, luas pulpa putih, indeks organ limpa, serta meningkatkan performa pertumbuhan yang meliputi berat badan ayam dan efisiensi pakan. Oleh karena itu, dapat disimpulkan bahwa ekstrak buah naga merah dapat meningkatkan struktur histologis usus halus, otot pektoralis, dan limpa, serta meningkatkan performa pertumbuhan ayam broiler, terutama pada ekstrak buah naga merah dengan konsentrasi 2%.

Kata Kunci: Ayam Broiler, Performa Pertumbuhan, Struktur Histologis, Ekstrak Buah Naga Merah, Usus Halus

ABSTRACT

Saragih HTSSG, Susanto A, Aditya NC, Damayanti SC, Firdaus ABI, Salsabila N, Nuriliani A. 2024. Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]. JITV 29(4):208-220. Doi:http://dx.doi.org/10.3443/jitv.v29i4.3445.

One of the advantages of broiler chickens is their short harvest period. Factors that influence their growth include the nutrient content of the feed. However, it will be difficult to get optimal growth only with conventional feed. Therefore, growth supplements are needed. Red dragon fruit water-extract contains flavonoids and vitamins that act as antioxidants. This research aimed to study the effect of red dragon fruit water-extract treatment in drinking water on the growth of broiler chickens. This research used 300 DOC male broiler chickens which were reared until they were 21 days old. This research was conducted using a completely randomized design, with 5 groups and 5 replications, each replication group consisting of 12 DOC. The five groups consisted of the control group (P0), P1 0.25%, P2 0.50%, P3 1%, and P5 2%. Parameters observed included the histological structure of the small intestine, pectoralis muscle, and spleen, as well as growth performance. The results showed that red dragon fruit water extract could increase villi length and area, ratio of villi length to crypt depth, number and area of goblet cells, fasciculus area, muscle fiber area and white pulp area, splenic organ index, as well as improving growth performance including chicken body weight and feed efficiency. Therefore, it can be concluded that red dragon fruit water extract can improve the histological structure of the small intestine, pectoralis muscle, and spleen, as well as increase the growth performance of broiler chickens, especially in red dragon fruit extract with a concentration of 2%.

Key Words: Broiler Chicken, Growth Performance, Histological Structure, Red Dragon Fruit Water-Extracted, Small Intestine

INTRODUCTION

Broiler chickens make a major contribution to the availability of meat in Indonesia, because it has a soft texture and relatively large size (Umam et al., 2015). The optimal growth of broiler chickens is influenced by nutritional factors from the feed consumed. However, it is not easy to get optimal growth performance if only relying on conventional feed. According to Saragih et al. (2018), adding feed supplements with the right concentration could have a positive influence on the quality of broiler chickens including the digestive system, immunity, and growth performance. However, feed supplements that act as antibiotics on the market have been banned in most countries in the world because of concerns about the accumulation of residues in livestock products and resistance to these types of antibiotics (Ravindran 2013).

A safe source of feed supplement can come from plants. One of them was using the ethanolic extract of cashew leaves as a supplement to chicken feed (Jingga et al. 2019). Red dragon fruit can also be used as a natural ingredient for animal feed supplements because it could positively impact the digestive tract by increasing the optimization of absorption in the digestive tract (Yuniarti et al. 2015). Several studies have explored the benefits of dragon fruit- flesh on the growth of Wistar rats and quail (Cantika et al. 2019; Heryani 2016; Prakoso et al. 2017; Yuniarti et al. 2015). Meanwhile, so far, research on the growth of broiler chickens has only used red dragon fruit-skin (Simanjuntak et al. 2014).

The color of red dragon fruit is caused by pigments that contain lots of anthocyanins. Anthocyanins are phenolic compounds in plants. One of the phenolic compounds in dragon fruit is flavonoids. Phenolic compounds in red dragon fruit flesh extract are the main contributors to antioxidant activity (Prakoso et al. 2017). Natural antioxidant compounds in plants are generally polyphenolic and phenolic compounds, including flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and others (Hossain et al. 2021). Flavonoids can play an active role as supplements in feed. The flavonoids in red dragon fruit-flesh are known to have anti-inflammatory, antioxidant, anticancer, antibacterial, antidiabetic, and hepatoprotective properties (Nuari et al. 2017; Fidrianny et al. 2017). The flavonoid content in red dragon fruit also has the potential to influence the chicken's immune system. Kamboh et al. (2015) stated that the epigallocatechin and cyanidin glycoside compounds in fruit can increase interleukin-2 secretion, lymphocyte proliferation, and NK (Natural Killer) cell activity. According to research by Jingga et al. (2019), the ethanolic extract of cashew leaves containing flavonoids can increase the organ index and splenic white pulp of super Javanese chickens, especially at a concentration of 2%. High immunity in chickens can reduce the risk of exposure to disease so that chickens can have optimal growth performance. Flavonoids in the form of glycosylation or methylation in plants have more stable structures, are easy to obtain, and have easy bioactivity. Flavonoids are belongs to the water-soluble polyphenol family (Arifin and Ibrahim 2018), so in this study, red dragon fruit was made as a water extract for broiler chicken supplements.

So far, there are limited studies that have been done on the growth of broiler chickens using water extract from red dragon fruit. The newest study was conducted by Darmawan et al. (2003) using drinking water containing dragon fruit peel juice in broiler chicken. If the chicken's digestive system works well, it is hoped that nutrient absorption from the intake provided will increase and improve the chicken's growth performance. Meanwhile, strong immunity will protect chickens from pathogens and can affect chicken growth. Therefore, this research was carried out to study the effect of red dragon fruit water-extracted on morphology of the small intestine, pectoralis muscle, spleen, and growth performance in broiler chickens.

MATERIALS AND METHODS

Ethics approval

Procedures for the care and use of test animals have been approved by the Ethics Committee for the Faculty of Veterinary Medicine, Gadjah Mada University with letter number 0024/EC-FKH/Eks./2020.

Red dragon fruit extract preparation

The red dragon fruit used in this study was aged 50 days after it had flowered. The fruit weighed 50 grams and was cut into cubes. The weighed dragon fruit was put in the jam bottle containing 100 mL of mineral water. The jam bottle was closed tightly and incubated at room temperature for 12 hours. After that time, the substance was filtered to extract the dragon fruit water. The protocol followed the preparation of an aqueous extract from Javed and Bashir (2012). The total flavonoid of red dragon fruit water-extracted (RDFWE) was examined at LPPT Unit II UGM.

Experimental design

Acclimation

DOC (day-old chicken) broiler obtained from PT Japfa Comfeed Indonesia Tbk. The chicks were acclimated for 3 days following movement to the cage at the Sawitsari Research Station, Faculty of Biology UGM.

Animal management

This study used 300 DOC which were divided into 5 groups. The group consisted of a control group (P0) which was given drinking water only. Whilst treatment groups P1, P2, P3, and P4 which were given drinking water mixed with water-extracted red dragon fruit at a concentration of 0.25%; 0.5%; 1%; and 2%. The research was carried out with 5 replications, each replication was containing 12 chicks.

Treatment with red dragon fruit extract

Red dragon fruit water-extracted treatment was carried out by mixing it with drinking water given at the same time as feeding with basal feed at 09.00 o'clock in the morning. Then, at 17.00 o'clock in the afternoon, the treated drink was replaced with plain water. The treatments and feeding begun at the age of 3 days and terminated at 21 days. The amount of drinking water was provided 30 mL/chick in the first week, 40 mL/chick in the second week, and 50 mL/chick in the third week. The provision of water extract of red dragon fruit based on the amount of drinking water consumed by broiler chickens is as follows (Table 1). The composition of the basal feed used is presented in Table 2.

Histological preparations of the small intestine, pectoralis muscle, spleen of broiler chickens, and spleen index measurements

DOC was euthanized using the decapitation method (PIC 2016). Three DOCs were taken per replication. Histological preparations of the small intestine, pectoralis muscle, and spleen were made using the paraffin method. The samples were fixed with Bouin's solution for 12 hours, dehydrated with graded alcohol, cleared with toluol, and embedded with paraffin at 57-60°C. Next, the preparation was sliced with a thickness of 5 µm. Small intestine preparations were stained with PAS-AB dye to measure villi length, crypt depth, and the number and area of goblet cells. Meanwhile, pectoral and splenic muscle preparations were stained with Hematoxylin-Eosin dye.

Observation of the fasciculus area, muscle fibers, and white pulp was carried out by examining 5 different fields of view in each coupe. Determining the area was done by finding the average value in each treatment and control group. The splenic organ index was calculated using the formula of Shin et al. (2020):

$$Organ\ index = \frac{OW\ (g)}{CBW\ (g)} \times 100\%$$

where OW is organ weight and CBW is chicken body weight.

Morphometry of the small intestine

Observation and measurement of the small intestine using ImageJ software consists of several stages.

Measurement of the length and area of the villi

The length, basal and apical width of the villi in the duodenum, jejunum, and ileum were measured from 5 fields of view on each preparation. The area of the villi (mm2) is calculated using the formula of Setiawan et al. (2018):

$$Villi\ area = \frac{VBW + VAW}{VAW} \times VH$$

where VBS is villi basal width, VAW is villi apical width and VH is villi height.

Table 1. The total volume of red dragon fruit water extract given to broiler chicken from the first to third week.

Week	Red dragon fruit extract (%)	Total volume of water- extracted dragon fruit (mL)/chick	Total Flavonoid concentration/chick/days (µg)
First week	P1 (0.25%)	0.075	50.71
	P2 (0.5%)	0.15	101.43
	P3 (1%)	0.3	202.86
	P4 (2%)	0.6	405.71
Second week	P1 (0.25%)	0.1	67.62
	P2 (0.5%)	0.2	135.24
	P3 (1%)	0.4	270.48
	P4 (2%)	0.8	540.95
Third week	P1 (0.25%)	0.125	84.52
	P2 (0.5%)	0.25	169.05
	P3 (1%)	0.5	338.01
	P4 (2%)	1	676.19

Crypt depth measurement

The depth of the crypts was observed from 5 fields of view on each histology preparation. The ratio between villi length and crypt depth is calculated using the formula of Fard et al. (2014):

$$\frac{Villi}{Crypt\ depth} ratio = \frac{villi\ height\ (\mu m)}{crypt\ depth\ (\mu m)}$$

Calculation of the number and area of goblet cells

The number of goblet cells was counted in small intestinal villi of 500 µm long. The area of goblet cells observed was measured from the edge of the membrane surrounding the goblet cells on a cross-section of the villi of the small intestine.

Calculation of Feed Conversion Ratio (FCR)

Feed Conversion Ratio is the comparison between the amount of feed consumed and the amount of weight produced. Feeding of broiler chicks was carried out from the age of 3 to 21 days. The feed that went into the cage and the remaining was weighed, to calculate the amount of feed consumed by chicks every day. The formula for calculating the Feed Conversion Ratio according to Umam et al. (2015) is as follows:

$$FCR = \frac{total\ feed\ consumed\ (gram)}{chicken\ weight\ gain\ (gram)}$$

Table 2. Basal feed formulation and nutrition content.

Composition of feed (%)	Single feed
Corn	49.0
Soybean meal	29.0
Rice bran	9.8
Full-fat soya	5.4
Crude palm oil	3.0
Dicalcium phosphate	2.37
Premix vitamin[a]	0.03
Premix mineral[b]	0.06
D, L-methionine	0.22
NaCl	0.32
Calcit	0.5
L-lysine HCl	0.1
L-threonine	0.04
Choline chloride 60%	0.16
Calculated composition[c]	
Metabolizable energy of poultry (kcal/kg)	2,904.02
Crude protein (%)	20.23
Fiber (%)	8.30
Lysine (%)	3.37
Methionine (%)	1.22
Methionine + cycteine (%)	0.53
Calcium (%)	0.86
Phosphorus, available (%)	1
Sodium (%)	0.95
Chloride (%)	0.5

[a]Premix vitamin provided the following per kilogram of diet (Vitamin A= 15000 IU, Vitamin D3= 3000 IU, Vitamin E= 22.5 mg, Vitamin K3= 3 mg, Vitamin B1= 3 mg, Vitamin B2= 9 mg, Vitamin B6= 4.5 mg, Vitamin B12= 30 mcg, biotin= 30 mcg, folic acid= 1.5 mg, niacin= 45 mg, pantothenic acid= 1.5 mg, Vitamin C= 0 mg, choline= 2090 mg and 1242 mg), [b]Premix mineral provided the following per kilogram of diet (Cu= 12 mg, Fe= 72 mg, Iodine= 0.9 mg, Mn= 84 mg, Se= 0.3 mg, Zn= 60 mg). [c]Proximate, amino acids, minerals, and metabolizable energy were obtained from calculated values of Haritadi et al. (2017)

Statistical analysis

The data obtained were quantitative data for variables of villi length, crypt depth, number and area of goblet cells, area of fascicles and muscle fibers, splenic index, and white pulp area. Each treatment was then analyzed using one-way ANOVA and Duncan's test to the level of $P<0.05$ significancy for each treatment.

RESULTS AND DISCUSSION

Total flavonoid examination

The examination result of total flavonoid could be seen in Table 3, with the concentration of total flavonoid is 676,19 µg/ml.

Histological structure of the duodenum, jejunum and ileum of broiler chickens

Based on the results of the research that has been carried out (Figure 1, Figure 2, and Table 4), it can be seen that the length of the villi for the duodenum and jejunum of broiler chickens increased significantly in the treatment group of red dragon fruit water-extracted (RDFWE) concentration of 0.25% and 1 % (P1 to P3) compared with the control group (P0), but there was no significant difference between the control group (P0) and the 2% RDFWE of treatment group 4 (P4). The length of the villi in the duodenum and jejunum showed the highest numbers in the RDFWE treatment group with a concentration of 0.5% (P2).

The depth of the crypts in the duodenum of broiler chickens showed a significant increase in the 0.25% RDFWE treatment group 1 (P1) compared to the control group (P0), which also differed significantly the group of 0.5% (P2) group of 1% (P3); and group of 2% (P4). The depth of the duodenal crypts was highest in the group of 0.25% (P1). Meanwhile, the depth of crypts in the jejunum of broiler chickens increased significantly in the group of 0.25% and 0.5% (P1 to P2) compared to the control group (P0), which was also significantly different from the group of 1% and 2%. (P3 and P4). The depth of the jejunal crypts was highest in the of 0.5% (P2). The ratio of villus length to duodenal crypt depth was highest in the group of 0.5% (P2) but was not significantly different from the control group (P0). The ratio in the jejunum was highest in the group of 0.25% (P1) and there was a significant difference between the control group (P0) and all RDFWE treatment groups.

The number of goblet cells in the duodenum and jejunum of broiler chickens showed a significant increase in the group of 0.25% (P1) to 0.5% (P2) compared to the control group (P0). In the duodenum, there was no significant difference between the number of goblet cells in the control group (P0) and in the group of 2% (P4), while in the jejunum it was significantly different from all treatment groups. The highest number of goblet cells in the duodenum and jejunum was found in the group of 0.5% (P2). The highest goblet cell area in the duodenum and jejunum of broiler chickens was found in the group of 0.5% (P2), and the control treatment (P0) was significantly different from all RDFWE treatment groups.

This study aims to determine the effect of giving red dragon fruit water-extracted on the development of the small intestine, pectoralis muscle, spleen and growth performance of broiler chickens. Red dragon fruit was very famous for its good antioxidant activity that could provide various health benefits (Tenore et al. 2012). In this study, the chemical compound examination that used for red dragon fruit extract is total flavonoid concentration. Red dragon fruit is known to contain flavonoids and phenols as antioxidants, which can affect the growth of broiler chickens. According to Huyut et al. (2017), phenol and flavonoid compounds are known to have antioxidant properties. Apart from flavonoids, red dragon fruit also contains protein, carbohydrates, vitamin C, crude fiber, minerals (phosphorus, potassium, calcium, magnesium, iron, zinc, sodium), essential fatty acids (linoleic acid, oleic acid), betacyanin, and phytoalbumin (Nurul and Asmah 2014; Luu et al. 2021).

The advantage of broiler chickens compared to other types of chicken is their fast growth. Growth efficiency in broiler chickens is related to the nutrients from the feed absorbed and is strongly influenced by the development of the digestive tract. The small intestine is part of the digestive tract which has the function of absorbing food nutrients and consists of the duodenum, jejunum, and ileum (Nasrin et al. 2015). The use of feed with high nutrition is very important in the process of raising broiler chickens. According to Sitompul et al. (2016), nutritional factors in feed, especially protein, can increase the value of pectoralis muscle deposition in chickens. Apart from that, the feed given to chickens also influences their lymphoid organs. In chickens, splenic function is very important considering that the lymph nodes and vessels are not as well developed as in mammals (Davison et al. 2008).

In the intestine, villi and crypts are parts of important role in the absorption of nutrients from feed or supporting supplements (Wang and Peng 2008). The growth of the length of the villi is related to the potential of the small intestine to absorb food juices. The longer the villi, the more effective the absorption of food essences in the small intestinal epithelium () (Lenhardt and Mozeš 2003). This is an expression of the smooth nutrient transportation system throughout the body. Based on the results of research that has been carried out (Figures 1 and 2, Table 4), the length of the

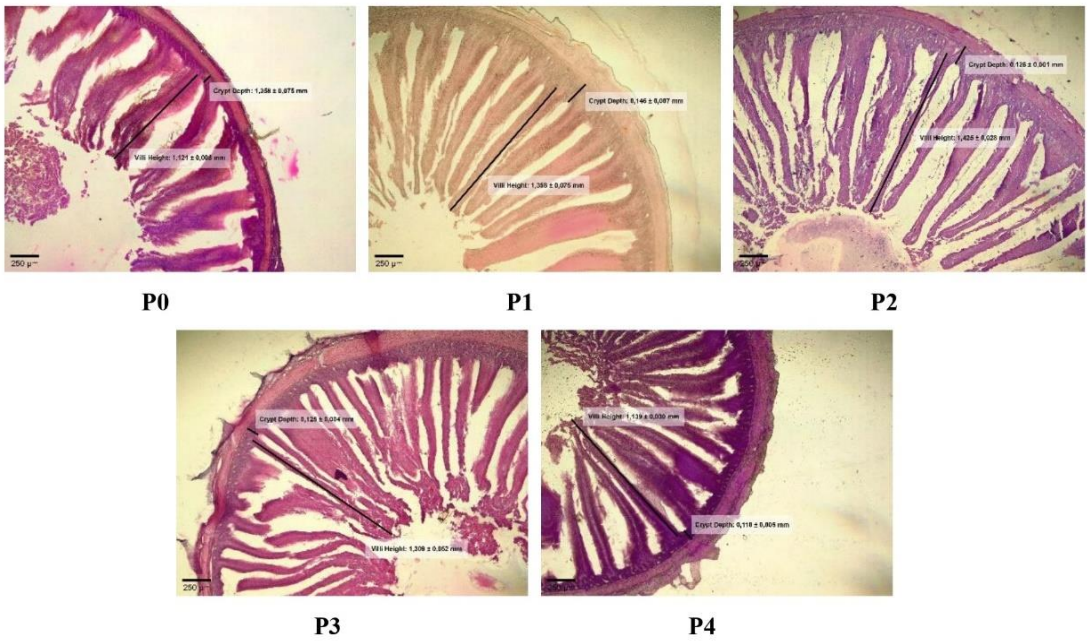

Figure 1. The images of villi length, crypt depth, and the number and area of goblet cells in the duodenum of 21 old broiler chicks treated with red dragon fruit water-extracted in their drinking water of Hematoxylyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

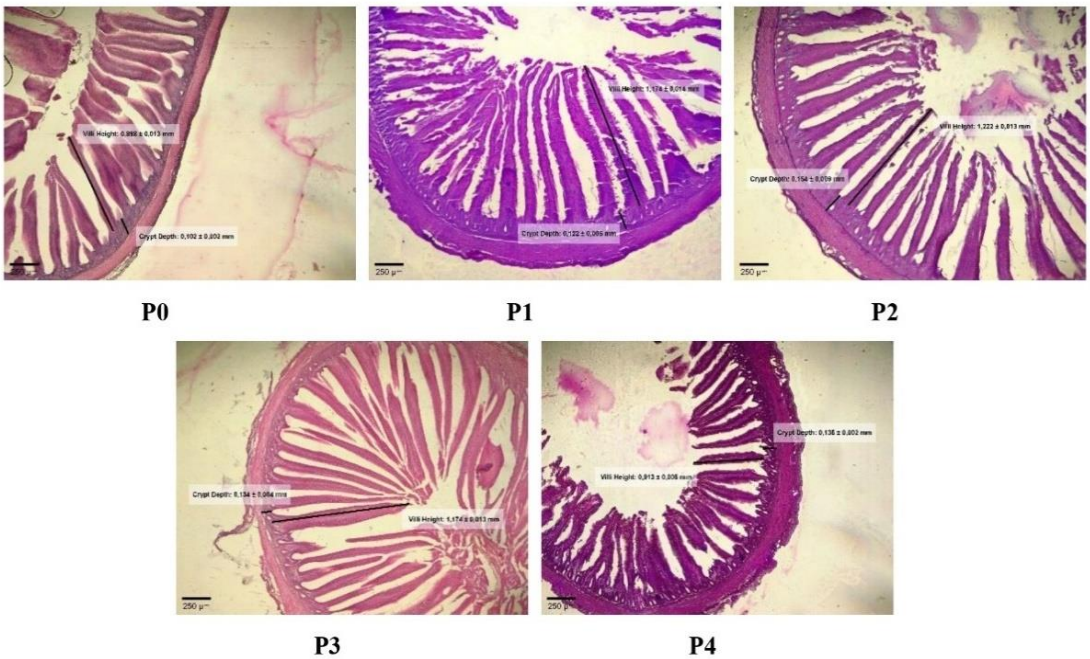

Figure 2. The image of villi length, crypt depth, and the number and area of goblet cells in the jejunum, of 21 old broiler chicks, treated with red dragon fruit water-extracted in their drinking water. Hematoxylyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

Table 3. Flavonoid total content of red dragon fruit water extract

Parameter	Result	Method
Flavonoid total	676.19 µg/mL	Spektrophotometry UV-vis

duodenal and jejunal villi of broiler chickens was highest in the red dragon fruit water-extracted (RDFWE) treatment group with a concentration of 0.5% (P2). The depth of the duodenal crypt was highest in the RDFWE treatment group with a concentration of 0.25% (P1), while in the jejunum was in the RDFWE treatment group of 0.5% (P2). The ratio of villous length and duodenal crypt depth was highest in the RDFWE treatment group at a concentration of 0.5% (P2) and in the jejunum was at the group of 0.25% (P1).

The red dragon fruit water-extracted used in this research had a flavonoid content of 676.19 µg/mL. Based on research conducted by Edi et al. (2018), flavonoid levels of 1286.9 µg/mL contained in teak leaf extract were able to improve growth performance in laying hens. The flavonoid content in red dragon fruit water-extracted has a role in increasing the length of the villi and the depth of the crypts in the duodenum of broiler chickens. Based on the measurement results, it shows that the length of the villi and the depth of the crypts in all treatment groups increased when compared with the control group. The flavonoid content in red dragon fruit water-extracted can stimulate the mitosis of duodenal villous epithelial cells. The longer villi in the treatment group compared to the control group were caused by the epithelial cells which is being active in mitosis. The depth of the crypt has a role in the center of villous cell regeneration due to the increase the length of the villi, which always requires new cells originating from crypt cell migration (Rajput et al. 2013). The ratio of villi length to crypt depth which increased in the treatment group, showing that the flavonoid content in red dragon fruit water-extracted makes the intestinal function healthier.

The goblet cells in the villi have the function of producing mucus. This mucus functions as a protector for the epithelial cells in the villi and can protect the brush border during the process of digestion of food. According to Wang and Peng (2008), goblet cells can increase the ability of nutrient absorption in chickens. The observation results (Table 4) showed that the number and area of goblet cells in the duodenum and jejunum were highest in the RDFWE treatment group with a concentration of 0.5% (P2). The highest number and area of goblet cells in P2 was probably to be caused by the flavonoid content in red dragon fruit water-extracted which acted as an antimicrobial (Tenore et al. 2012). Its role as an antimicrobial can protect epithelial cells from pathogenic bacteria found on mucosal surfaces. Epithelial cells in good and healthy condition will reduce goblet cell mucus secretion, so that the group treated with dragon fruit water-extract had a larger size and number of goblet cells than the control. When goblet cells release mucus in the lumen, glycoproteins from the mucus will be secreted form a gel that protects the epithelial cells. Goblet cells are cells that must always be renewed to prevent damage to epithelial cells (Setiawan et al. 2018).

Flavonoids as an antibiotic compound are more easily absorbed by the small intestine (Arifin and Ibrahim 2018). Flavonoids can also prevent damage to goblet cells. Apart from that, flavonoids function to protect the digestive tract from damage due to stress or pathogens and can protect and prevent erosion of epithelial cells due to pathogens on the surface of the villi. Based on research by Fard et al. (2014) and Setiawan et al. (2018), giving flavonoids to chickens can increase the length of the villi. In previous study, Albab et al. (2022) stated that flavonoid content in date water extract could protect the small intestine of broiler chicken and maintain its good condition. Flavonoids in red dragon fruit water-extracted play a role in maintaining intestinal health by stimulating the growth of beneficial bacteria and inhibiting the growth of pathogenic bacteria (Cardona et al. 2013).

Pectoralis muscle growth at the cellular level was determined by measuring the fasciculus area and muscle fiber area. The administration of red dragon fruit water-extracted in drinking water showed a significant difference between the fascicle area of the treatment and control. The results indicated that the fascicle area and pectoralis muscle fiber area of broiler chickens showed the highest numbers in the RDFWE treatment group with a concentration of 2% (P4). An increase in skeletal muscle mass is caused by an increase in the area of muscle fibers (Glass 2005; Saragih et al. 2018). Based on Saragih et al. (2024), the administration of marine macroalgae (*Chaetomorpha limun*) which has flavonoid content has positive effects on the *pectoralis major* muscle growth.

Area of fascicles and pectoralis muscle fibers

Based on the results of research that has been carried out (Figure 3 and Table 5), it can be seen that the area of the fasciculus in the pectoralis muscle of broiler chickens increased significantly in the treatment groups of 0.25% to 2% (P1 to P4) compared with the control group (P0). The fascicle showed the highest area in the group of 2% (P4). The area of pectoralis muscle fibers in broiler chickens showed a significant increase in the groups of 0.5% to 2% (P2 to P4) compared to the control group (P0), but there was no significant difference between control group (P0) and group of 0.25% (P1). The area of pectoralis muscle fibers was highest in the group of 2% (P4).

White spleen pulp index and area

Based on the results of research that has been carried out (Figure 4 and Table 6), the splenic index of broiler chickens showed that there was no significant

Table 4. The average villi length, crypt depth, ratio of villi length to crypt depth, number of goblet cells, and goblet cell area in the intestines of broiler chicks given red dragon fruit water-extracted in drinking water at the age of 3 to 21 days

Variable	Treatment Groups									
	Duodenum					Jejunum				
	P0	P1	P2	P3	P4	P0	P1	P2	P3	P4
Villi Length (mm)	1.121±0.008^{a}	1.358±0.075^{bc}	1.425±0.028^{c}	1.309±0.052^{b}	1.139±0.030^{a}	0.898±0.013^{a}	1.174±0.014^{b}	1.222±0.013^{c}	1.174±0.013^{b}	0.913±0.005^{a}
Crypt Depth (mm)	0.107±0.001^{a}	0.146±0.007^{c}	0.126±0.001^{b}	0.125±0.004^{b}	0.110±0.005^{a}	0.102±0.002^{a}	0.122±0.005^{b}	0.154±0.009^{d}	0.134±0.004^{c}	0.135±0.002^{c}
Villi: Crypt Ratio	10.443±0.077^{b}	9.296±0.725^{a}	11.283±0.285^{b}	10.453±0.427^{b}	10.503±0.734^{b}	8.780±0.104^{a}	9.663±0.447^{d}	7.980±0.555^{b}	8.790±0.216^{c}	6.750±0.079^{c}
Total Goblet Cells/250 µm	33 ± 0.882^{a}	42.667±0.665^{b}	55.556±1.389^{c}	43.890±3.083^{b}	34.110±0.840^{a}	28.667±1.527^{a}	36.113±3.501^{b}	58.223±5.013^{c}	36.446±1.072^{b}	36.446±2.267^{b}
Goblet Cell Area (μm^2)	261.680±6.206^{a}	366.976±10.601^{c}	522.123±8.916^{d}	333.126±10.916^{b}	371.576±2.378^{c}	256.266±7.951^{a}	357.380±3.474^{t}	576.723±16.201^{d}	432.353±17.255^{c}	343.340±8.726^{b}

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Table 5. The average area of fascicles and muscle fibers of broiler chickens given red dragon fruit water-extracted at the age of 3 to 21 days.

Groups	Fascicle Area (µm2)	Muscle Fibers Area (µm2)
P0	46611.55±8683.09^{a}	310.02±42.35^{a}
P1	75010.85±12052.18^{c}	339.10±45.49^{a}
P2	65888.85±12233.24^{b}	454.62±39.37^{c}
P3	60704.45±8324.49^{b}	406.25±40.57^{b}
P4	86961.67±9154.03^{d}	598.35±75.88^{d}

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

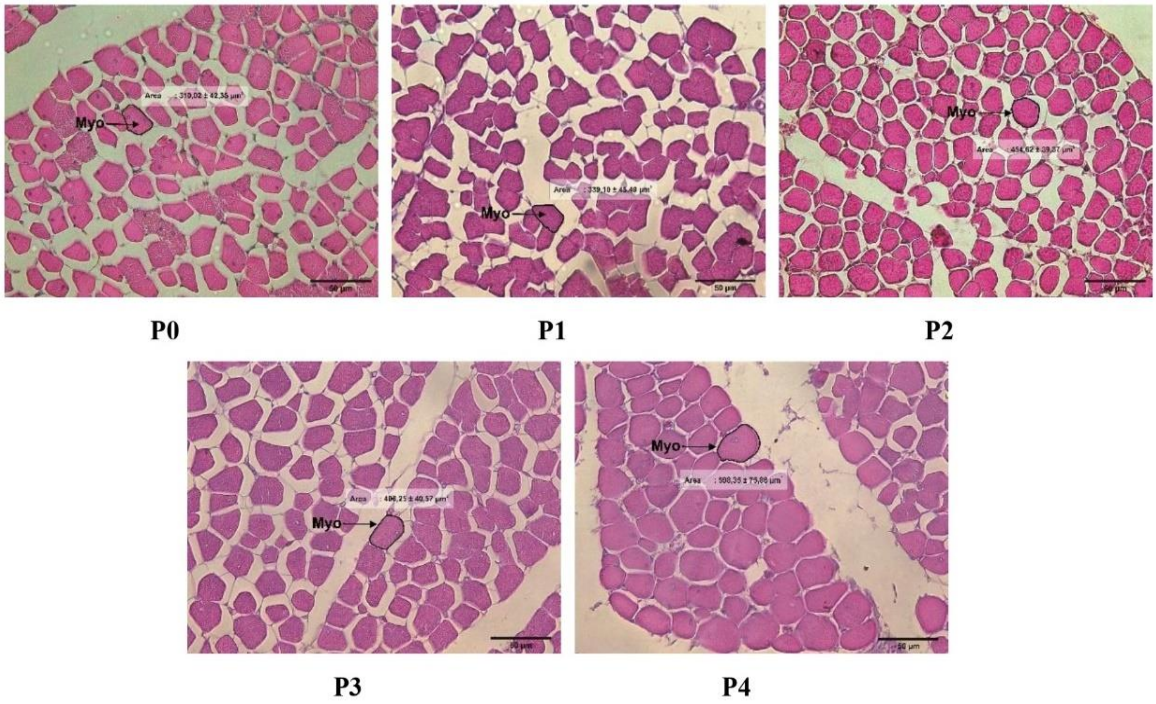

Figure 3. Cross section of pectoralis muscle fibers under magnification of 40 x 10 of 21-day-old broiler chicks treated with red dragon fruit water-extracted in their drinking water, Hematoxilyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract, Myo = muscle fiber. Scale bar: 250 µm.

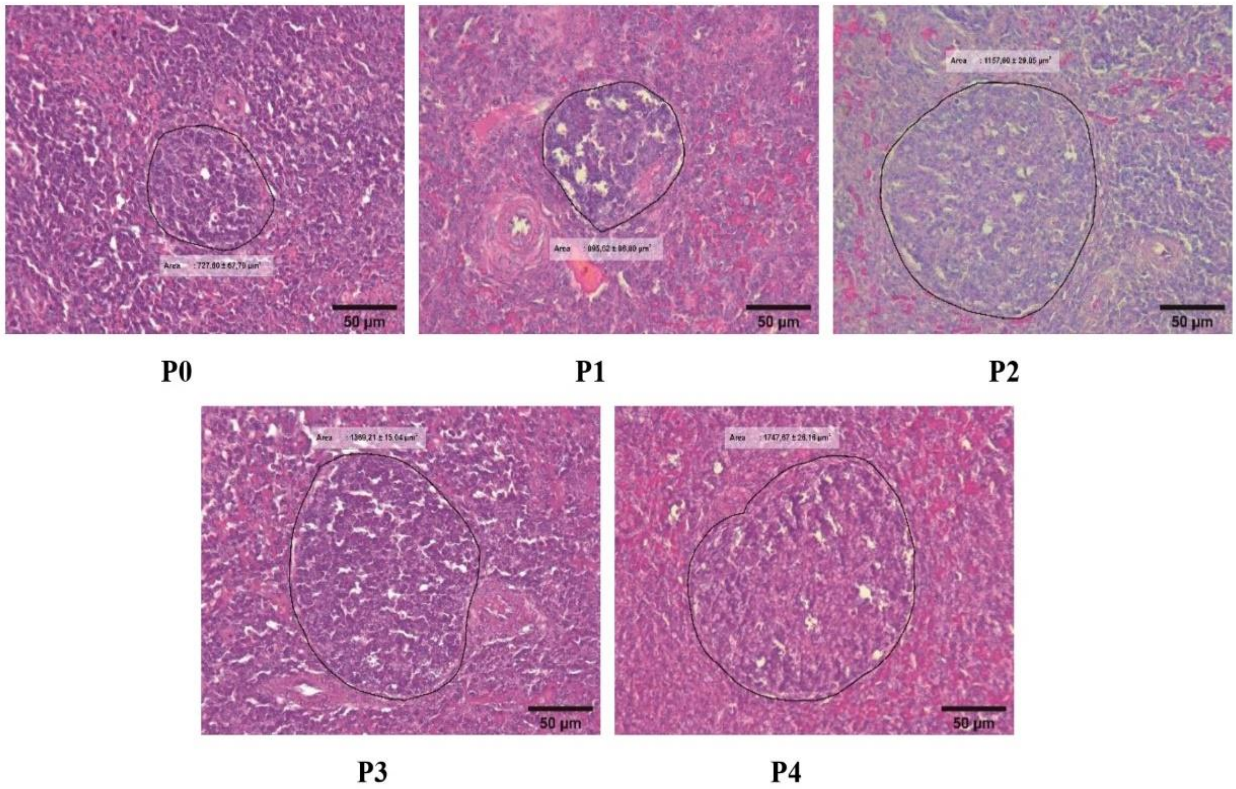

Figure 4. Structure of the white splenic pulp of 21-day-old broiler chicks treated with dragon fruit water-extracted in their drinking water. Hematoxylin-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

difference between the control group (P0) and all groups (P1 to P4). Meanwhile, the white pulp area showed a significant increase from group of 0.25% (P1) up to 2% (P4), which was also significantly different from the control group (P0). The highest white pulp area of the splenic organ of broiler chickens were shown in the RDFWE treatment group with a concentration of 2% (P4).

In Table 6 it can be seen that the splenic index of broiler chickens that were given red dragon fruit water-extracted tended to be the same as chickens that were not given water-extracted dragon fruit. Based on statistical analysis, the results obtained did not show any significant differences between treatment and control. According to Jingga et al. (2019) intake of flavonoids from ethanol extract of cashew leaves can increase the splenic index and bursa Fabricius of super Javanese chickens as the concentration increases. Zhou et al. (2019) revealed that intake of the flavonoid baicalein in broiler chicken diets significantly increased the splenic index and bursa of Fabricius compared to controls. However, this statement is at odds with research conducted by Goliomytis et al. (2014) who stated that administration of the flavonoid quercetin did not have a significant effect on the splenic index compared to controls. This statement is also supported by Martínez et al. (2021) who stated that consumption of flavonoids from cashew leaf flour did not affect the relative weight (index) of the bursa of Fabricius, thymus, and spleen of broiler chickens. Research by Yang et al. (2019) also strengthens the statement above which states that the combination of cinnamon oil intake with bamboo leaf flavonoids did not affect the weight of the spleen, thymus, and bursa of Fabricius of broiler chickens on days 21 and 42. There was no significant difference in the splenic index between the treatment and the control caused by an increase in chicken body weight accompanied by an increase in organ weight.

The data in Table 6 shows that the administration of red dragon fruit water-extracted increases the area of white splenic pulp. Based on the results of statistical tests, administering red dragon fruit water-extracted had a significant effect on increasing the area of white pulp in the treatment group. The 2% RDFWE group (P4) had the largest white pulp area and the control group (P0), which had the smallest area. Based on the results obtained, it can be assumed that there is an increase in the proliferation of immune cells in the spleen (Jingga et al. 2019). In Figure 4 it can be seen that the splenic white pulp area in the treatment group has a larger area compared to the control.

Broiler chicken growth

Broiler chickens are a type of superior breed of chicken resulting from cross-breeding, selection, and genetic engineering from chicken breeds that have high productivity in meat production (Tamalluddin 2014). The growth of broiler chickens depends on several factors, namely food, environmental temperature, and rearing methods (Rasyaf 2012). Based on the research that has been carried out, the following results were obtained.

Results presented in Table 7 showed that the body weight of broiler chickens at the 21st day old after RDFWE treatment of 0.25% up to 2% (P1 up to P4) was t shown highest in group of 2% (P4) and was significantly different from control group (P0). Feed intake in the control group (P0) was not significantly different compared to all RDFWE treatment groups. The highest weight gain was shown in the of 2% (P4), which was significantly different from the control group (P0). The highest figure of FCR was in the control group (P0) and was significantly different from the group of 0.25%; 0.5%; and 2% (P1, P2, and P4). The results of the one-way ANOVA feed intake test did not show any significant differences between treatment and control. Meanwhile, one-way ANOVA weight gain test analysis showed that there were significant differences between the treatment and control groups.

The total flavonoid in red dragon fruit water-extracted can increase the proliferation activity of immunocompetent cells in the spleen organ (Grigore 2017; Martínez et al. 2019; Zhou et al. 2019). Flavonoids such as quercetin, chrysin, and wogonin can modulate proliferation and induce B-cell differentiation and antibody production (Martínez et al. 2019). The flavonoid baicalein can increase the production of gamma interferon (IFN-γ) from cytotoxic and helper T cells thereby inducing the proliferation of surrounding immune cells (Zhou et al. 2019). Proliferation of lymphocyte cells is very important as the initial step of the immune response to produce effector lymphocytes and memory lymphocytes (Desforges et al. 2016).

The growth performance of broiler chickens can be seen through body weight and FCR. The results of the study showed that giving red dragon fruit water-extracted in drinking water could increase the growth of broiler chickens (Table 7). The difference in growth between treatment groups and control group began to occur at the 10th day and lasted until the 21st day. The feed used in this research was basal. The basal feed used has a crude protein of 20.58% (Table 3), the composition of the crude protein is not much different from research by Ahmed et al. (2015) which used a crude protein of 20.89% for chickens aged 0-21 days. Providing feed with sufficient crude protein content is useful for protein synthesis and deposition in muscles (Susbilla et al. 2003).

Providing red dragon fruit water-extracted containing antioxidant has the benefit of suppressing the production of free radicals from the body's metabolic processes in broiler chickens. According to

Table 6. Organ index and splenic white pulp area (x10^2 µm^2) in broiler chickens after treatment with red dragon fruit water-extracted in drinking water at the age of 3 to 21 days

Groups	Spleen Index	White Pulp Area (µm^2)
P0	0.127±0.004^a	727.60±67.70^a
P1	0.121±0.012^a	895.62±86.00^b
P2	0.126±0.011^a	1157.80±29.05^c
P3	0.118±0.024^a	1369.21±15.04^d
P4	0.113±0.009^a	1747.67±26.16^e

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Table 7. Average growth of broiler chickens given red dragon fruit water-extracted at the age of 3 to 21 days.

Parameter	Age (Days)	Treatment Groups				
		P0 (0%)	P1 (0.25%)	P2 (0.5%)	P3 (1%)	P4 (2%)
Body live weight (g/chick)	1	48.00±1.52^a	48.50±1.07^a	47.44±1.64^a	47.39±1.72^a	48.67±0.81^a
	4	67.00±1.53^a	66.11±3.27^a	65.72±1.96^a	67.78±1.51^a	67.67±1.17^a
	7	107.61±3.22^a	106.67±3.10^a	106.89±3.96^a	106.94±1.39^a	108.55±2.49^a
	10	155.05±7.87^a	161.22±6.83^a	163.61±14.05^{ab}	163.78±4.87^{ab}	173.06±5.42^b
	13	247.45±7.05^a	252.50±7.37^a	247.61±8.42^a	250.50±8.49^a	261.50±1.50^b
	16	338.94±9.52^b	353.72±6.99^c	337.67±11.03^{ab}	327.83±7.44^a	359.55±5.88^c
	19	406.83±7.83^a	442.61±17.03^{bc}	429.05±9.49^b	444.78±13.59^c	468.72±11.48^d
	21	443.44±19.73^a	507.33±17.69^b	498.22±7.59^b	501.06±18.62^b	551.50±12.2^c
Feed Intake (g/chick/day)		30.67±2.96^a	29.66±2.30^a	29.19±1.04^a	31.22±2.63^a	32.57±1.54^a
Body Weight Gain (g/chick/day)		18.83±2.16^a	22.56±1.41^b	21.47±1.98^{ab}	21.60±1.27^b	23.95±2.45^b
FCR		1.63±0.06^b	1.32±0.08^a	1.37±0.15^a	1.45±0.13^{ab}	1.36±0.11^a

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. a-d Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Zhang & Kim (2020), using quercetin to supplement the growth of broiler chickens can improve growth performance. Based on research by Shilov et al. (2020), feed that is high in antioxidants has been proven to increase the appetite of broiler chickens. The addition of red dragon fruit water-extracted increased the appetite of broiler chickens.

According to Saeed et al. (2017) the use of flavonoids in poultry can cause various biological activities, including growth promoter, anti-inflammatory, antioxidant and antibacterial. So, broiler chickens in the treatment group are thought to have more optimal nutritional absorption compared to the control.

CONCLUSION

Water-extracted red dragon fruit at a concentration of 2% has the effect of improving the histological structure of the intestine, pectoralis muscle, spleen, and growth performance of broiler chickens.

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Growth Response and Carcass Yield of Male Japanese Quail Fed Diets Contained Fermented Rubber (*Hevea brasiliensis*) Seed Meal

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ABSTRAK

Hertamawati RT, Suryadi U, Prasetyo AF, Rahmasari R, Imam S, Asrianto N. 2024. Respon Pertumbuhan dan Produksi Karkas Puyuh Jepang Jantan yang Diberi Pakan Tepung Biji Karet (*Hevea brasiliensis*) Fermentasi. JITV 29(4):221-226. DOI: http://dx.doi.org/10.14334/jitv.v29i4.2450.

Penelitian ini mengevaluasi pemberian biji karet fermentasi menggunakan ragi tempe dalam pakan terhadap performa pertumbuhan dan produksi karkas puyuh jantan. Penelitian ini menggunakan metode percobaan Rancangan Acak Lengkap (RAL) dengan menggunakan sekitar 260 ekor puyuh jantan umur 1-5 minggu yang dibagi menjadi 4 kelompok perlakuan dengan taraf pemberian tepung biji karet fermentasi (FRSM) menggunakan ragi fermentasi yang berbeda yaitu R0= pemberian pakan tanpa FRSM (kontrol), R1= pemberian FRSM 4%, R2= pemberian FRSM 8%, dan R3= pemberian FRSM 12%. Masing-masing perlakuan diulang sebanyak 5 kali sehingga diperoleh 20 unit yang masing-masing unit berisi 13 ekor. Parameter yang diamati adalah konsumsi pakan, pertambahan bobot badan, konversi pakan, dan produksi karkas. Proses fermentasi dengan ragi tempe menurunkan kadar kandungan asam sianida (HCN) biji karet berkurang dari 158,64 ppm menjadi 17,84 ppm Hasil pengamatan menunjukkan penambahan tepung biji karet fermentasi pada pakan tidak memberikan pengaruh yang nyata (P>0,05) terhadap konsumsi pakan, mempertahankan pertambahan bobot badan, konversi pakan, bobot akhir badan, bobot karkas, dan persentase karkas. Kesimpulan penelitian ini adalah penambahan tepung biji karet yang difermentasi (FRSM) hingga 12% tidak mengganggu kinerja pertumbuhan dan hasil karkas puyuh.

Kata Kunci: Asam Sianida, Fermentasi, Biji Karet, Ragi Tempe, Karkas Puyuh

ABSTRACT

Hertamawati RT, Suryadi U, Prasetyo AF, Rahmasari R, Imam S, Asrianto N. 2024. Growth response and carcass yield of male Japanese quail-fed diets contained fermented rubber (*Hevea brasiliensis*) seed meal. JITV 29(4):221-226. DOI: http://dx.doi.org/10.14334/jitv.v29i4.2450.

This paper discusses the previous research on the use of fermented rubber seed meal using tempeh yeast (Rhizopus oligosporus) (FRSM) given to male quail from 1 to 5 weeks of age on the growth performance and carcass production. The research used a Completely Randomized Design (CRD) experimental method using about 260 male quail aged 0-5 weeks divided into 4 treatment groups with the level of giving fermented rubber seed meal (FRSM) using different fermented yeast, namely R0= feeding without FRSM (control), R1= feeding of 4% FRSM, R2 = feeding of 8% FRSM, and R3= feeding of 12% FRSM. Each treatment was repeated 5 times, making 20 units of 13 male quails. The parameters observed were feed consumption, body weight gain, feed conversion, and carcass production. Rubber seed fermentation in this research showed that rubber seeds' cyanide acid (HCN) content was reduced from 158.64 ppm to 17.84 ppm. The experimental results showed that adding fermented rubber seed meal to the diets did not decrease feed consumption or prevent weight gain, feed conversion, final body weight, carcass weight, and carcass percentage. This research concluded that adding fermented rubber seed meal up to 12% did not harm male quail's growth performance and carcass yield.

Key Words: Cyanide Acid, Fermentation, Rubber Seed, Tempeh Yeast, Quail Cascass

INTRODUCTION

The nutritional requirements for the development of quail are high, especially for the fulfillment of protein feed. It takes feed with a protein content of 23 to 25% to get optimal growth and productivity of quail during the growth period (Hertamawati et al., 2019). The protein source that is often used in commercial feed is fish meal. The relatively high price of fish meal makes the price of commercial feed expensive, so it is necessary to do research using alternative raw materials that are cheaper and have abundant and sustainable availability.

Rubber seeds have been researched as an alternative raw material suitable for use as a protein source

substitute on layers (Lu et al. 2021), broilers (Aguihe et al. 2017), chickens (Syahruddin et al. 2016), and ducks(Boye et al. 2019). The advantage of rubber seed flour, which is produced from the seeds of the rubber plant, is that it is the most widely grown plantation crop in Indonesia, so its availability in large quantities is relatively guaranteed.

Previous researchers have found that *Hevea brasiliensis* dry seeds contain 17-25% protein (Oluodo et al. 2018); the composition includes 50.2% crude fat, 6.5% crude fiber, 3.6% ash, and 18.2% carbohydrates, as well as moderate levels of trace minerals (Udo et al. 2018). Additionally, other research (Syamsunarno et al. 2014) the analysis revealed that rubber seeds consist of 92.22% dry matter, with nutrient contents including 19.20% crude protein (CP), 47.20% crude fat, 6% crude fiber, 3.49% ash, and 24.11% nitrogen-free extract (NFE). These nutritional values can vary based on the seed variety, harvest age, soil type, processing techniques, and storage conditions. Although rubber seed meal has an adequate protein content, it also contains anti-nutritional factors (ANFs), particularly cyanogen glycosides, which can convert into hydrogen cyanide and adversely affect physiological and metabolic processes (Oyewusi et al. 2007). Fresh rubber seeds contain various anti-nutritional factors, including tannins (0.07%), saponins (0.76%), oxalates (0.18%), and phytates (0.51%), as well as a toxic compound. These substances can cause gastrointestinal problems and decrease metabolic activity when the seeds are used directly in animal feed (Agbai et al., 2021). The content of rubber seed hydrocyanic acid is 24.89 % (Udo et al. 2018), similar to cassava cyanic acid (Montagnac et al. 2009). Fresh rubber seeds contain 1,200 ppm of HCN and 27 ppm of rubber seed cake meal (Agbai et al. 2021).

Rubber seed processing using fermentation technology is one way to reduce HCN content (Syamsunarno et al. 2014). Fermentation can enhance the digestibility of feed ingredients by breaking down complex substances through enzymes produced by fermenting microbes (Oluodo et al. 2018), (Novita et al. 2019). One of the inoculants that can be used in the fermentation of rubber seeds is tempeh yeast. Fermented rubber seeds with Rhizopus oligosporus could reduce HCN by 18 times (573.72 ppm to 30.75 ppm) (Syahruddin et al. 2016). Tempeh yeast comprises four mold species: *Rhizopus oligosporus, R. orizae, R. stolonifer*, and *R. arrhizus*. Additionally, it includes various bacteria, such as *Klebsiella, Bacillus species, Lactobacillus species, Pediococcus species, and Streptococcus* species, along with other bacteria that produce vitamin B12 (Fadhilah, 2018). The fungus *Rhizopus sp.* contained in tempeh yeast can help hydrolyze the substrate, making it simpler and easier to absorb in the digestive tract.

Limited research has been done on using fermented rubber seed meal in quail diets. This study evaluated how incorporating fermented rubber seed meal, treated with tempeh yeast, affects male quails' growth and carcass yield.

MATERIALS AND METHODS

The source and processing methods of rubber seeds

The rubber seeds utilized in the study were sourced from a rubber plantation in Jember, East Java, Indonesia. All the seeds were collected fresh. They were cracked open to retrieve the contents and then cut into smaller pieces. To lower the cyanide acid levels in the seeds, they were soaked in water for 36 hours, followed by a 30-minute boil without a cover (Rachmawan 2008). Subsequently, the rubber seeds are steamed for 10 minutes and mixed evenly with 200 grams of yeast by stirring. The seeds are then wrapped in plastic with air circulation and stored for 7 days. The resulting rubber seed tempeh is dried and ground into flour, making it ready for use.

Experimental birds and management

The study's methods for handling and caring for the birds were approved by the Animal Ethics Committee at the Polytechnic State of Jember in East Java, Indonesia. No. 02/PL17.4/PG/2024, September 4th 2023. The experiment involved 260 male day-old quails. Following a completely randomized design, the birds were randomly assigned to one of four dietary treatment groups, each consisting of five replicates containing thirteen birds. The birds were reared in a quail battery cage (50x50x16 cm). Each battery cage is equipped with one long trough feeder and manual drinker. The experiment spanned 35 days, during which each group had unrestricted access to its designated diet and clean water.

Experimental diets

Four distinct feed rations were formulated to fulfill the nutritional requirements of growing quails. The R0 diet, the negative control, did not contain any tempeh rubber seed meal (FRSM). In contrast, the R1 diet, the positive control, incorporated 5% FRSM. The R2 and R3 diets included 10% and 15% FRSM, respectively; all diets were formulated to similar levels of calculated ME and CP(Abbas et al. 2016), as outlined in Table 1. The specific nutrient composition of the fermented rubber seed meal is presented in Table 2. The quails were given diets in the morning and afternoon.

Data collection

Feed conversion data was taken from the division between one week's feed consumption and body weight gain for one week; the data was taken once a week. At

Table 1. Ration formulations and nutrient contents of diet treatments

Ingredient	Treatment			
	R0	R1	R2	R3
Yellow corn (%)	48.9	40.5	34.49	28
Rice bran (%)	2	5.4	4	12,3
Layer concentrate*(%)	49	50.01	50.7	45.5
Fermented rubber seed meal (FRSM)(%)	0.0	4	8	12
Mineral (%)	0.1	0.09	2.81	2.2
Total	100	100	100	100
Nutrient				
Metabolize energy (kcal/kg)**	2901.15	2910.80	2901.32	2912,80
Crude Protein (%)***	24.00	24.74	24.99	24.00
Crude Fat (%)***	3.92	5.11	6.13	7.39
Crude Fiber (%)***	4.40	4.74	5.79	7.02
Calcium (%)	1.14	1.16	1.34	1.19
Phosphor (%)	0.73	0.76	0.74	0.77

*PT. Wonokoyo Jaya Corp; **ME, Ca & P content based the calculation; ***proximate analysis

Table 2. Nutrient of fermented rubber seed meal based on proximate analysis

Nutrient content	Total
Dry matter (%)	95.47
Ash (%)	2.03
Crude Protein (%)	16.18
Crude Fiber (%)	17.59
Extract ether (%)	32.43
Contains of HCN	
Rubber seed flour (ppm)	158.64
Fermented Rubber seed flour (ppm)	17.84

the end of the research (35 days old), the percentage of carcasses were collected by comparing the carcass weight with the live weight.

Statistical analysis

The data were examined using Analysis of Variance (ANOVA) within a fully randomized design. Duncan's multiple range test (DMRT) was utilized to pinpoint differences among the treatment groups.

RESULTS AND DISCUSSION

Growth performance

Table 3 shows the average growth performance of quails from day 1 to day 35. The analysis of variance revealed that adding 12% fermented rubber seed meal (FRSM) to their diet did not significantly affect their feed intake, body weight gain, or feed conversion ratio ($P>0.05$).

Dietary fermented rubber seeds meal showed no significant influence on quail feed intake and palatability. According to the opinion (Lamichhane et al. 2018), the organoleptic, such as taste, smell, and texture, reflects the palatability. The findings of this study are consistent with those reported by (Olawoyin 2010) that feeding rubber seed flour in quail does not affect ration consumption due to the palatability of the ration. The average feed intake in this study was 141.91-143.28 g/bird, as reported by (Hertamawati et al. 2019), that quail feed intake during the grower period averaged 131-154 g/bird.

Another factor that affects feed intake is the nearness of anti-nutritional substances in the ration; rubber seed flour contains antinutrient substances such as hydrogen

cyanide (HCN), which are toxic and harmful to the quail. The alternative to reducing these toxins could also be soaked and fermented (Matho et al. 2021). This research showed that fermentation with tempeh yeast reduced HCN levels from 158.64 ppm to 17.85 ppm. Quail and other poultry have some capacity to detoxify HCN through the enzymatic conversion of cyanide to thiocyanate, which is then excreted. However, this mechanism has a limit, and if the HCN content exceeds a certain threshold, the detoxification capacity is overwhelmed, leading to toxicity symptoms. The safe limit of HCN in poultry feed is generally recommended to be below 100 mg/kg to avoid adverse effects on carcass quality and bird health (Devi and Diarra 2021). According to (Aguihe et al. 2017), rubber seed meal treated with soaking and fermentation processes will reduce the HCN content in rubber seeds. The increase in body weight was related to feed intake (Nnadi et al. 2022), an important factor affecting body weight gain.

The feed conversion value with FRSM was higher than the control feed, which indicates a decrease in feed efficiency. Several factors affect feed conversion, namely digestibility, body weight gain, and feed consumption (Varkoohi et al. 2010), which stated that the factors affecting feed conversion include digestibility of feed quality, body weight gain, and feed intake.

Carcass yield

Table 4 illustrates the average body weight, carcass weight, and carcass percentage. According to the variance analysis, adding fermented rubber seed meal (FRSM) to the diet did not have a significant effect on the final body weight, carcass weight, or carcass percentage (P>0.05). The average final body weight of quail in this study was 160.4 to 161.0 g/bird. The final body weight that did not differ in each treatment was thought to be due to the consumption of the same feed from all the treatments carried out, resulting in a relatively equal average live weight because one of the factors that affect live weight is feed consumption (Novita et al. 2019; Kouassi et al. 2020).

This study demonstrated that the administration of fermented rubber seeds did not affect the carcass quality of quail, as the HCN content in the rubber seeds had decreased and was digestible by the quail. Reducing HCN levels in rubberseed-based feeds through fermentation can mitigate the adverse effects on carcass quality, leading to healthier, more robust birds with better meat yield and texture (Oluodo et al. 2018). The average carcass weight obtained ranged from 107.39 to 111.77 g/bird. The quality and production of carcasses are closely related to live weight. The live weight of quail in this study was higher than the results of research conducted by (Karim 2015), which stated an average live weight of 131 to 139 grams/bird. Factors affecting carcass weight include live weight, species, genetics, and the same slaughter age. The study by (Basri and Sulastri 2019) also concluded that including rubber seed meal in the diet did not significantly affect carcass weight or the average carcass weight of quails. The average carcass percentage for each treatment was 69.59%, .68.58%, 66.947% and 67.97%. This result is similar to the results of studies by (Nnadi et al. 2022) and (Karim 2015), which stated that quail carcasses ranged from 62.26% to 75.75%. The percentage of carcass is determined by several factors, one of which is the live weight produced. The rate of the carcass is affected by live body weight, and the rate of the carcass begins from the development rate demonstrated by the increment in body weight, which influences the coming about of live weight (Sabow 2020).

Table 3. Growth performance of quail feeding with different levels of fermented rubber seed meal

Feeding Treatments	Feed intake (g/bird)	Body weight gain (g/bird)	Feed Conversion
R0 (0% FRSM)	491.90±2.04	149.76±5.21	3.29±0.11
R1 (4% FRSM)	496.47±2.60	144.71±3.35	3.43±0.08
R2 (8% FRSM)	493.32±2.05	144.71±3.25	3.41±0.09
R3 (12% FRSM)	496.30±2.46	143.00±4.98	3.47±0.09

FRSM= fermented rubber seed meal; R0= feeding without FRSM (control); R1= feeding of 4% FRSM; R2 = feeding of 8% FRSM; R3= feeding of 12% FRSM

Table 4. Carcass yield of quail feeding with different levels of fermented rubber seed meal

Feeding Treatment	Final body weight (g/bird)	Carcass weight (g/bird)	Percentage of Carcass (%)
R0 (0% FRSM)	160.4±4.62	107.39±4.53	66.94±1.57
R1 (4% FRSM)	160.4±4.72	110.02±5.08	68.58±2.14
R2 (8% FRSM)	161.0±6.16	109.45±5.96	67.97±2.42
R3 (12% FRSM)	160.6±3.29	111.77±3.55	69.59±0.94

FRSM= fermented rubber seed meal; R0= feeding without FRSM (control); R1= feeding of 4% FRSM; R2 = feeding of 8% FRSM; R3= feeding of 12% FRSM

CONCLUSION

It can be concluded that the male quail-fed diets containing fermented rubber seed meal (FRSM) up to 12% did not reduce growth performance and carcass yield.

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Supplementation of Selenium-enriched Black Soldier Fly (*Hermetia illucens*) Larvae Meal on Growth Performance, Blood Parameters, and Immune Function in Broiler Ducks

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ABSTRAK

Kurniawan D, Widodo E, Susilo A, Sjofjan O. 2024. Suplementasi tepung larva lalat tentara hitam (*Hermetia illucens*) yang diperkaya selenium terhadap performa pertumbuhan, parameter darah, dan fungsi imun pada itik pedaging. JITV 29(4):227-235. DOI: http://dx.doi.org/jitv.v29i4.3452.

Penelitian ini bertujuan untuk mengevaluasi pengaruh dari tepung larva *Hermetia illucens* yang diperkaya dengan Se (Se-BSF) terhadap penampilan produksi, biokimia darah, dan status kekebalan tubuh itik pedaging. Sebanyak 200 ekor itik pedaging persilangan berumur satu hari tanpa dibedakan jenis kelamin dibagi secara acak ke dalam empat kelompok perlakuan, masing-masing dengan lima ulangan yang terdiri dari 10 ekor itik. Itik pedaging diberi pakan dengan pakan kontrol, pakan dengan 5% dan 7,5% Se-BSF, serta pakan kontrol positif dengan 10 mg/kg Se-Yeast dengan lama pemeliharaan selama 49 hari. Hasil penelitian menunjukkan bahwa performa produksi, karakteristik karkas, dan organ dalam itik pedaging dipengaruhi secara signifikan (P<0,001) oleh suplementasi Se-BSF. Pemberian pakan yang mengandung 7,5% Se-BSF menunjukkan tren penurunan konsumsi pakan, bobot badan akhir, pertambahan bobot badan, dan efisiensi pakan. Pengaruh pemberian Se-BSF berbeda nyata (P<0,001) antara semua perlakuan pada bobot hidup, bobot karkas, persentase karkas, daging dada, daging paha, hati dan limpa. Penambahan Se-BSF pada pakan itik pedaging tidak menunjukkan perbedaan yang signifikan antara semua perlakuan pada organ jantung, ampela, lemak abdominal, dan bursa fabricius. Pemberian pakan yang mengandung 5% sampai 7,5% Se-BSF berpengaruh secara signifikan (P<0,001) terhadap kadar HDL, LDL, Kolesterol, Trigliserida, Ig-A, SOD, dan IL-6 dalam serum dibandingkan dengan kontrol dan 10 mg/kg Se-yeast. Hasil ini menunjukkan bahwa pemberian Se-BSF dalam pakan meningkatkan parameter biokimia darah dan fungsi kekebalan tubuh itik pedaging, yang mengindikasikan potensi manfaat dari penggunaan Se-BSF sebagai aditif pakan untuk unggas.

Kata Kunci: Tepung Larva BSF, Penampilan Produksi, Imunitas, Selenium Organik

ABSTRACT

Kurniawan D, Widodo E, Susilo A, Sjofjan O. 2024. Supplementation of selenium-enriched black soldier fly (*Hermetia illucens*) larvae meal on growth performance, blood parameters, and immune function in broiler ducks. JITV 29(4):227-235. DOI: http://dx.doi.org/jitv.v29i4.3452.

This study aimed to evaluate the effects of Se-enriched *Hermetia illucens* larvae meal (Se-BSF) on production performance, blood biochemistry, and immune status of broiler ducks. Two hundred one-day-old hybrid broiler ducks without sex were randomly allocated into four groups, each with five replications of 10 ducklings. Broiler ducks were fed diets with a controlled diet, an experimental diet with 5% and 7.5% Se-BSF, and positive control with 10 mg/kg Se-Yeast, respectively, daily for 49 days with drinking water. The results showed that the performance production on the carcass traits and visceral organs of broiler ducks was significantly affected (P<0.001) by Se-BSF supplementation. Dietary 7.5% Se-BSF showed trends in decreasing the feed intake, final body weight, average daily gain, and feed efficiency ratio. The effect of dietary Se-BSF was significant differences (P<0.001) between all treatments in live body weight, carcass weight, dressing, breast meat, thigh meat, liver, and spleen. The addition of Se-BSF to broiler ducks' diet showed no significant differences between all heart, gizzard, abdominal fat, and bursa treatments. The effects of dietary 5% to 7.5% Se-BSF had significantly affected (P<0.001) HDL, LDL, Cholesterol, Triglyceride, Ig-A, SOD, and IL-6 levels in serum compared with control and 10 mg/kg yeast-Se. These results indicate that dietary Se-BSF improves broiler ducks' blood biochemistry and immune function, suggesting potential benefits from using Se-BSF as a feed additive for poultry.

Key Words: BSF Larvae Meal, Growth Performance, Immunity, Organic Selenium

INTRODUCTION

Poultry meat and eggs are among the most popular animal-derived consumables worldwide, regardless of culture, tradition, or religion. Duck meat is highly nutritional because it contains all required amino acids and has a healthy fatty acid composition (Ismoyowati & Sumarmono 2019). The Food and Agriculture

Organization (2022) reported that in 2020, the worldwide population of ducks (*Anas spp.*) reached 1.15 billion and 1.0 billion (89 per cent) were in Asia. Duck meat production in Indonesia has averaged 37,878 tons/per year over the last 5 years, contributing less than 1% of the meat production of all livestock species nationally (Directorate General of Animal Husbandry and Animal Health, 2022). The livestock industry is expected to rise to the challenge of developing innovative and sustainable methods in the future. Meeting the need for safe and resource-efficient livestock products, resulting in quality products that can compete globally (Barragan-Fonseca et al. 2017), the use of natural feed additives as an alternative to antibiotics (Ismita et al. 2022), the use of alternative sources of animal protein are environmentally friendly and sustainable that do not compete with humans (Cullere et al. 2019) and increase the nutritional value of poultry meat products produced (Jachimowicz et al. 2022).

Selenium (Se) is one of the indispensable nutrients for human health and livestock growth that plays a role in various physiological functions, such as antioxidant activity, immune response, and metabolism (Ferro et al. 2021). Se in the form of selenoproteins contains 21 amino acids and has 25 forms in the livestock body (Dalgaard et al. 2018). Se can improve antioxidant status through the enzymatic redox system by forming Se-Cysteine at the cellular level (Gu & Gao 2022). Se also plays a role in DNA formation, immune response to oxidative stress, maintenance of endoplasmic reticulum integrity, and nutrient metabolism (Silva et al. 2019). In nonruminant livestock such as poultry and pigs, Se in the form of selenoproteins regulates oxidative stress, redox mechanisms, and other critical cellular processes involved in innate and adaptive immune responses (Qiu et al. 2021).

There are two types of Se additions in animal feed: organic and inorganic. Organic Se (selenoprotein and Se-yeast) is more effective than inorganic Se (sodium selenite) in enhancing production performance, antioxidant status, and Se concentration deposited in animal body tissues, and plays a role in improving livestock meat quality (Ferrari et al. 2022). It has been observed that using organic sources of Se in broiler diets improves the physical and chemical properties of meat while also extending shelf life (Silva et al. 2019). However, some Se sources exhibit shortcomings that include a low content of organic Se, a long production period, and a high cost (Zhang et al. 2014). As a result, a novel Se-enriched technique that enables a high yield of organic Se in a brief production time and at a reasonable cost is still needed (Dong et al. 2021). Selenium conjugated insect protein (SCIP) is a new type of organic Se source obtained through two biotransformation steps of microbial fermentation and synthesis of insect larvae to possibly guarantee high bioavailability, biosafety, and high biological value. SCIP has been shown to benefit the health of laying hens and improve the safety of Se-fortified eggs (Qiu et al. 2021).

Black soldier fly /BSF (*Hermetia illucen* L.) has been researched for their capacity to transform organic waste into high-quality protein, suppress some dangerous bacteria and insect pests, supply possible chemical precursors to produce biodiesel, and be fed to a variety of livestock (Barragan-Fonseca et al. 2017). BSF larvae contain high levels of protein (37-63%), dry matter, and other macro and micronutrients essential for animal feed. Existing research on BSF larvae in poultry, pigs, and fish feed shows that BSF larvae can only partially replace conventional feed ingredients (Cullere et al. 2019). (Ferrari et al. 2022) investigated the enrichment of Se in BSF larvae and found that the average total Se content obtained was produced more than five times that of the control group. Efforts are needed to explore non-toxic sources of Se as feed additives to reduce the stressors effect on poultry during production. Our study's objective was to investigate the potential of Se-enriched black soldier fly larvae meal (Se-BSF) as feed ingredients on production performance, blood biochemistry, and immune system of broiler ducks.

MATERIALS AND METHODS

Ethical approval

The Research Ethics and Animal Care Committee at the Institute of Biosciences, Brawijaya University, Indonesia, approved the experimental methodology (No 004-KEP-UB-2024).

Animals, diets, and experimental design

Two hundred one-day-old Hybrid broiler ducks were obtained from a commercial hatchery (Blitar, East Java, Indonesia). The ducklings were randomly grouped into four feeding treatments with 5 replicates and 10 birds in each group and housed in communal cages measuring 100 cm wide × 200 cm long × 60 cm high. The diet consisted of a control diet, 5% and 7.5% Se-BSF, and positive control with 10 mg/kg Se-Yeast. Dried and bio-synthesized selenium-enriched BSF larvae (Se-BSF) meals were supplied from the Department of Poultry Product Processing, Community College State of Putra Sang Fajar Blitar, Indonesia. The procedure for producing Se-BSF meal through two-step biotransformation using sodium selenite refers to the study of (Qiu et al. 2021). First, under optimal conditions, BSF larval growth media fermentation enriched with sodium selenite using yeast *Saccharomyces cerevisiae*. Bran and soybean meal are added as raw materials, and 400 mg/kg sodium selenite

Table 1. Composition (%) of the experimental broiler duck diets containing different levels of Se-BSF

Item	Starter (0-21 days)				Finisher (21-42 days)			
	Control	Se-BSF 5%	Se-BSF 7.5%	Se-Yeast	Control	Se-BSF 5%	Se-BSF 7.5%	Se-Yeast
Corn	10.05	10.05	9.67	10.05	19.03	19.03	18.65	19.03
Soybean meal	29.05	29.05	26.93	29.05	20.07	20.07	17.95	20.07
Rice bran	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Fish meal	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Premix	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
BSF meal	5.00	-	-	5.00	5.00	-	-	5.00
Se-BSF meal	-	5.00	7.50	-	-	5.00	7.50	-
Calculated composition								
Metabolizable energy (kcal/kg)	2,759	2,759	2,758	2,759	2,860	2,860	2,859	2,860
Crude Protein (%)	23.00	23.00	23.00	23.00	20.00	20.00	20.00	20.00
Crude Fat (%)	7.80	8.02	8.85	7.80	7.96	8.17	9.00	7.96
Crude Fiber (%)	3.36	3.36	3.23	3.36	3.00	3.00	2.87	3.00
Calcium (%)	0.62	0.62	0.61	0.62	0.60	0.60	0.59	0.60
Phosphorus (%)	0.38	0.38	0.36	0.38	0.33	0.33	0.31	0.33
Lysine (%)	1.82	1.82	1.82	1.82	1.58	1.58	1.57	1.58
Methionine (%)	0.69	0.69	0.70	0.69	0.65	0.65	0.66	0.65

Se-BSF= selenium-enriched black soldier fly

is added (Kurniawan et al. 2024). Second, fermented growth media (Se-rich protein yeast) is given as BSF larval feed. Finally, selenium-enriched BSF larvae are dried and ground into a Se-BSF meal, then stored in a dry place until the following process. The experimental diets were formulated using ground yellow maize, rice brand, and a soybean meal (Table 1) in two phases: the starter phase on days 1 to 21 and the finisher phase on days 21 to 49. All birds were in the same condition.

Growth performance and relative weights of organs

Weight recording was conducted weekly to determine average body weight gain (BWG) along with recording feed intake (FI) and calculating feed conversion ratio (FCR). At the age of 49 days, representative ducks were slaughtered and randomly selected from each replicate that had previously been fed for 12 hours. Slaughter was carried out by manual slaughtering using a sharp knife, scalded in a hot water bath, and mechanically plucked feathers. Then the weight of the carcass, breast and thighs, abdominal fat, and internal organs such as liver, heart, gizzard, bursa Fabricius, and spleen were determined by weighing and calculating the relative weight as a percentage of the live body weight to determine the carcass traits of broiler ducks.

Biochemical analysis of blood serum

At the end of the finishing period (7 weeks), the representative ducks from each replicate were collected as blood samples. Blood samples were drawn into sterile, non-heparinized tubes to separate the serum and centrifuged for 15 minutes at 4°C at 3000 rpm. After collected, the serum was transferred to Eppendorf tubes

and kept cold (-20°C) for further biochemical examination. The amount of cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum was determined using a commercial kit on a spectrophotometer. Superoxide dismutase (SOD) assay was determined with a commercial kit (Khan et al. 2023). IgA ELISA quantification kits explicitly designed for chickens were used to measure the levels of plasma IgA (Abdel-Moneim et al. 2022).

Intestinal immunohistochemistry

Intestinal immunohistochemistry was performed using the same slaughtered broiler ducks. The immunohistochemistry protocol refers to the study reported by (Khan et al. 2020). An automated tissue processor was used to process the pre-fixed ileum samples for a night. The processed ileum samples were embedded in paraffin blocks using an embedding apparatus. We sectioned ileum specimens into 4 mm thick pieces using a rotary microtome. After that, the portions were placed on highly frosted glass slides. They were run through distilled water, xylene, and ethanol at varying concentrations to de-paraffinize the paraffinized sections. They were microwave-heated in citrate buffer for 10 minutes to prime the sections for effective antigen retrieval. For immunohistochemistry, we used a DAB Detection kit. IL-6 was employed as the main antibody for the proinflammatory cytokines. Primary antibodies were first applied to the sections and added to the DAB substrate. Lastly, Harris Haematoxylin counterstaining was applied to the sections to examine them under a light microscope. Calculating the number of expression antibodies is performed using the ImageJ program to analyze the stained preparation immunohistochemistry (IHC) image to see the amount of expression-specific antibodies that can be downloaded online. The expression of IL-6 antibodies in the intestine can be calculated by describing the brown colour expressed in the preparation. Results of IL-6 expression calculation using ImageJ of the form percentage. Expression assessment was carried out on 5 fields, a different view. This observation was performed with a light microscope (Nikon Eclipse type Ei) and an Optilab Microscope Camera connected to a computer.

Statistical analysis

All data were presented as mean ± standard deviation and analyzed using one-way ANOVA with a completely randomized design in SPSS software. To compare the means, differences between means were determined using the least significant difference (LSD) tests (Mattjik and Sumertajaya 2013). For all tests, P-values <0.05 indicated significant differences.

RESULTS AND DISCUSSION

Effect of Se-BSF on the production performance

The effects of feeding Se-BSF compared to Se-yeast on the production performance of broiler ducks are presented in Table 2. There was no mortality during the experiment. From 1 to 49 d, the FBW, ADG, FI, FCR, FER, PI, PER, EI, and EFR of broiler ducks were influenced by Se-BSF supplementation (P<0.001). Dietary Se-BSF 7.5% showed trends in decreasing the FI, FBW, ADG, and FER and increasing the FCR. These data revealed that dietary Se-BSF 5% did not negatively influence broiler ducks' performance production. The effect of feeding Se-BSF on broiler ducks' carcass and internal organ traits showed significant differences (P<0.001) in Dressing, BM, TM, Liver and Spleen. Adding Se-BSF to the broiler duck diet resulted in no significant differences in heart, gizzard, abdominal fat, or bursa.

Growth performance characteristics are utilized to assess the financial advantages of additives, such as Se-BSF supplementation. Compared to Se-yeast, the current study showed that dietary Se-BSF significantly impacted the growth performance of broiler ducks. Feeding selenium improved body weight, feed consumption, and feed conversion ratio. According to (Al-Quwaie 2023) the addition of *Bacillus subtilis* selenium nanoparticles (BseNPs) to the feed considerably raised the chicks' final body weight, reduced their feed intake, and improved their FCR value. Similarly, (Rehman et al. 2022) discovered that adding SeNP-MOS to the broiler diet enhanced feed consumption, FCR, weekly BWG, and final body weight of broilers that were ready for the market. The SeNP supplementation had greater body weight, weight gain, and performance indicators after 38 days of the feeding trial. Some research showed that selenium had no significant effect on growth and performance.

Bami et al. (2022) reported that different levels of green synthesized nano selenium (GNS) had no significant effect on broiler chicken growth. The discrepancies in results could be attributed to variances in Se levels, experimental setups, or chicken breeds used. The dietary Se-BSF showed significant differences in CW, BM, TM, Liver, and Spleen. Still, there was no significant difference among all treatments in dressing, heart, gizzard, abdominal fat, and bursa. The previous findings (Al-Quwaie 2023) demonstrated that feeding BSeNPS to broiler diets significantly decreased fat content and increased breast muscle. Still, there were no significant differences in the carcass properties. (Elkhateeb et al. 2022) reported that broilers fed diets fortified with different sources of selenium had higher percentages of dressing and abdomen fat but no effect on internal organs among all treatments. (Bakhshalinejad et

al. 2019) reported similar findings, demonstrating that Se source, Se inclusion rate, and their interaction did not affect carcass traits.

Effect of Se-BSF on the blood biochemistry

The effect of Se-BSF feeding on blood biochemical parameters and the immune status of broiler ducks are presented in Table 4. After 49 days, the serum levels of HDL, LDL, Cholesterol, Triglycerides, Ig-A, SOD, and IL-6 were significantly (P<0.001) affected in ducks with 5% to 7.5% Se-BSF compared to controls and Se-yeast. In addition, compared to controls, ducks in Se-BSF 7.5% had significantly decreased HDL, LDL, triglycerides, and cholesterol levels. Regarding immunological status parameters, ducks fed between 5% and 7.5% Se-BSF showed significantly greater serum Ig-A levels than the control group. In terms of oxidative stress characteristics, ducks treated with 5% and 7.5% Se-BSF exhibited significantly lower serum SOD levels than ducks in the control group.

Ileum immunohistochemistry of broiler ducks

The immunohistopathology of the ileum of broiler ducks showed immunostaining for IL-6 (Figure 1). The results of the semi-quantitative analysis of immunostaining for different proinflammatory cytokines are summarized in Table 4. Dietary Se-BSF significantly increased interleukin 6 (IL-6) levels in broiler ducks compared to the control group (P<0.001).

Expression IL-6 antibodies in the intestine can be calculated through the description brown colour expressed in the preparation (Arrow). Results of IL-6

Table 2. Effect of dietary Se-BSF on the growth performance, carcass traits, and visceral organ of broiler ducks from 0 to 49 d of age

Parameters	Control	Se-BSF (%)		Se-Yeast	SEM	P-value
		5	7.5			
Growth performance						
IBW (g)	47.00±1.22	46.88±1.21	46.58±0.83	46.6±0.82	0.1907	0.942
FBW (g)	2,052±93^{b}	1,999±74^{b}	1,666±107^{a}	1,690±82^{a}	80.83	<0.001
ADG (g)	2,005±94^{b}	1,952±74^{b}	1,620±107^{a}	1,656±87^{a}	78.85	<0.001
FI (g)	5,245±283^{b}	4,937±384^{ab}	4,680±236^{a}	4,66±93^{a}	155.28	<0.001
FCR	2.77±0.13^{a}	2.69 0.11^{a}	3.11±0.24^{b}	2.89±0.18^{ab}	0.082	<0.001
FER	36.20±1.64^{b}	37.21±1.50^{b}	32.33±2.56^{a}	34.70±2.07^{ab}	0.827	<0.001
PI	1,029±51^{b}	972±69^{ab}	918±42^{a}	914±17^{a}	32.70	<0.001
PER	1.96±0.09^{bc}	2.10±0.08^{c}	1.76±0.14^{a}	1.81±0.18^{ab}	0.046	<0.001
EI	15,522±791^{b}	14,662±1074^{ab}	13,908±660^{a}	13,872±317^{a}	488.91	<0.001
EFR (%)	7.71±0.36^{ab}	7.50±0.31^{a}	8.67±0.68^{b}	8.42±0.92^{b}	0.246	<0.001
Carcass traits						
Dressing (%)	63.37±2.86	65.04±1.29	63.18±2.83	62.74±4.63	0.669	0.019
BM (%)	18.93±1.16^{b}	18.58±2.48^{b}	16.95±1.00^{a}	16.31±1.08^{a}	0.369	<0.001
TM (%)	12.71±0.67^{a}	13.92±0.48^{ab}	14.42±1.08^{b}	14.13±0.95^{b}	0.236	0.011
Visceral organ						
Liver %	2.26±0.18^{a}	2.53±0.40^{ab}	2.78±0.52^{ab}	3.09±0.62^{b}	0.116	0.010
Heart %	0.53±0.03	0.55±0.11	0.57±0.03	0.54±0.03	0.012	0.387
Gizzard %	2.63±0.47	2.71±0.20	2.65±0.53	3.10±0.54	0.105	0.440
AF %	1.36±0.56	1.55±0.22	1.40±0.33	1.57±0.49	0.101	0.907
Spleen %	0.21±0.10^{b}	0.09±0.01^{a}	0.10±0.05^{a}	0.13±0.05^{ab}	0.013	0.040
Bursa %	0.11±0.02	0.15±0.04	0.11±0.04	0.15±0.03	0.007	0.175

Data are presented mean ± SD. *P* value ≤0.05 indicates significant difference. Lowercase latter ($^{a-c}$) in the same row indicates significant difference at P value ≤0.05. Se-BSF= selenium-enriched black soldier fly, IBW= Initial body weight, FBW= Final body weight, ADG: Average daily gain, FI= Feed intake, FCR= Feed conversion ratio, FER= Feed efficiency ratio, PI= Protein intake, PER= Protein efficiency ratio, EI= Energy intake, ERR= Energy efficiency ratio, Dressing, BM= Breast muscle, TM= Thigh muscle, AF= Abdominal fat

Table 3. Effect of dietary Se-BSF on the blood biochemistry and immune status of broiler ducks from 0 to 49 d of age

Parameters	Control	Se-BSF (%)		Se-Yeast	SEM	*P*-value
		5	7.5			
Blood biochemistry						
HDL (mg/dl)	147.96±0.21^{d}	93.01±0.50^{b}	100.90±1.57^{c}	89.40±0.40^{a}	7.10	<0.001
LDL (mg/dl)	152.89±1.47^{d}	89.78±0.84^{a}	93.94±1.74^{b}	107.72±2.54^{c}	7.56	<0.001
Cholesterol (mg/dl)	166.00±2.61^{b}	90.90±0.43^{a}	88.47±2.68^{a}	88.83±1.10^{a}	10.01	<0.001
Triglyceride (mg/dl)	36.93±0.13^{c}	36.83±0.66^{c}	35.32±1.11^{b}	33.58±0.14^{a}	0.44	<0.001
Blood immune						
Ig-A (mg/ml)	1.83±0.04^{a}	6.53±0.02^{b}	13.23±0.40^{d}	12.51±0.01^{c}	1.41	<0.001
SOD (U/ml)	37.99±0.66^{a}	39.07±0.95^{a}	52.20±0.79^{b}	82.39±0.66^{c}	5.41	<0.001
Immunohistochemistry						
IL-6 (%)	19.62±1.01^{a}	23.12 ± 2.16^{a}	27.89±3.44^{b}	33.96 ± 3.17^{c}	1.35	<0.001

Data are presented mean ± SD. *P* value ≤ 0.05 indicates significant difference. Lowercase latter ($^{a-d}$) in the same row indicates significant difference at *P* value ≤ 0.05. HDL: High-density lipoproteins, LDL: Low-density lipoproteins, Ig-A: Immunoglobulin A, SOD: Superoxide dismutase, IL-6: Interleukin 6.

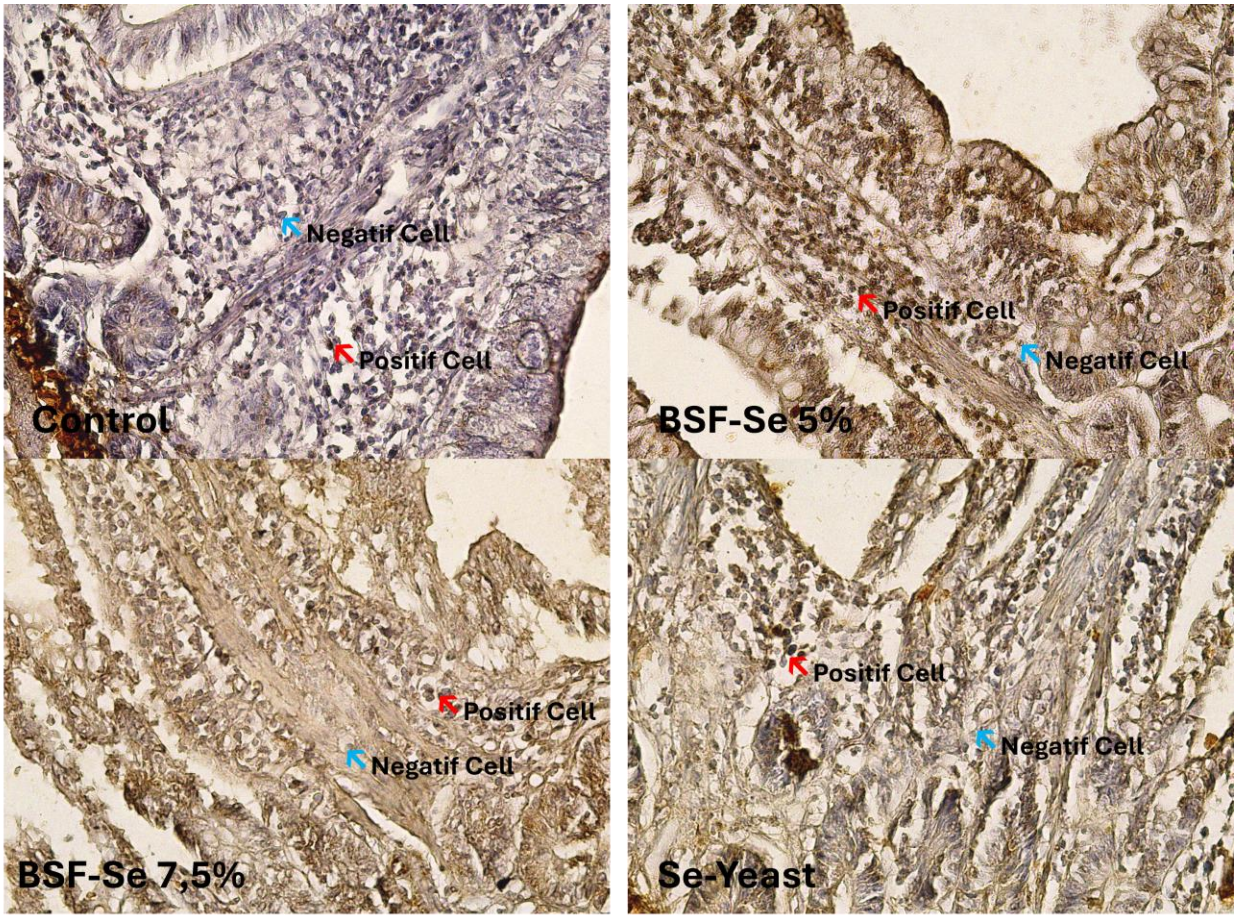

Figure 1. Immunohistochemistry of IL-6 in ileum of broiler duck treated with Se-BSF

expression calculation using ImageJ of the form percentage. Biochemical indicators are positively connected with the animals' nutritional status and can be used to evaluate the animals' general and physiological health. The dietary Se-BSF had significant differences (P>0.001) in serum HDL, LDL, Cholesterol, and Triglyceride compared with the control. This agrees with previous findings that feeding broilers with Se had significantly affected the plasma concentration of total cholesterol and triglyceride. (Abdel-Moneim et al. 2022) reported that birds fed diets enriched with Spirulina platensis (SP), selenium nanoparticles (SeNPs), or their mixtures significantly reduced blood cholesterol, triglycerides, and LDL-cholesterol. (Sun et al. 2020) Se-enriched earthworm powder (SEP) downregulated triglycerides, total cholesterol, glucose, and nitric oxide. Serum total cholesterol and triglycerides are two extensively used indices for assessing body lipid metabolism; an abnormal increase of either indicates a lipid metabolism disorder. SE-BSF reduced serum total cholesterol and triglycerides, suggesting that BSF meals enhanced with Se play an essential role in regulating body lipid metabolism and lowering blood fat of broiler duck.

In the current investigation, the dietary Se group had significantly decreased SOD level in serum compared to control. Previous research (Qiu et al. 2023) found that Se-BS supplementation improved the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), peroxidase (POD), and total antioxidant capacity (T-AOC) in plasma as compared to the broiler control group. (Sun et al. 2020) found that selenium-enriched earthworm powder with 1.0 mg Se per kg significantly improved the antioxidative enzyme activity of glutathione peroxidase and superoxide dismutase. Oxidative stress is an imbalance in the ratio of reactive oxidative species to antioxidants. The organism's antioxidant defence mechanism relies mainly on GSH-Px, SOD, and catalase to remove excess free radicals such as O_2, H_2O_2, and ROO_2. Increased reactive oxygen species generation and high levels of oxidative stress on blood metabolites are associated with selenium deficiency (Liu et al. 2022).

B cell-produced antibodies and immunoglobulins are two key indications of humoral immunity. Besides its antimicrobial and antiviral properties, IgA is also involved in the "barrier" function of the body's mucosal immunity in the digestive and respiratory systems. (Dalia et al. 2018) Se supplementation from inorganic and bacterial organic sources boosted immunity in broiler chickens by increasing IgA, IgG, and IgM levels. In this investigation, the dietary Se-BSF had increasing Ig-A levels in the serum of the broiler duck at day 42. This could be explained by Se's role in protecting and activating B-lymphocyte cells, which produce immunoglobulin. Our investigation found that selenium might alter cytokine release in broiler duck ileum, which is consistent with earlier findings. (Zhang et al. 2022) found that low selenium levels lowered IL-6 and IL-10 levels in mouse serum, but high selenium levels enhanced IL-6 content. The findings revealed that low and high selenium levels can modify immune cell composition via modulating cytokine release, creating inflammatory injury, poor immune response in the mouse spleen, and inflammatory damage to other organs.

CONCLUSION

The dietary Se-BSF 5% did not have a negative influence on the performance production of broiler ducks. These findings suggest that feeding broiler ducks dietary Se-BSF enhances their blood biochemistry and immune system, suggesting possible advantages for using Se-BSF as a feed supplement for poultry.

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Qualitative Traits of Local Bambu Apus Rabbits

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ABSTRAK

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Kelinci lokal Bambu Apus merupakan kelinci persilangan yang memiliki sifat kualitatif yang beragam. Banyak diantaranya menyerupai kelinci Rex dan New Zealand White. Sehingga perlu dilakukan perbandingan sifat kualitatif kelinci lokal Bambu Apus dengan kelinci Rex dan kelinci New Zealand White. Total sampel yang diamati sebanyak 94 ekor kelinci lokal Bambu Apus, 89 ekor kelinci Rex, dan 89 ekor kelinci New Zealand White. Penelitian ini bertujuan untuk mengevaluasi sifat kualitatif kelinci lokal Bambu Apus sebagai kelinci yang telah mampu beradaptasi dengan lingkungan DKI Jakarta sehingga diharapkan dapat menjadi galur kelinci pedaging untuk mendukung urban farming di DKI Jakarta. Variabel yang diamati meliputi sifat-sifat kualitatif seperti tipe kepala, tipe telinga, warna mata, warna tubuh dominan, pola warna tubuh, warna belang, penyebaran belang, karakteristik bulu, tipe tubuh, dan ukuran tubuh. Analisis data dilakukan dengan menggunakan software SAS versi 9.4 dengan prosedur PROC FREQ untuk frekuensi dan persentase masing-masing variabel, kemudian dilakukan analisis Multiple Correspondence Analysis (MCA) antar variabel kategori dengan menggunakan prosedur PROC CORESP. Hasil penelitian menunjukkan kelinci lokal Bambu Apus memiliki sifat kualitatif yang berbeda dengan kelinci Rex dan New Zealand White. Adapun sifat kualitatif yang dapat dijadikan pembeda diantaranya keberadaan warna mata biru dan heterochromia, warna dominan cokelat dan pola warna harlequin yang lebih banyak, adanya variasi tipe tubuh compact, karakteristik bulu lion, dan tipe telinga lop. Kelinci lokal Bambu Apus memiliki tipe tubuh komersial dan ukuran medium, menunjukkan potensi genetik sebagai kelinci pedaging adaptif iklim tropis untuk mendukung urban farming di DKI Jakarta.

Kata Kunci: Adaptabilitas, Kelinci Bambu Apus, Kelinci Pedaging, Sifat Kualitatif

ABSTRACT

Nuraini H, Islami AK, Aditia EL, Brahmantiyo B, Handiwirawan E. 2024. Qualitative traits of local Bambu Apus rabbits. qualitative traits of local Bambu Apus rabbits. JITV 29(4): 236-250. DOI:http://dx.doi.org/10.14334/jitv.v29i4.3464.

The local Bambu Apus rabbit is crossbred with diverse qualitative traits, many resembling Rex and New Zealand White rabbits. Hence, it is necessary to compare the qualitative traits of local Bambu Apus rabbits with Rex and New Zealand White rabbits. The total samples observed were 94 local Bambu Apus rabbits, 89 Rex rabbits, and 89 New Zealand White rabbits. This study aims to evaluate the qualitative traits of local Bambu Apus rabbits as rabbits that have adapted to the environment of DKI Jakarta, so they are expected to become broiler rabbit strains that support urban farming in DKI Jakarta. Variables observed included qualitative traits such as head type, ear type, eye color, predominant body color, body color pattern, stripe color, stripe distribution, fur characteristics, body type, and body size. Qualitative traits observed included head type, ear type, eye color, body color, color pattern, stripe characteristics, fur type, body type, and size. Data were analyzed using SAS 9.4 with PROC FREQ for frequency and percentage of each variable and Multiple Correspondence Analysis (MCA) using PROC CORESP. Results showed that Bambu Apus rabbits exhibit distinct traits, including the presence of blue and heterochromia eye color, predominantly light brown color and harlequin color pattern that is more prevalent in Bambu Apus rabbits, variations in compact body type, lion fur characteristics, and lop ear type. With a commercial body type and medium size, these traits highlight their genetic potential as tropical climate-adaptive broiler rabbits supporting urban farming in DKI Jakarta.

Key Words: Adaptability, Bambu Apus Rabbit, Broiler Rabbit, Qualitative Traits

INTRODUCTION

In late 2003 and early 2004, an avian influenza (AI) outbreak caused by the H5N1 virus spread rapidly in Southeast Asia, including Indonesia. In Indonesia, the AI virus spread very quickly to all parts of Indonesia, including DKI Jakarta. The rapid spread of the disease prompted the government to take precautionary measures to reduce casualties and limit the spread (Sarah 2019; Kencana et al. 2021). As an effort by the DKI Jakarta government to prevent and mitigate AI outbreaks, a policy was issued in Regional Regulation

No. 4/2007 that regulates the Control of Poultry Rearing and Distribution, where people are not allowed to keep poultry freely to prevent AI outbreaks. With the enactment of this regulation, the DKI Jakarta urban farming program also adjusted the livestock commodities that can be developed.

Rabbits are a potential livestock commodity that can be used to produce meat. However, not only meat, fur, and skin can also be utilized, which makes it a livestock with high economic value. As stated by (Brahmantiyo et al. 2014), commercially produced rabbits are very profitable because they can produce quality meat with low production costs. Rabbits have a high growth rate, efficient feed consumption, do not require large areas of land, and a short cultivation process where if adequately maintained (Fischer et al. 2012; Tembachako and Mrema 2016; Jiang et al. 2020; Marín-García et al. 2021). A total litter averaging 40-60 kits per year is produced by a rabbit (approximately 8-12 kits/parity). Another advantage is that the doe can mate immediately after kindling or some days later because of high receptivity at this particular period (Dalle Zotte 2014).

Since October 2020, the Bambu Apus Rabbit Park has been opened under the Services Centre for Animal Health and Livestock (Pusyankeswannak). The Bambu Apus livestock park is devoted to raising livestock in the form of rabbits. Rabbits can be introduced as prospective and alternative livestock for the people of DKI Jakarta who want to participate in supporting the sustainable integrated urban farming program (Margatama et al., 2023). The Service Centre for Animal Health and Livestock (Pusyankeswannak) plans to develop new strains of local Bambu Apus rabbits that are adaptable to the environment and climate of Jakarta by paying attention to breeding according to GFP (good farming practice) and GBP (good breeding practice). Breeding of local Bambu Apus rabbits in Pusyankeswannak is expected to be carried out sustainably, and adequately as a source of animal protein hope from broiler rabbits whose growth is expected to continue to increase in line with the DKI Jakarta Urban Agriculture Grand Design Target in 2030, there are 1.000 rabbits spread and maintained in the community.

Apart from the advantages of rabbit farming, there is a challenge, particularly in that rabbits have an ideal temperature range of around 15-25ºC (Liu et al. 2022). When heat stress occurs, they will attempt to reduce the excess heat through different mechanisms, such as thermoregulatory responses. These responses impact rabbits by interfering with their physiological processes (Mutwedu et al. 2021; El Sabry et al. 2021; Liang et al. 2022). The DKI Jakarta area has an average temperature of 28.8ºC with the maximum temperature reaching 35ºC (Badan Pusat Statistik 2021). Even though it is reared in an unsuitable environment, the local Bambu Apus rabbits can grow and reproduce. Local Bambu Apus rabbits were raised in 2018 in the field owned by the livestock business and promotion unit before establishing the Bambu Apus Rabbit Park in 2020. Pusyankeswannak received 85 rabbits, 75 ready-for-breeding New Zealand White does, and 10 Flemish Giant bucks. The rabbits were then bred, and their offspring have been distributed across five administrative cities in DKI Jakarta. The total population is 109 local rabbits in the Bambu Apus Rabbit Park as of May 2024; this shows the local Bambu Apus rabbit's adaptability to the environment of DKI Jakarta, as evidenced by its population, which still reproduces and survives today.

A strain is a group of individual animals in a breed with specific characteristics used for breeding or cultivation purposes (Setiadi 2017). One of the requirements for the concession of strains stipulated in Article 6 of Permentan Number: 117/Permentan/SR.120/10/2014 is that the characteristics referred to are qualitative traits covering the characteristics of a strain that can be distinguished from other strains. Local Bambu Apus rabbits have diverse qualitative traits, and many resemble Rex and New Zealand White rabbits, requiring further research. New Zealand White rabbits are bred for their meat, and Rex rabbits are bred for their fur and meat. Both breeds can be utilized as broiler rabbits. Therefore, the characterization of qualitative traits of local Bambu Apus rabbits is needed as a basis for the concession of rabbits specific to DKI Jakarta, whose utilization is as broiler rabbits adaptable to the environment of DKI Jakarta.

MATERIALS AND METHODS

This research was conducted at Bambu Apus Rabbit Park, Service Center for Animal Health and Animal Husbandry (Pusyankeswannak) DKI Jakarta, and Poultry and Miscellaneous. Livestock Instrument Standard Testing Centre (BPSI-UAT Ciawi). All work procedures in this study have met the Ethical Clearance standards of the Balitbangtan Animal Welfare Commission (KKHB) of the Agricultural Research and Development Agency, with registration number: Balitbangtan/Livestock Research Centre BRIN/NRm/01/2022.

Sample and materials

For comparison, this study used 94 local Bambu Apus rabbits from Bambu Apus Rabbit Park, 89 Rex rabbits (Rexsi Agrinak), and 89 New Zealand White rabbits from BPSI-UAT Ciawi. The rabbits' age and sex were not considered. The equipment used in this study included a digital thermo-hygrometer, digital scales, a vernier caliper, measuring tape, a posing table, and a posing mat.

Figure 1. Body type of rabbit as (a) Arch, (b) Semi Arch, (c) Compact, (d) Commercial, (e) Cylindrical (ARBA 2000)

Qualitative traits data measurement

Qualitative data measurement is done by direct observation of the rabbit. Qualitative traits variables observed were: (1) Predominant body colour, determined by looking at the colour that dominates throughout the rabbit's body; (2) Body colour pattern, categorised by the number of colour variations on the rabbit's body; (3) Striped colour, determined by colour variations other than the predominant colour on the rabbit's body; (4) The spread of stripes, determined by the extent of the spread of stripes on the predominant colour of the rabbit's body; (5) Fur characteristics, categorised if the rabbit has hair with short size and smooth texture then the rabbit has normal fur characteristics, if the rabbit has hair with short size and upright right on the skin and smooth hair texture then the rabbit has rex fur type, if the rabbit has long, thin and smooth hair then the rabbit has angora fur characteristics, if the rabbit has long hair on the neck and groin then the rabbit has lion fur characteristics, if the rabbit has hair with a smaller diameter and the outer layer is transparent, shiny and slippery then the rabbit has satin fur characteristics (Brahmantiyo et al. 2021); (6) Eye colour, determined by the colour of the iris; (7) Ear shape, determined by the shape of the ears standing upright/lop, if only one ear is lop-shaped it is categorised as standing upright ears; (8) Body type, seen from the shape of the body when in a stationary position, calm and the rabbit's body is on a flat medium. Figure 1 shows the 5 body types of rabbits: (9) Head type, determined based on the index value. The head shape is oval if the head index value is <0.5. If the head index value is >0.5, then the head shape is round (Brahmantiyo et al. 2021); (10) Body size, categorized by weight. If the weight is <2.7 kg, it is categorized as small. If the weight is 2.7-4.1 kg, it is categorized as medium size; if the weight is 4.1-5 kg, it is categorized as large; if the weight is >5 kg, it is categorized as giant size.

The qualitative traits observed were recorded as (1) Predominant body color, 1= White, 2= Light brown, 3= Dark brown, 4= Grey, 5= Black; (2) Body color pattern, 1= One color, 2= Two color mix, 3= Three color mix; (3) Stripe color, 0= None, 1= White, 2= Light brown, 3= Dark brown, 4= Grey, 5= Black, 6= Harlequin; (4) Stripe distribution, 0= 0%, 1= 1 - 10%, 2= >10 - 20%, 3= >20 - 30%, 4= >30 - 40%, 5= >40 - 50%; (5) Fur characteristics, 1= Normal, 2= Rex, 3= Satin, 4= Lion, 5= Anggora; (6) Eye color, 1= Black, 2= Grey, 3= Brown, 4= Pink, 5= Red, 6= Blue, 7= heterochromia; (7) Ear type, 1= Upright, 2= Loop; (8) Body type, 1= Arch, 2= Semi Arch, 3= Compact, 4= Commercial, 5= Cylindrical; (9) Head type, 1= Round, 2= Oval; and (10) Body size, 1= Giant, 2= Large, 3= Medium, 4= Small.

Data analysis

In the data analysis of qualitative traits, the frequency and percentage of each qualitative trait variable were calculated using the PROC FREQ procedure through SAS software version 9.4 to cross-tabulate the qualitative traits observed in different rabbit breeds. Multiple Correspondence Analysis (MCA) between categorical variables was then conducted using the PROC CORESP procedure in SAS software version 9.4. The results will plot the output data in a graphical representation of the relationship between the category variables. The model used in MCA is additive (additional model):

$$Y_{ij\ldots n} = \bar{Y} + a_i + b_j + \ldots + \varepsilon_{ij\ldots n} \quad \ldots\ldots (1)$$

where $Y_{ij\ldots.n}$ is Observation scores for individuals in the i-th category of variable a, the j-th category of variable b, and so on; $\bar{Y}$ is grand mean (overall average) of the dependent variable; a_i is influence or effect of the grand mean of the i-th category of variable a; b_j is influence or effect of the grand mean of the j-th category of variable b; and $\varepsilon_{ij\ldots n}$ is residual for the individual corresponding to $Y_{ij\ldots n}$, where

$$\bar{Y} = \frac{\sum_{k=1}^{n} Y_k}{n} \quad \ldots\ldots (2)$$

with $Y_k$is x-th, the individual's value is the dependent variable, and n is the number of observations.

RESULTS AND DISCUSSION

General conditions of the research site

Local Bambu Apus rabbits were raised at the Bambu Apus Rabbit Park, DKI Jakarta. The Rex and New Zealand White rabbits used in this study were raised at the Poultry and Miscellaneous Animal Instrument Standard Testing Centre, Ciawi. Table 1 presents the average temperatures, humidity, and THI of both locations.

Table 1. Daily temperature and humidity averages and THI values at Bambu Apus Rabbit Park, DKI Jakarta, and Poultry and Miscellaneous Animal Instrument Standard Testing Centre, Ciawi

Location	Temperature (°C)	Humidity (%)	THI
Bambu Apus Rabbit Park, DKI Jakarta	30.99±4.95	60.30±23.02	28.63±3.30
Poultry and Miscellaneous Animal Instrument Standard Testing Centre, Ciawi	28.07±2.62	80.67±17.86	27.19±2.23

Bambu Apus Rabbit Park in DKI Jakarta has higher temperatures and lower humidity than the Poultry and Miscellaneous Animal Instrument Standard Testing Centre in Ciawi. The THI index at Bambu Apus Rabbit Park in Jakarta is higher than at the Poultry and Miscellaneous Animal Instrument Standard Testing Centre in Ciawi. According to (Sugiono et al. 2016), the temperature relative humidity index (THI) is a numerical representation of the combined impact of air temperature and humidity on the degree of heat stress.

The correspondence between THI values and heat stress categories has been defined by (LPHSI 1990) whereby THI is classified as follows: 27.8= No heat stress; 27.8-28.9= Slight heat stress; 29.0-30.0= Moderate heat stress; >30.0= Severe heat stress. According to the provisions of (LPHSI 1990), Ciawi Poultry and Miscellaneous Animal Instrument Standard Testing Centre is within slight or no heat stress conditions, while conditions in Bambu Apus Rabbit Park may potentially result in moderate heat stress.

In tropical and subtropical areas with high environmental temperatures, livestock animals like rabbits experience heat stress. Because of their thick fur coats and dysfunctional sweat glands, which inhibit their ability to eliminate excess body heat, rabbits are susceptible to heat stress at exceptionally high temperatures (Fadare 2015; Verga et al. 2007). When heat stress occurs, they will attempt to reduce the excess heat through different mechanisms, such as thermoregulatory responses. These responses impact rabbits by interfering with their physiological processes and behavior (Mutwedu et al. 2021; El Sabry et al. 2021; Liang et al. 2022). Increased rectal temperature, decreased feed intake and daily gain, and increased water consumption in meat rabbits are physiological indicators of heat stress that can lead to higher energy consumption and reduced production and reproductive performance (Liu et al. 2022).

Qualitative traits in Bambu Apus rabbits

Qualitative traits of local Bambu Apus rabbits are divided into head, body, and color and fur characteristics. Table 2 presents the results of observations of head characteristics.

Observations of the head characteristics of local Bambu Apus rabbits had the most significant proportion of oval head types at 94.7%. In comparison, round head types were also found in a smaller percentage at 5.3%. The round head and oval head of local Bambu Apus rabbits can be seen in Figure 2. The rounded head type exhibits the "brachycephaly" trait, which is characterized by a reduced facial region of the head. This phenotype can also be considered pathological (Geiger et al. 2021). Indeed, brachycephaly has been described to occur in many domesticated animals (Herre & Röhrs 1990).). In anthropology, brachycephaly was used to define the shape of the cranial vault in dorsal view, based on the measurements of the length and width (Retzius 1850; Rosenberg 1966; Lüps 1974). Domesticated ancient rabbit breeds are known to have short-faced brachycephalic, including the Polish Netherland Dwarf, Dwarf Fox, and Dwarf Rex.

Nevertheless, none of the reviewed studies use the term "brachycephalic" for Dwarf rabbits. However, none of the studies mentioned the term "brachycephalic" in describing Dwarf rabbits (Geiger et al. 2021). Figure 3 shows the jaw structure of animals with brachycephaly.

However, an oversized lower jaw can only be seen in certain strains, varieties, and breeds of rabbits, as this is a malformation that leads to dental malocclusion and an improper arrangement of teeth and jaws (Geiger et al., 2021). Another factor contributing to malocclusion is the limited available space in the oral cavity for growth tooth growth, which is packed. This condition is relatively common in rabbits with rounded head shapes, particularly in lop and dwarf rabbits. The health condition of rabbits that suffer from dental malocclusion will certainly affect their productivity because it will influence feed intake.

Another indicator of head characteristics is ear type. In general, ear types in rabbits are divided into upright ears and lop ears. Most of the local Bambu Apus rabbit population has an upright ear type, 84%, and 16% have a lop ear type. Figure 3 shows the ear types in local Bambu Apus rabbits. The large percentage of upright ears in local Bambu Apus rabbits is thought to be related to the primarily upright-eared parents. Compared to body weight, which develops more slowly, ear length is the body component that matures sooner and achieves its maximum development rate far faster (Dunlop & Hammond 1937). Rabbit breeds, in general, have a strong correlation between ear length and overall

Table 2. Head characteristics of local Bambu Apus rabbits

Variables	Percentage (%)		
	Local Bambu Apus	Rex	New Zealand White
Head type			
Oval	94.7[a]	100[b]	100[c]
Round	5.3	0	0
Ear type			
Upright	84[a]	100[b]	100[c]
Lop	16	0	0
Eye color			
Black	0	0	0
Brown	62.8[a]	78,7[b]	0
Pink	25.5	0	100[c]
Blue	6.4	0	0
Red	2.1	11,2	0
Grey	1.1	10,1	0
Heterochromia	1.1	0	0

Superscripts in different rows indicate the predominant character for each indicator. (a) for local Bambu Apus, (b) for Rex, (c) for NZW

(a) (b)

Figure 2. Ear types in local Bambu Apus Rabbit. (a) Round head type, (b) Oval head type

body size; this is also the case in lop-eared rabbits; however, in pure lop-eared rabbits, gene mutations result in ear lengths that are almost twice as long as in common rabbits of equivalent body size (Castle & Reed 1936). Rabbits use their respiratory rate and ear surfaces as the primary means of heat release, followed by body position because the rabbit has thick insulator hair on their skin, preventing them from losing heat through their skin (Oladimeji et al. 2022). The upright ear type in local Bambu Apus rabbits indicates that the physiological condition of the rabbit's body has adapted to the higher temperature environment of Jakarta. The rabbit ear pinna

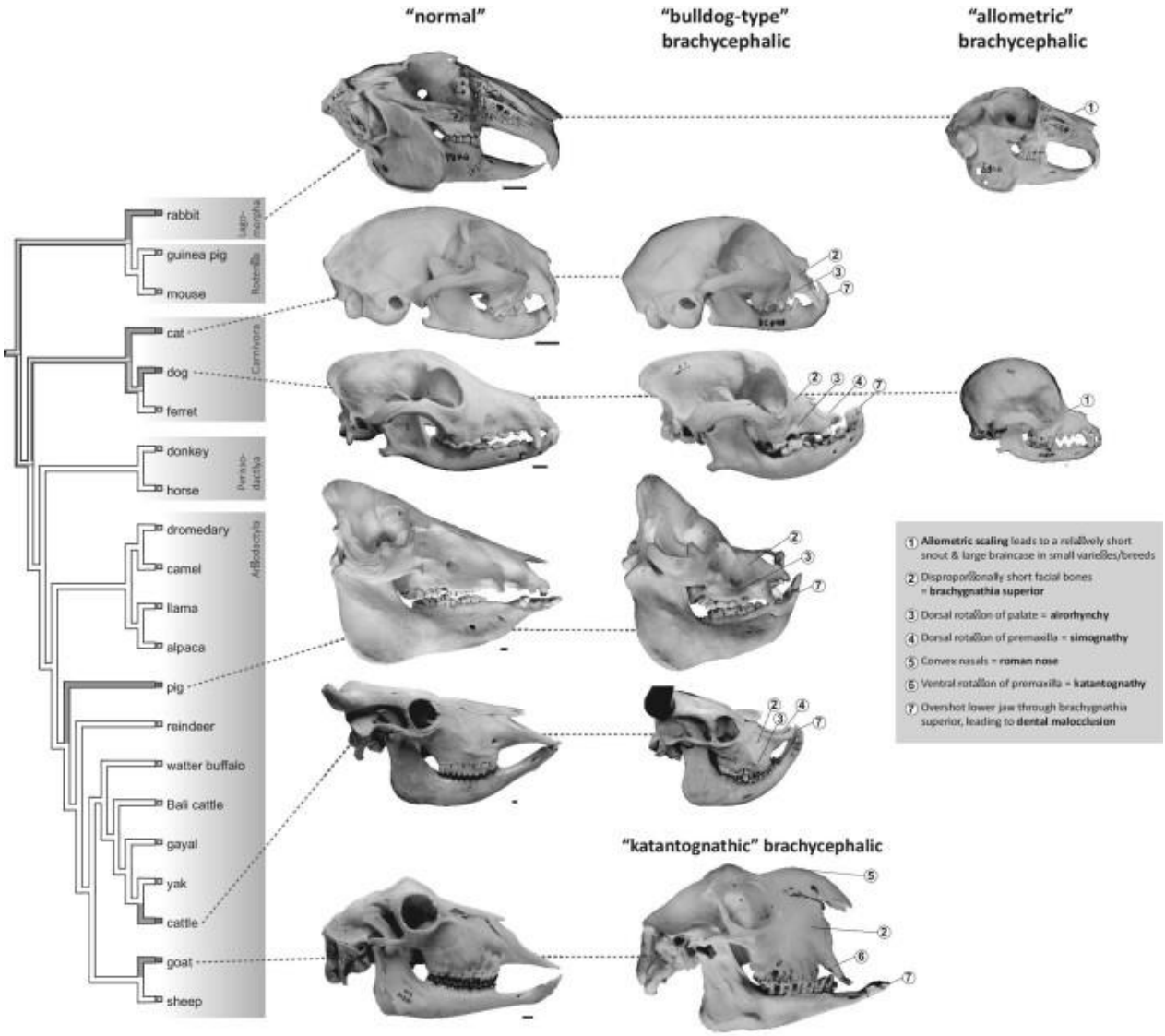

Figure 3. An overview of the different types of brachycephalic in domesticated mammals (Geiger et al. 2021)

(a) (b)

Figure 4. Ear types in local Bambu Apus Rabbit. (a) Lop ear type, (b) Upright ear type

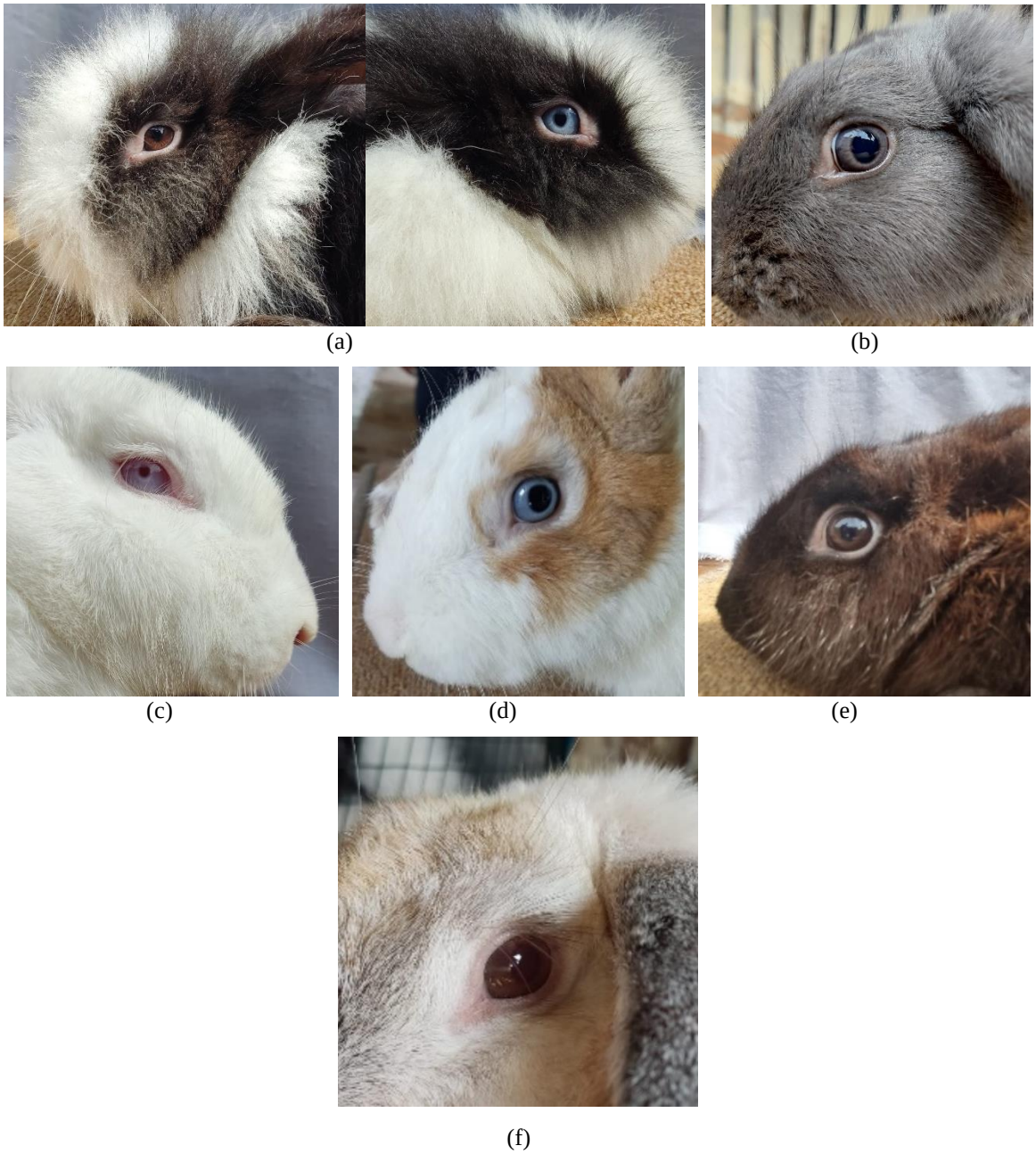

Figure 5. Eye color in local Bambu Apus Rabbit. (a) Heterochromia, (b) Grey, (c) Pink, (d) Blue, (e) Brown, (f) Red

also has hairless skin that acts as a heat exchanger between the body and the environment and thus plays a significant role in body temperature regulation by changing blood flow to the skin (cutaneous) vascular bed during physiological responses such as warm- or cold-defence. The blood vessels swell (vasodilatation) when the rabbit is overheated and contract (vasoconstriction) in cold weather, so much so that they are almost invisible in cold weather (Ootsuka & Tanaka 2015; Quesenberry et al. 2021). Through this, rabbits with upright ear types allow maximum heat release compared to those with lop ear types. In some conditions, the central mechanisms of cutaneous blood flow control are inhibited by the signal from another system, including the thermoregulatory and alerting Ootsuka & Tanaka (2015), which can help the rabbit monitor the environment and predators.

Through a study conducted by Johnson & Burn (2019), lop-eared rabbits showed higher rates of stenosis/narrowing of the ear canal occurrence of cerumen/earwax build-up and erythema/reddish patches on the skin due to dilation of blood vessels and more frequent potential pain responses during ear examinations compared to erect-eared rabbits. There were also statistically significantly more incisor problems, overgrowth of molars, sharpness of molars, spurs on molars, and veterinary dental history in lop-

eared rabbits than in upright-eared rabbits. The domesticated wild rabbits have led to the development of at least nine breeds with lop ears, as the characteristics of lop ears are hereditary (Castle & Reed 1936; British Rabbit Council 2021). Lop-eared breeds have 3-5 mm of soft tissue at the proximal acoustic meatus cartilage ring and distal ear canal tragus cartilage, leading to the formation of the 'pinnae' becoming pendulous (Csomos et al. 2016; Harcourt-Brown et al. 2013).

The color of the iris determines eye color. The iris is composed of two anatomical layers that contain pigments. The sphincter muscle fibers are located near the pupillary margin and are slightly anterior to the pigmented epithelium of the iris. It surrounds the iris's center and narrows the pupil in high light through pupillary light reflex or while adjusting focus. The iris regulates the quantity of light reaching the retina by adjusting the pupil size (Lui & Stokkermans 2023; Bloom et al. 2023).

The eye color found in local Bambu Apus rabbits shows a lot of variation, ranging from brown to heterochromia, where there is more than one variant of eye color in one individual, as seen in Figure 5. Eye color is an expression of the rabbit genotype. Melanocyte and melanosome pigment quantity and distribution inside the iris define the color of the eye (Brahmantiyo et al. 2021). The many eye variations in local Bambu Apus rabbits can be attributed to uncontrolled breeding. However, the most significant percentage of eye color was brown at 62.8%, followed by pink at 25.5%. According to research results by Andriansyah (2023) and Awalia et al. (2016), this is due to inherited traits from both parents, Rex rabbits and New Zealand White rabbits. Rex rabbits have predominantly dark or brown eye color in 90% of the population studied, while New Zealand White rabbits have pink eye color and plain white bodies. Referring to Lukefahr et al. (2022), pink eyes in plain white rabbits express the cc gene that causes albino on the body surface and pink eyes.

A study by Ioshimoto et al. (2018) also revealed that albino rabbits are more sensitive to the retina than rabbits with colored eyes; this is revealed by the ERG of pink-eyed albino rabbits, where the amplitudes are higher under scotopic conditions and more so under photopic and blinking conditions. The more significant receptoral and post-receptoral activity can be accounted for by light gain from scattering and reflections on the layers of the retina. However, a larger ERG is an unfavorable sign that shows worse visual health than usual. The operational and clinical means also reveal an inverse correlation between the depth of color depigmentation and visual acuity. Amblyopia, nystagmus, refractive errors, and foveal hypoplasia cause these effects.

The albino condition, which has poorer vision than pigmented rabbits, will significantly impact the survival and productivity of rabbits living in the wild; this relates to predators' survival mechanisms and finding food. However, this will have little impact on farmed rabbits, as rabbits will be kept in cages that minimize the threat of predators and have readily available feed.

Based on the results of the entire population of local Bambu Apus rabbits observed, 77.7% were identified as medium-sized, while 22.3% were identified as small-sized. Body size can describe the ability of rabbits to produce meat, with the majority of the local Bambu Apus rabbit population classified as medium-sized; this indicates that local Bambu Apus rabbits have the potential to be developed as broiler rabbits.

Besides size, another indicator of body characteristics is body type. Three body types are found in local Bambu Apus rabbits: commercial, compact, and cylindrical. From the three body types found, most (59.6%) local Bambu Apus rabbits have a commercial body shape. A total of 39.4% of the total population of local Bambu Apus rabbits observed had a compact body type. In addition to commercial and compact body types, 1% of the population had a cylindrical body type. The large

Table 3. Body characteristics of local Bambu Apus rabbits

Variable	Percentage (%)		
	Local Bambu Apus	Rex	NZW
Body size			
Large	0	0	100[c]
Medium	77.7[a]	100[b]	0
Small	22.3	0	0
Body type			
Commercial	59.6[a]	100[b]	100[c]
Compact	39.4	0	0
Cylindrical	1	0	0

Superscripts in different rows indicate the predominant character for each indicator. (a) for local Bambu Apus, (b) for Rex, (c) for NZW

Table 4. Comparison of carcass productivity of local Bambu Apus, New Zealand White rabbits, and Rex rabbits

Variables	Sex	Breeds		
		Local Bambu Apus	New Zealand White	Rex
Slaughter Weight (g)	M	2393.80±237.90	2576.80±316.13	2711.44±232.72
	F	2441.60±209.25	2452.50±300.52	3017.18±257.13
	Avg	2417.70±33.80[b]	2514.69±87.95[b]	2864.32± 216.19[a]
Carcass Weight (g)	M	1251.60±218.15	1295.62±185.34	1408.55±125.72
	F	1326.80±139.16	1285.00±84.25	1544.43±140.33
	Avg	1289.20±53.17[b]	1290.31±7.51[b]	1476.49±96.08[a]
Carcass Percentage (%)	M	52.07±5.12	50.18±2.35	51.95±1.55
	F	54.28±1.56	52.58±2.98	51.19±1.78
	Avg	53.18±1.56	51.38±1.70	51.57±0.57
Total Meat Weight (g)	M	832.20±160.96	980.63±151.93	1102.17±107.50
	F	849.80±77.43	945.00±91.92	1188.69±83.94
	Avg	841.00±12.45[b]	962.81±25.19[b]	1145.43±61.18[a]
Total Bone Weight (g)	M	280.00±24.37	302.50±32.07	334.17±29.12
	F	273.60±32.53	335.00±7.07	353.13±24.96
	Avg	276.80±4.53[b]	318.75±22.98[a]	343.65±13.41[a]
Meat-to-Bone Ratio	M	3.00±0.71	3.26±0.50	3.30±0.17
	F	3.14±0.47	2.82±0.33	3.37±0.19
	Avg	3.08±0.10	3.04±0.30	3.34±0.05

Margatama et al. (2023). M= Male, F= Female, Avg= Average

population with a commercial body type is the result of inheritance from the characteristics of the parents of local Bambu Apus rabbits. The compact and cylindrical body types may be influenced by the inherited characteristics of the local Bambu Apus rabbit parents that are not explicitly identified. The weighing of medium-sized local Bambu Apus rabbits and commercial body types in local Bambu Apus rabbits can be seen in Figure 6. Ioshimoto et al. (2018) state that broiler-type rabbits own the commercial body type with a larger and fuller body size. Table 4 compares the carcass productivity of local Bambu Apus rabbits, New Zealand White rabbits, and Rex (rex agrinak) rabbits.

The carcass yield percentage of Local Bambu Apus rabbits is an average of 53.18%, which is not significantly different from the carcass yield of New Zealand White rabbits (51.38%) and Rex rabbits (51.57%). A rabbit carcass is defined as the body part of the rabbit after slaughter, minus the blood, head, skin, feet, tail, digestive tract, and its contents and the contents of the chest cavity (Brahmantiyo et al. 2017). Carcass percentage is a crucial variable in determining the carcass performance of the rabbits produced. The higher the carcass percentage interprets the economic value of rabbit livestock (Wahyono et al. 2021). According to Gillespie (2004), the percentage of good carcasses is 50-59%, so looking at the percentage of carcasses produced by local Bambu Apus has the potential to be used as broiler rabbits. Moreover, the meat-to-bone ratio of local Bambu Apus rabbits (3.08±0.10) is not significantly different from New Zealand rabbits (3.04±0.30) and Rex rabbits (3.34±0.05). The meat-bone ratio is between a rabbit's meat and bone weight (Al-Amin et al., 2020). By comparing the weight of the meat to the weight of the bones, it is possible to determine how much meat is produced compared to the bone (Wibowo et al. 2014).

There are five parameters to indicate color and fur characteristics. The fur colors found were classified into five, as presented in Table 5. The predominant body color (40.4%) of the observed local Bambu Apus rabbit population was white. The pigment melanin is the source of all hair, skin, and eye color in livestock. In mammals, there are two types of melanin: black melanin (eumelanin) and red melanin (phaeomelanin). The colors that appear in livestock combine these two pigments (Caro & Mallarino 2020). Besides one of the known parents is the New Zealand White breeds, the dominance of white color is thought to be a form of adaptation of local Bambu Apus rabbits to the temperature of DKI Jakarta, which has an average temperature of 28.8ºC with

(a) (b)

Figure 6. Body traits in local Bambu Apus Rabbit. (a) Medium-sized, (b) Commercial body type

(a) (b)

(c) (d)

(e)

Figure 7. Fur characteristics in local Bambu Apus Rabbit. (a) Normal, (b) Satin, (c) Rex, (d) Angora, (e) Lion

Table 5. Color and fur characteristics of local Bambu Apus rabbits

Variable	Percentage (%)		
	Local Bambu Apus	Rex	NZW
Predominant body color			
White	40.4[a]	37.1[b]	100[c]
Light brown	12.8	9	0
Dark brown	10.6	10.1	0
Grey	9.6	21.3	0
Black	26.6	22.5	0
Body colour pattern			
1 colour	37.2[a]	37.1[b]	100[c]
2 colours	47.9	57.3	0
3 colours	14.9	5.6	0
Stripe colour			
No stripes	37.2[a]	37.1[b]	100[c]
White	29.8	29.2	0
Light brown	4.26	4.5	0
Dark brown	6.4	5.6	0
Grey	1.1	10.1	0
Black	6.4	7.9	0
Harlequin	14.9	5.6	0
Spread of stripes			
0%	37.2 [a]	37.1[b]	100[c]
1–10%	29.8	9	0
>10–20%	4.3	29.2	0
>20–30%	7.4	11.2	0
>30–40%	6.4	6.7	0
>40–50%	14.9	6.7	0
Fur characteristics			
Normal	72.3[a]	0	100[c]
Lion	16	0	0
Rex	5.3	100[b]	0
Angora	5.3	0	0
Satin	1	0	0

Superscripts in different rows indicate the predominant character for each indicator. (a) for local Bambu Apus, (b) for Rex, (c) for NZW

a maximum temperature reaching 35ºC (Badan Pusat Statistik 2021). Color may affect an animal's body temperature since dark surfaces absorb higher solar energy than bright surfaces, which is then converted to heat (Stuart-Fox et al. 2017).

The predominant color pattern in local Bambu Apus rabbits is two (47.9%), and most (37.2%) do not have stripes. Five characteristics were found while observing local Bambu Apus rabbits: normal fur, lion fur, rex fur, angora fur, and satin fur. Most (72.3%) of the local

Bambu Apus rabbit population had normal fur characteristics. Various fur characteristics of local Bambu Local are shown in Figure 7. Rabbits have different fur types, and the difference between these types is the influence of genotype. Rabbits with long fur are angora, which can produce wool, and Rex rabbits have short, fine hair. Rex rabbits have a unique breed phenotype, which is determined by the minimal presence of the guard and awn hairs that are rather distinguishing from the fur, created from short- and soft-haired coats (Fontanesi 2021). The large variety of fur characteristics in local Bambu Apus rabbits is because they are crossbred from various local Bambu Apus rabbit parents, each with different fur characteristics.

Rabbit breeds correspond with qualitative traits

Local Bambu Apus rabbits have parents from various breeds. Some known parents include New Zealand White and unidentified rabbit breeds. Some local Bambu Apus rabbits show the characteristics of Rex rabbits. Therefore, a correspondence analysis of local Bambu Apus rabbits with Rex rabbits and New Zealand White rabbits was conducted. Correspondence analysis determines the qualitative traits that have a relationship with one of the rabbit breeds and distinguishes between breeds based on the traits of qualitative traits owned by the breed.

The correspondence graph of the rabbit breeds with the qualitative traits in Figure 8 shows that each rabbit breed occupies a different quadrant. Local Bambu Apus rabbits are in quadrant I, New Zealand White rabbits are in quadrant III-IV, and Rex rabbits are in quadrant II. Thus, the three rabbit breeds based on qualitative traits are different and have qualitative characteristics that can be distinguished. The same method conducted by Hayanti et al. (2021) successfully distinguished the district origin of Bali cattle in Jambi Province. Table 6 implements the correspondence graph (Figure 8) of breeds with qualitative traits associated with each breed.

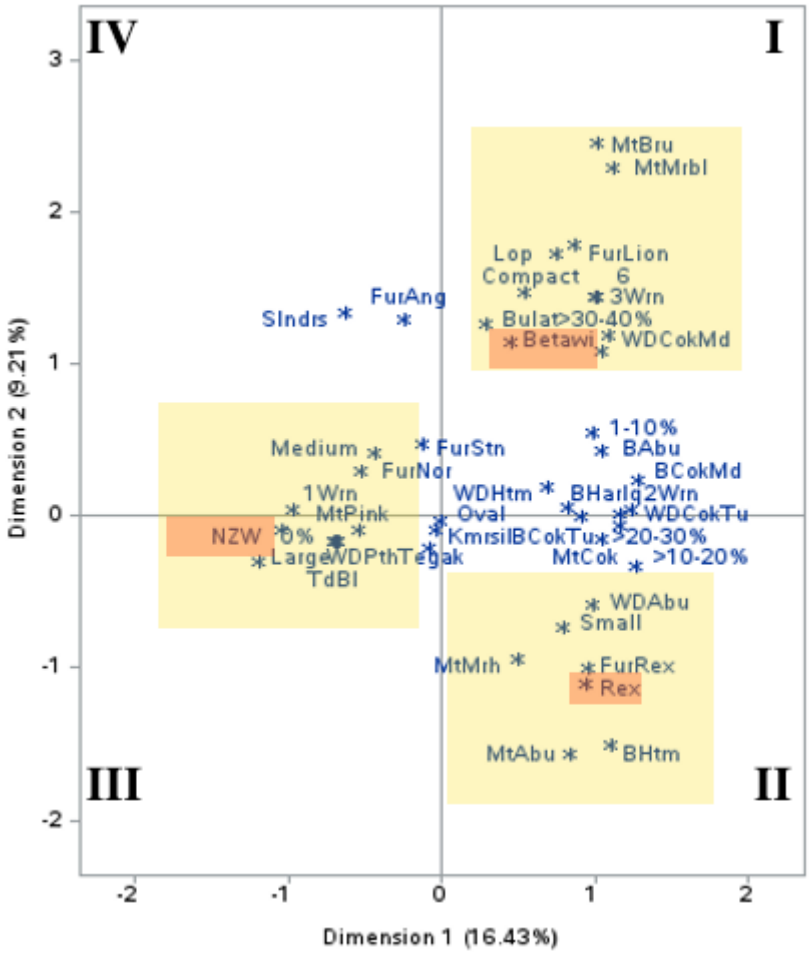

Figure 8. Correspondence graph of rabbit breeds with qualitative traits. Betawi= Local Bambu Apus rabbit, NZW= New Zealand White rabbit, REX= Rex rabbit, Round= Roundhead, Oval= Oval head, Large= Large size, Medium= Medium size, Small= Small size, MtBru= Blue eyes, MtMrb= Heterochromia eyes, MtMrh= Red eyes, MtPink= Pink eyes, WDPth= White predominant colour, WDCokMd= Light brown predominant colour, WDAbu= Ash predominant colour, 1Wrn= 1 colour pattern, 3Wrn= 3 colour pattern, TdBl= No stripes, BHtm= Black stripe colour, BHarlq= Harlequin stripe colour

Table 6. Correspondence graph implementation

Rabbit Breeds		
Local Bambu Apus Rabbit	Rex	New Zealand White
Predominantly light brown color	Predominantly grey colour	Predominantly white color
Blue and heterochromia eye color	Grey and red eye color	Pink eye color
Stripe spread >40-50%	Stripe spread >20–30%	Stripe spread 0%
Harlequin striped color	Black striped color	No striped color
Compact body type	Small size	Large and medium size
Lion fur	Rex fur	Upright ear type
3-color pattern		1 color pattern
Lop ear type		

Table 6 describes the traits that cause the grouping of each breed in different quadrants, as shown in Figure 8. Of the 10 variables of qualitative traits, 8 variables can differentiate local Bambu Apus rabbits from Rex and New Zealand White rabbits. The differentiators include the local Bambu Apus rabbit having a predominant light brown color, the presence of blue and heterochromia eye color, stripe spread >40-50%, harlequin color pattern, and 3-color pattern. As well as fur lion characteristics and lop ear type, which is only seen in the local Bambu Apus rabbit, and the variation of compact body type, which is not found in New Zealand White and Rex rabbits during observation. The local Bambu Apus rabbit is the result of crossing several breeds whose genetic composition is unknown, making it a separate breed different from its parent breeds.

CONCLUSION

Local Bambu Apus rabbits have qualitative traits that differ from New Zealand White and Rex rabbits. Local Bambu Apus rabbits have a predominantly light brown color, variations in blue eye color and heterochromia, stripe spread >40-50%, 3-color pattern as well as harlequin stripe color, variations in commercial body type, fur lion, and lop ears. Genetically, local Bambu Apus rabbits also show potential to develop as broiler rabbits that are adaptable to the environment of DKI Jakarta; this can be shown through the medium size and commercial body type of local Bambu Apus and carcass yield percentage, which is not significantly different from New Zealand White and Rex rabbits as broiler rabbits. Regarding qualitative traits and genetic potential, local Bambu Apus rabbits meet the basic requirements for concession strain broiler rabbits to support the urban farming program in DKI Jakarta.

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Key Words Index

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Penentuan tingkat pertumbuhan dan kelangsungan hidup anak kambing Saanen di peternakan kambing susu UniSZA (Determination of growth and survival rates of Saanen goat lamb in UniSZA dairy goat farm)

(Org: Eng)

JITV 29(1): 1-8

Live weight is one of the parameters for evaluating milk and meat production of livestock. Live weight is an important indicator for improving production traits. The present study was conducted to measure growth performances based on two factors; sex differences and litter size and observation of survival between these factors during the sample period. Data from 22 Saanen lamb were recorded for 6 weeks. Results showed that male lamb had higher live weight than female lamb (P>0.05), with a difference of 3.3% in the percentage of live weight gained over 6 weeks of sampling. This resulted in a body gain of 0.68 kg/day for male lamb and 0.48 kg/day for female lamb. The current study also showed that twin lamb had a higher average live weight than single lamb (P>0.05). The average size of single-born Saanen lamb was not significantly different from twin-born Saanen goat lamb (P>0.05). Twin-born Saanen lamb gained 9.9% more live weight in 6 weeks than singleton- born. Survival was 86.4%, with all deaths attributable to singleton-born lamb. In this study, preweaning kid survival was not affected by sex or litter size, but was affected by maternal age. Other factors such as pellet deficiency should be considered as they affect the overall production of the animals. The data collected in this study could potentially lead to more accurate evaluation of breeding value, and improvements in feeding and management.

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Key Words: Goat Lamb, Growth, Saanen, Survival Rates

UDC: 636.32

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Estimasi pengaruh factor non genetic pada sifat reproduksi strain Afec-Assaf di Peternakan Bani Naim, Palestina (Estimate the effect of non-genetic factors on the reproductive traits of Afec-Assaf strain in Bani Naim Farm, Palestine)

(Org: Eng)

JITV 28(1):9-15

The aim of the study was to evaluate the reproductive performance of the Afec-Assaf strain in intensive farming. The research was carried out in Bani Naim, Hebron city, Palestine. Data collected from 450 ewes and 1660 lambs born over the period 2019 to 2022 were used to compile the data for this study. Linear models with fixed effects were used to estimate the influence of year of birth and parity on litter size born alive (LS), litter size at weaning (LSAW), and lambing interval (LI). The most influential component in LS was parity, which resulted in an average of 1.81 ± 0.9. There was a significant difference between the size of litters born in the first and fourth parities, with the smallest litters born in the first parity (1.75±0.04) and the largest born in the fourth (2.25±0.14). LSAW lamb averaged 1.75±0.82. The LSAW was significantly influenced by parity (P<0.05). LI was 250.60±77.59 days on average. There was no significant (P>0.05) relationship between parity and LI. The longest LI was observed at third parity (251.56±6.15 days). According to the findings, the birth type of a lamb is significantly affected by the parity. Non-genetic variables have a substantial impact on the diversity of reproductive traits in Afec-Assaf.

(Author)

Key Words: Afec-Assaf, Litter Size, Lambing Interval, Parity, Sheep

UDC: 616.995.7

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Inovasi ekstrak terstandar biji bengkuang dan daun sirih merah sebagai bioinsektisida miasis (Innovation of standardized extract of yam bean seed and red betel leaf as bioinsecticide for myiasis treatment)

(Org: Eng)

JITV 29(1): 16-28

Myiasis causes severe economic and production losses in animals. Chemicals are frequently employed to prevent or control myiasis in animals, however long-term exposure to chemical-based pesticides can have severe effects. This study aims to assess the effectiveness combination of ethanol extract of yam bean seeds and red betel leaf in vitro as a preparation capable of killing all larval stages of Chrysomya bezziana, the primary agent responsible for myiasis. This study consisted of eleven treatment groups. Twenty instar 1 (L1), 2 (L2), and 3 (L3) C. bezziana larvae were used for in vitro testing utilizing plastic pots containing larval media and ethanol extract of yam bean seeds, red betel leaf, and their combination as well as a combination of 0.5%, 1%, and 2% concentrations of ethanol extract of yam bean seeds and red betel leaf. Asuntol and sterile distilled water were utilized as positive and negative controls, rerspectively. The results demonstrated that the combination of 2% ethanol extract of yam bean seeds and red betel leaf was able to induce 100% larval death and 100% pupal failure. The L3 test demonstrated that the combination of yam bean seed ethanol extract and red betel leaf reduced hatchability at all doses. The L1 and L2 tests suggested that the extract was efficient as a stomach poison, whilst the L3 test indicated that it was also a contact poison. Yam bean seeds and red betel leaf show larvicidal efficacy against multiple species of Chrysomya bezziana insect larvae.

(Author)

Key Words: Bioinsecticide, *Chrysomya bezziana*, Myiasis, Red Betel Leaf ,Yam Bean Seeds

UDC: 633.2

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Perbandingan komposisi nutrisi Lalat Tentara Hitam yang dikembangbiakkan pada sampah organik dengan yang dikembangkanbiakan pada pakan campuran pelet komersial dengan dedak padi (Comparison of the nutritional composition of Black Soldier Fly Bred on organic waste and bred on commercial pellet mixed with rice bran)

(Org: Eng)

JITV 29(1): 29-35

The black soldier fly (BSF, *Hermetia illucens*) is an insect often used as animal feed that is easy, inexpensive, and fast to breed. However, these flies have never been used for the poultry diet. This study compares the nutritional content of black soldier flies, third-instar larvae, and pupae fed with organic waste and a mixture of commercial chicken pellets with rice bran. The method used in this study uses proximate analysis of the nutritional content of black soldier flies for dry matter, ash, crude protein, crude fat, crude fiber, calcium, nitrogen-free extract, and gross energy.

(Author)

Key Words: Animal Nutrition, Black Soldier Fly, Dipterans, Swiftlet

UDC: 636.2.033

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Pengaruh substitusi silase rumput dengan fodder jagung hidroponik terhadap konsumsi nutrient, kecernaan nutrient, parameter rumen dan metabolit darah pedet sapi Ongole x Brahman lepas sapih (The effect of replacing grass silage with hydroponic maize fodder on nutrient intake, nutrient digestibility, rumen parameters and blood metabolites of weaned Ongole x Brahman calves)

(Org: Eng)

JITV 29(1): 36-44

Hydroponic maize fodder (HMF) may replace grass silage as an alternative green forage for ruminants during dry seasons. An *in vivo* experiment was conducted to investigate the effect of replacing grass silage with HMF on nutrient intake, nutrient digestibility, rumen parameters and blood metabolites of weaned calves. Four weaned Ongole x Brahman calves (BW= 127±17.45 kg) were fed 70% grass silage + 30% concentrates (G; control), 35% grass silage + 35% HMF + 30% concentrates (GCF1), 17,5% grass silage + 52,5% HMF + 30% concentrates (GCF2), and 70% HMF + 30% concentrates (GCF3) in a 4 x 4 Latin Square Design experiment. Calves fed HMF had lesser total dry matter intake (DMI) ($P<0.05$), total organic matter intake (OMI), and total crude fibre intake (CFI) compared with calves fed only grass silage. There were no differences ($P>0.05$) between treatments for total crude protein (CP) intake, dry matter digestibility (DMD), crude protein digestibility (CPD), crude fibre digestibility (CFD), digestible crude fibre intake (DCFI), digestible crude protein intake (DCPI) and ruminal pH. Calves provided HMF in their diet had the greatest ($P < 0.05$) ammonia-nitrogen (NH3-N) and total volatile fatty acids (TVFA) concentration compared with calves fed only grass silage. There were no differences between treatments ($P>0.05$) for the concentration of acetate, propionate, butyrate, iso-butyrate, iso-valerate, blood glucose or blood urea of calves. Hence, hydroponic maize fodder could be used as a replacement for silage to improve the rumen fermentation of weaned Ongole x Brahman calves, although it might decrease the total dry matter intake.

(Author)

Key Words: Hydroponic Maize Fodder, Nutrient Intake, Nutrient Digestibility, Ongole X Brahman Calves

UDC: 575.16

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Keragaman morfometrik dan polimorfisme gen melanocortin-4 receptor (MC4R) pada Ayam Kedu Merah dan Kampung (Morphometric diversity and polymorphism of melanocortin-4 receptor (MC4R) gene in Red Kedu and Kampung Chickens)

(Org: Eng)

JITV 29(1): 45-55

The aim of this research was to compare morphometric differences and to determine the presence of MC4R gene polymorphisms in Red Kedu and Kampung chickens. This research used a total of 98 Red Kedu and Kampung chickens. The 37-week-old chickens were subjected to experimental study with morphometric measurements. PCR used a pair of MC4R primers based on GenBank access number AB01221 to amplify the PCR targets 221 base pairs long. Data analysis used the t-test to compare the morphometrics between Red Kedu and Kampung chickens. The genotype frequency, gene frequency, heterozygosity, and genetic distances determine the presence of polymorphisms. Analysis of variance to determine the effect of genotype on body weight and shank length. The results showed significant differences ($P<0.05$) between male and female Red Kedu and Kampung chickens in terms of body morphometric parameters. Sequencing of the PCR product found SNP in base pair 54G>C. GC and GG genotype frequencies of Red Kedu chicken were 0.51 and 0.49, while those of Kampung chicken were 0.32, 0.50, and the CC genotype was 0.18. Allele frequency for G and C of Red Kedu and Kampung chickens were 0.74 vs. 0.26 and 0.66 vs. 0.34, respectively, and the heterozygosity was 38% and 45%, respectively. The genetic distance between Red Kedu and Kampung chickens showed a close kinship of 0.42. Conclusively, the association of the MC4R gene had no significant effect ($P>0.05$) on body weight and shank length, and therefore, the MC4R gene could not be used as a marker assisted selection.

(Author)

Key Words: Kampung Chickens, MC4R Gene, Morphometric, Polymorphism, Red Kedu Chickens

UDC: 581.145

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Kondisi alginat dan kalsium klorida yang optimal dan toleran untuk enkapsulasi semen sapi Pasundan (Optimal and tolerant conditions for alginate and calcium chloride for the semen encapsulation of Pasundan bull)

(Org: Eng)

JITV 29(2): 56--66

The study aimed to determine the tolerance of Pasundan bull's sperm with different alginate and calcium chloride (CaCl2) concentrations to identify optimal conditions for sperm encapsulation. Semen samples were collected weekly with artificial vaginas from pasundan bulls. Pooled semen was divided into 9 equal volumes. The first sample was diluted with tris egg yolk extender (control), 4 samples were diluted with tris egg yolk extender supplemented with different concentrations of alginate (0.25, 0.5, 0.75, and 1%), and 4 other samples with CaCl2 (2.5, 5, 10, and 20 mM). Evaluation of semen was determined after 5 min incubation at room temperature and after overnight storage at 5 °C. Results showed that after 24h of refrigerated storage, the values for total motility and progressive motility in the groups with 2.5 mM CaCl2 and 0.25% alginate showed no difference compared to the control group. In contrast, a significant difference ($P<0.05$) was found between the other groups with CaCl2 and alginate. The progressive motility value in the group with alginate concentration greater than 0.25% decreased significantly ($P<0.05$). There was no difference between the groups (both alginate and CaCl2) in the spermatozoa viability and plasma membrane integrity variable. In conclusion, sperm with 2.5 mM calcium chloride and 0.25 % alginate was more tolerant of appropriate prolonged exposure and the sperm encapsulation process.

(Author)

Key Words: Alginate, Calcium Chloride, Sperm Tolerance, Encapsulation, Pasundan Bull Semen

UDC: 57.085

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Pengaruh berbagai waktu penyimpanan dan metode koleksi oosit terhadap kuantital, kualitas dan maturasi *in vitro* kerbau (The effect of different ovarian storage times and oocyte collection methods on the quantity, quality, and maturation of buffalo oocytes *in vitro*)

(Org: Eng)

JITV 29(2): 67-78

This study aimed to ascertain the impact of oocyte harvest methods and storage times on the amount, quality, and rate of buffalo oocyte maturation in vitro. Ovaries were collected from slaughterhouse at four different time storages (0, 3, 6, 9 and 12 h) before processing with three collection techniques slicing, aspiration, and slicing plus the aspiration of oocytes. According to the morphology of cumulus cell layers closely attached to the zona pellucida of the oocytes and the cytoplasmic appearance of the oocytes, the oocytes were divided into 4 groups (A, B, C, and D). Cumulus-oocyte-complexes (COCs) were transferred into in vitro maturation (IVM) medium of TCM- 199 (Sigma) for 24 h.

The stages of nuclear maturation were examined by staining the enlarged COCs from each group. The result found that the average quantity of oocytes per ovary ranged from 8.08±1.28 to 9.83 ±1.89 and no significant effect (P>0.05) on the quantity of oocytes per ovary. The average of oocytes quality per ovary ranges from 0.2 to 5.00 was a highly significant effect of storage time on quality oocytes (P<0.01). The maturation rate in the M-II stage ranges from 1.33 to 3.92 per ovary, and storage time is a significant effect (P<0.05) on the maturation rate of local buffalo. The collection methods were highly significant (P<0.01) on the quantity of oocytes between slicing and aspiration, also between aspiration plus slicing, and no significant (P>0.05) between slicing and aspiration plus slicing. The Average oocyte quality ranged from 0.10 to 4.95 per ovary and the highest in slicing plus aspiration methods (P>0.05). The average maturation rate in M-II stage was ranged from 1.00 to 3.85 oocytes per ovary, and the methods of oocytes collection were significant (P<0.05) on the maturation rate of local buffalo. In conclusion that the storage time of up to 6 h and aspiration plus slicing is suitable on in vitro maturation of local buffalo.

(Author)

Key Words: Buffalo, Oocyte, Ovary Storage Time, Collection Techniques, *In Vitro* Maturation

UDC: 636.087.6

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Pengaruh level tepung ikan yang berbeda terhadap sifat karkas dan kualitas daging Sapi Bali jantan (Effect of supplementing ground leaf of misai (. Effects of different levels of fish meal in the diet on carcass traits and meat quality of Bali cattle)

(Org: Eng)

JITV 29(2): 79-90

This study aims to ascertain how fattened Bali bulls' carcass characteristics and meat quality are affected by using fishmeal as a protein source in full feed. Fifteen male Bali cattle, ages two to three and with beginning body weights ranging from 180 to 200 kg, were the animals employed. Livestock were divided into three groups, with 5 animals replicated in each treatment. The cattle were split into three groups: T1 cattle were fed a complete diet containing 4% fishmeal; T2 cattle were fed a complete diet containing 8% fishmeal; and T3 cattle were fed a complete diet containing 12% fishmeal. The ANOVA method was used to analyze the data. The findings demonstrated that compared to T2, the T3 treatment's slaughter weight, carcass weight, and non-carcass weight were higher (P<0.05), although T1 was essentially equal to T2 and T3. Across the three treatments, the percentages of meat and non-meat, weight and meat percentage, moisture content, and meat protein were all comparatively close. While the beef fat content of the T2 treatment was comparatively equivalent to that of the T1 and T3 treatments, it was higher than that of the T1 treatment. T1 meat had a higher (P<0.05) collagen concentration than T3. However, the T2 treatment was identical to the T1 and T3 treatments. While the pH, cooking shrinkage, water binding capacity, and meat softness were all somewhat consistent across treatments, the amount of cholesterol in the meat of treatments T1 and T2 was significantly lower (P<0.05) than that of treatment T3. Based on the high carcass weight generated with the best possible physical and chemical quality of meat, it can be determined that adding 12% more fishmeal to the entire feed improves animal performance.

(Author)

Key Words: Bali Cattle, Carcass, Feed, Fish Meal, Meat Quality

UDC: 636.087

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Penggunaan limbah sagu yang difermentasi dengan *Neurospora* sp. sebagai alternatif pengganti jagung terhadap produksi itik petelur (Utilization of sago waste fermented by *Neurospora* sp. as alternative corn substitution on laying duck production)

(Org: Eng)

JITV 29(2): 91-96

The corn stock as a feed energy metabolism source in the Moluccas region is still an obstacle, so there is a need to find a substitution. One of the alternative feeds used is sago waste fermented by *Neurospora* sp. The study aimed to determine the effect of sago waste fermented by *Neurospora* sp. as alternative feed productive for laying ducks. The material used was 120 local Moluccas laying ducks aged 22 weeks divided into 5 treatments. Each treatment consisted of 5 replications of 6 laying ducks in each treatment unit. There were five dietary treatments with the inclusion of fermented sago weste: T0 (0%), T1 (10%), T2 (20%), T3 (30%), and T4 (40%). A feeding trial was carried out for 16 weeks. Results showed that the treatments using fermented sago waste by *Neurospora* sp. significantly affected (P<0.05) consumption, production, and feed conversion efficiency. At the same time, egg quality regarding egg weight, yolk carotene content, and odor were not significantly influenced. In conclusion, the maximum level of the use of Neurospora sp. fermented sago was 30% in feed without reducing the general performance of the ducks.

(Author)

Key Words: Laying Duck, *Neurospora* sp., Sago Waste

UDC: 616.34

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Efek gastroprotektif dari *Curcuma zedoria* terhadap kejadiaan gastritis ulcerative pada tikus yang diinduksi dengan etanol (Gastroprotective effects of *Curcuma zedoaria* in ethanol-induced gastritis ulcers of experimental rats)

(Org: Eng)

JITV 29(2): 97-102

Gastritis is one of the most common health problems of humans in the world. Gastric ulcer is mostly characterized by inflammation of the epithelial cells lining the gastric mucosa. Stomach injury may occur due to excessive secretion of stomach acid, pepsin, *Helicobacter pylori*, stress, smoking habit, alcohol consumption, irregular eating pattern, infection, and the use of non-steroidal anti-inflammatory drugs. This study aimed to explore the gastroprotective effects of *Curcuma zedoaria* infusion (CZI) on HCl/ethanol-induced gastric mucosal damage in rats. A total of 16 Sprague Dawley rats were randomly divided into 4 groups: negative control (without treatment), positive control (treated with omeprazole), P1 (CZI 30%), and P2 (CZI 60%). Several endpoints were evaluated, including gastric mucosal lesions, cellular degeneration, intracellular damage, and pH value. The gastric mucosal injury and ulcer score were determined by evaluating the inflamed gastric mucosa and by histopathological examination. After the treatment animal with CZI significantly ($P<0.05$) decreased the ulcer index by preventing gastric mucosal lesions, erosion, and cellular degeneration, and the value of pH value was increased ($P<0.05$). These results demonstrate that CZI has significant gastroprotective properties which support its use in traditional medicine.

(Author)

Key Words: *Curcuma zedoaria*, Gastritis, White Turmeric

UDC: 581.145

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Peningkatan stabilitas penyimpanan dan kinematik enkapsulasi *solid bead* alginat pada sperma sapi Pasundan (Improving storage stability and kinetics of Pasundan bull sperm encapsulation using alginate solid beads)

(Org: Eng)

JITV 29(2): 103-113

The study aims to develop a pasundan bull sperm encapsulation process and investigate whether alginate solid bead encapsulation improves sperm lifetime and survivability in cold storage. In order to make sperm encapsulation, 0.5% and 0.25% sperm-alginate droplets were added to a solution containing 1.5% dissolved calcium chloride ($CaCl_2$) in physiological saline, and droplets were allowed to settle for one minute, resulting in sperm embedded in solid beads of alginate matrix. Solid beads and unencapsulated sperms in a diluent of tris egg yolk with 0.25% and 0.5% alginate were assessed following 0 h, 24 h, 48 h, 72 h, and 96 h of refrigerated storage at 5°C. The observed parameters, in addition to sperm viability and membrane integrity, also include total motility, progressive motility, and sperm kinematics, which were measured using computer-assisted sperm analysis (CASA) systems. To determine if differences amongst data were statistically significant, analysis of variance was used, and the Duncan Multi Range Test was performed. The result showed that although the initial process of encapsulation resulted in a decrease in total motility, progressive motility, and kinematics value, alginate solid bead encapsulation was found to be more stable than unencapsulated sperm during storage at 5 °C for up to 4 days. The movement of spermatozoa is restricted to the viscosity of the alginate medium without disturbing the membrane's viability and integrity. It can be concluded that alginate solid bead encapsulation in pasundan bull semen can improve sperm stabilization during refrigerator storage.

(Author)

Key Words: Alginate, Kinematics, Pasundan Bull Sperm, Preservation, Solid Bead Encapsulation

UDC: 575.17

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Estimasi situasi genetik subpopulasi kelinci lokal Mesir Hulu menggunakan penanda mikrosatelit (Estimating the genetic situation of native Upper Egypt subpopulations of rabbits using microsatellite markers)

(Org: Eng)

JITV 29(2): 114-124

This study aimed to explore genetic diversity in four native upper Egypt subpopulations of rabbits using microsatellite markers. A total of 247 biological samples were collected from unrelated individuals of native Upper Egypt rabbit (NUER) subpopulations across 77 rural villages and were genotyped via 31 microsatellite loci. Four hundred ninety-six alleles were recorded among the 4 NUER subpopulations, with about 43% being private. Luxor's subpopulation exhibited the most significant values of the mean number of alleles, which was 19.012, allelic richness was 8.009, and private alleles were 133. The negative values of the inbreeding coefficient were recorded in Qena and Luxor (-0.084 and -0.134, respectively). About 45% of loci gave highly polymorphic information content (PIC), and 58% were insignificant in Hardy –Weinberg equilibrium (HWE). The overlapping between Asyut and Sohag has appeared in the discriminant analysis of principal components (DAPC). Generally, we concluded that the classification is based on geographical directions to southern subpopulations (Qena and

Luxor) and northern (Asyut and Sohag). Except that, the southern subpopulations (Qena and Luxor) showed high genetic variation. This study could be used as supporting documents for researchers in rabbit breeding and agriculture at national and regional levels.

(Author)

Key Words: Genetic Diversity, Native Rabbits, Upper Egypt, Microsatellite

UDC: 575.17

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Keragaman gen monoamine oxidase A pada sapi pedaging (Diversity of the monoamine oxidase A gene in beef cattle)

(org: Eng)

JITV 29(3): 127-134

Monoamine Oxidase A (MAOA) is a gene that controls aggressive traits. The MAOA gene plays a role in encoding the monoamine oxidase A enzyme, which plays a role in the catabolism of neurotransmitters, including dopamine, norepinephrine, and serotonin. This study aims to identify the diversity of the MAOA SNP gene in beef cattle using sequencing methods. This research used 127 cattle DNA samples to identify diversity, including Bali, Limousin, Wagyu, PO, Madura, and Wagyu-Bali (F1) cattle. MAOA gene polymorphisms in the promoter and exon 1 were analyzed using sequencing methods. Genotype frequencies, allele frequencies, heterozygosity values, and Hardy-Weinberg balance were calculated using the PopGen32 program. The results showed that the MAOA gene in the promoter region has six SNPs, one of which is SNP g.385G>A, while the MAOA gene in exon 1 is monomorphic. The PCR-RFLP method was used to investigate the SNP g.385G>A MAOA gene polymorphism using the RSaI restriction enzyme. The MAOA gene was detected in 3 genotypes: GG, GA, and AA. SNP g.385G>A is polymorphic in Bali, PO, Madura, and Wagyu-Bali cross (F1) cattle while monomorphic in Limousin and Wagyu cattle. Further studies are necessary to explore the functional implications of SNP g.385G>A and their relationship to aggressive behaviors in cattle.

(Author)

Key Words: Beef Cattle, Genetic Diversity, MAOA Gene, Single Nucleotide Polymorphism

UDC: 575.16

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Peningkatan kualitas sperma sexing sapi bali melalui penambahan air buah lontar (*Borassus flabellifer* Linn.) dalam pengencer sitrat-kuning telur (Improve the sexed sperm quality of Bali bulls by adding palmyra (*Borassus flabellifer* Linn.) fruit water to citrate-egg yolk extender)

(Org: Eng)

JITV 29(3): 135-142

Sperm sexing is a technique designed to separate sperm carrying the X and Y chromosomes, which are then used for artificial insemination to generate offspring with the sex the breeder desires. However, the sexing process causes a decrease in sperm quality caused by oxidative stress due to an excessive increase in free radicals. Thus, to counteract the detrimental effects of free radicals on sperm life, antioxidants must be added to the diluent. This study was designed by adding palmyra fruit water (PFw) to citrate-egg yolk (CEy) diluent. This study aimed to evaluate the potency of PFw as a natural antioxidant supplement in CEy diluent to improve the quality of sexing sperm in Bali bulls. Sperm were collected using the artificial vagina method from three Bali bulls aged 3-4 years. Good quality sperm (motility ≥70%, abnormality ≤20%) were sexed with a three-layer albumin gradient method (5, 10, and 15 percent) for 20 minutes, and sperm that were on the bottom albumin layer were preserved in CEy, PFw, PFw-egg yolk (PFw-Ey), or CEy-PFw. The results showed that sperm preservation in the CEy-PFw diluent resulted in higher sperm quality ($P<0.05$) compared to the other three diluents, except for the sperm abnormality parameter ($P>0.05$). It was concluded that adding PFw into the CEy diluent could potentially improve the sexed sperm quality of Bali bulls.

(Author)

Key Words: Bali Bulls, Citrate, Egg Yolk, Palmyra Fruit Water, Sperm Sexing

UDC: 636.38

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Studi kesejahteraan domba dalam kondisi pengangkutan yang disuplementasi dengan meionin hidroksi dan dekstrosa (Animal welfare study in sheep transported with methionine hydroxy analog and dextrose supplementation)

(Org: Eng)

JITV 29(3): 143-150

Livestock transportation adversely affects animal welfare and performance during subsequent production periods. This research aimed to evaluate the effects of methionine hydroxy analog (MHA) and dextrose supplementations before transportation on the animal welfare status of sheep. The study used 27 thin-tailed male sheep with an average body weight of 21,8±2,0 kg, 10-12 months of age, which were divided randomly into three treatments: sheep without transportation and additional supplement (K: Control); sheep transported without additional supplement (P1); sheep transported with additional supplement (P2: MHA 0.5 g/kg body weight and dextrose 0.5 g/kg body weight). Sheep in groups P1 and P2 were transported for 8 hours using commercial vehicles usually used by local farmers. The results showed that sheep in the P2 group had significantly

lower body weight loss than those in the P1 group and faster recovery time (P<0.05). Sheep in the P2 group also demonstrated better post-transportation behavior than those in P1, with no significant difference from the control group. The experimental sheep in the P2 group had a significantly lower N/L ratio than those in P1 and was comparable to the control group (P<0.05). The P2 group also has significantly lower cortisol hormone and blood glucose concentrations than P1, which indicates improved sheep welfare status in P2 and Control groups. Therefore, it can be concluded that supplementation of MHA and dextrose before departure improves sheep's welfare status under transportation.

(Author)

Key Words: Animal Welfare, Dextrose, Methionine Hydroxy Analogue, Sheep, Transportation

UDC: 613.287.5

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Profil biofilm bakteri *coagulase negative Staphylococci* dari isolat susu sapi perah mastitis subklinis (Biofilm profile of coagulase-negative *Staphylococci* bacteria from milk isolates dairy cows with subclinical mastitis)

(Org: Eng)

JITV 29(3): 151-160

Staphylococcus sp. is a pathogenic bacteria that causes subclinical mastitis. These bacteria are divided into coagulase-negative *Staphylococci* (CoNS) and coagulase-positive Staphylococci (CoPS). CoNS bacteria are a group of normal flora on human and animal skin. However, several studies have proven that CoNS bacteria are the most commonly isolated microorganisms from the milk of dairy cows with subclinical mastitis. The ability to form biofilms is an important virulence factor for CoNS bacteria. Detection of biofilm formation was carried out on 54 samples of CoNS bacteria in the form of Stored Biological Material (BBT), which were isolated from the milk of dairy cows with subclinical mastitis with positive California mastitis test (CMT) 2 (++). Detection of biofilm formation was performed qualitatively by Congo red agar (CRA) and test tube (TT) methods. Phenotypic confirmation results showed that 54 isolates (100%) were CoNS bacteria. Biofilm formation detection results showed that 51 out of 54 isolates (94.44%) were positive for biofilm formation. Thus, it can be concluded that CoNS bacteria have the ability to form biofilms as a form of self-protection and virulence factor.

(Author)

Key Words: Biofilm, Coagulase-negative Staphylococci., Subclinical Mastitis, Virulence Factor

UDC: 636.01

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Pengaruh program penyuluhan terhadap peningkatan pengetahuan peternak di wilayah pesisir penyempitan Laguna Segara Anakan, Indonesia (Effect of extension program on improving farmers' knowledge in the narrowing Segara Anakan Lagoon coastal area in Indonesia)

(Org: Eng)

JITV 29(3): 161-171

Segara Anakan Lagoon sedimentation has caused many people in Kampung Laut to change their profession from fishery and start other profession to survive. Sheep farming became an alternative to generate the family income. The extension program is one of the efforts to adapt and improve farmers' knowledge regarding sheep farming. This study aimed to analyze the effect of extension on improving the knowledge of farmers. The study instrument were used to measure the knowledge level of farmers was a questionnaire of pretest and post-test. Respondents were ramdomly sampled resulting in 215 farmers who participated in extension program. The differences of the farmers' knowledge level before and after extension program implemented were analized using t-test. Multiple regression analysis were carried out to determine the effect of characteristics on farmers' knowedge. Results showed that before the extension program was running no farmers had sufficient knowledge of forage preservation and scabies prevention. The trend indicated that the number of farmers with a high level of knowledge increased after the extension intervention. Improving knowledge on scabies prevention was higher than forage preservation (P≤0.01). Farmers' education and experience in sheep farming significantly influenced their knowledge on forage preservation (P≤0.01 and P≤0.05). This study also found that farmers' age would affect their knowledge on scabies prevention (P≤0.10). It can be concluded that farmers' knowledge on forage preservation and scabies prevention improved after participating in the extension program.

(Author)

Key Words: Extension, Farmers, Forage Preservation, Scabies Prevention

UDC: 57.017.5

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Distribusi folikel pada fase estrus dan peningkatan performa estrus sapi PO dara yang distimulasi dengan PMSG dosis non-superovulasi (The follicular distribution during the estrus phase and the enhanced estrus behavior of crossed Ongole heifers stimulated with a non-superovulation dose of PMSG)

(Org: Eng)

JITV 29(4): 172-180

The experiment's objective was to study the follicle distribution during the estrus phase and the estrus performance of the Ongole-crossed heifer by injecting a non-superovulation (NSO) dose of PMSG. Nine PO heifers were randomly assigned to one of three treatment groups, each receiving a different dose of PMSG injection, i.e., 0, 0.5, and

1.0 IU/kg BW, respectively; this was done at the early second follicular phase. The evaluation was conducted during the estrus phase to assess follicle distribution and estrus performance and to collect the reproductive tract for biometrics evaluation. The results demonstrated that PMSG injection at doses of 0.5 and 1.0 IU resulted in an increase in mean follicle diameter size above 0.8 cm by 7.08 % (P>0.05) and 15.04 % (P<0.05), respectively. However, the total number of follicles above 0.8 cm at 1.0 IU/kg BW dose remains high (5.33±2.52, P<0.05), indicating a continued propensity for multiple calving. The results demonstrated that the estrus performance score was enhanced with the administration of increasing doses of PMSG, which was accompanied by an increase in the biometrics of the ovaries (P<0.05) and the diameter of the uterine body, uterine horn, and cervix (P<0.05). It was concluded that the NSO dose of PMSG could facilitate follicle development and enhance estrus quality without increasing the risk of multiple calving.

(Author)

Key Words: Follicle Distribution, Ongole-crossed Heifers, Ovary, PMSG, Reproductive-tract Biometrics

UDC: 633.25

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Karakterisasi degradasi protein pada bahan pakan untuk ternak perah tropis dengan metode *in sacco* (Characterization of protein degradation in tropical dairy feedstuff using the *in sacco* method)

(Org: Eng)

JITV 29(4): 181-192

A study was conducted to determine the protein degradation characteristics of 27 tropical feedstuffs for dairy rations. Twenty-two tropical feedstuffs were grouped into A1 (local low fiber and low protein sources: corn, rice bran, cassava, cassava waste, wheat, pollard), A2 (local low fiber and low protein sources: palm kernel meal, tofu waste, tempe waste), and A3 (local high fiber sources: acacia, alfalfa, narra, gliricidia, indigofera, calliandra, bauhinia, leucaena, albizia, agati, piper, moringa, jack leaves), and compared to A4 (imported low fiber high protein sources: soybean, roasted soybean, DDGS, CGM, CGF) using the in sacco method. The study revealed that A1, A2, and A3 had lower protein content but higher crude fiber than A4. Protein solubility (a) was higher in A1 and A2, while the potentially degraded fraction (b) was higher in A2 and A3. A1 and A2 had higher RDP fractions than A3 and A4. High RDP feedstuffs include pollard, wheat, soybean, CGF, tempe waste, alfalfa, gliricidia, indigofera, agati, and moringa. In contrast, high RUP feedstuffs include corn, palm kernel meal, narra, calliandra, leucaena, albizia, tamarind, piper, jack leaves, roasted soybean, soybean meal, and CGM. Tropical feedstuffs exhibit diverse protein degradation characteristics, making them valuable for strategic ration formulation in dairy cattle.

(Author)

Key Words: Concentrate, Dairy Cattle, Forages, Rumen Degradable Protein, Rumen Undegradable Protein

UDC: 636.2.034

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Pengaruh sistem rasio pakan lengkap terhadap konsumsi bahan kering dan nutrisi serta awal estrus pada sapi perah yang sedang tumbuh (Effect of total mixed ration feeding system on dry matter intake, nutrient intake, and onset of estrus in growing dairy cattle)

(Org: Eng)

JITV 29(4): 193-200

The objective of the study was to compare the advantages of total mixed ration (TMR) feeding versus component (COMP) feeding concerning dry matter intake (DMI) and nutrient intake in terms of crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) intake, and the onset of estrus. A total of ten growing Holstein Frisian (HF) x Jersey breeds were randomly assigned to participate in five months of feeding trials at the Dairy Training and Research Institute. The design structure employed a randomized complete block design (RCBD) comprising five blocking factors under two treatment groups. The TMR and COMP were composed of the same feed ingredients, including corn silage, Napier grass, concentrate mash, mixed legumes, molasses, and iodized salt, and they had the same quantity and nutrient requirements. The TMR was prepared and mixed in advance, prior to the commencement of the feeding trial. In contrast, the components of the COMP were offered separately to the cattle following the specified feeding schedule. The data were collected from the daily intake of the rations offered. The feed refusal was weighed daily before the morning feeding periods, while the body weight gain (BWG) was recorded every two weeks. While samples of the offered and refused feeds were collected once a week for nutritional analysis, the data were subjected to the ANOVA procedure of SAS Statistical Software. The results demonstrated that DM, CP, NDF, and ADF intake was higher in the TMR than in the COMP group. However, the differences in means for BWG, feed conversion ratio, and the onset of estrus were not significantly different. In conclusion, TMR is expected to be beneficial in raising growing dairy cattle.

(Author)

Key Words: Component Feed, Feeding System, Growing Dairy Cattle, Total Mixed Ration

UDC: 619.616

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Tantangan: resistensi methicillin dan vancomycin terhadap *Staphylococcus aureus* akibat infeksi saluran kemih pada domba betina di Provinsi Diyala, Irak (Emerging challenges: Methicillin and vancomycin resistance in *Staphylococcus aureus* from urinary tract infections in ewes of Diyala Governorate, Iraq)

(Org: Eng)

JITV 29(4): 201-207

Methicillin resistant s. aureus is a rare cause of urinary tract infections (UTIs). Current study aims to Isolates and identify *S. aureus* from urine samples of ewes with UTIs and determination of antimicrobial sensitivity pattern for methicillin-Vancomycin resistant. A total of 71 urine samples were collected. S. aureus was isolated using mannitol salt agar and confirmed by VETEK2 system and conventional PCR, using (Staur 4, 6) and (mecA) primers. *S. aureus* was isolated from (9.85%). All *S. aureus* isolated from ewes, (100%) have resistance for Penicillines, cephalosporins and methicillin resistance was detected by cefoxitin screen test and confirmed by detection of MecA gene. Resistance of s. aureus to polypeptides antibiotics was detected in 100% for vancomycin and (85.72%) for Teicoplanin. Resistance of S. aureus to macrolides and Lincosamides antibiotics was detected in (14.28%) for Azithromycin and Clindamycin respectively. *S. aureus* isolated from ewes have absolute sensitivity for aminoglycosides, quinolones, macrolides (Erythromycin), oxazolidinone, tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides. The study found a significant prevalence of MRSA among the isolated strains of S. aureus from ewes with UTIs. This indicates a high level of resistance to beta-lactam antibiotics, posing challenges for effective treatment. The study also identified vancomycin resistance among the MRSA isolates. Vancomycin is often considered a last-line defense against resistant bacteria, so the presence of vancomycin-resistant S. aureus is concerning and limits treatment options.

(Author)

Key Words: Ewes, *Staphylococcus aureus*, Urinary Tract Infection

UDC: 636.58.033

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Performa pertumbuhan ayam broiler yang disuplementasi dengan ekstrak buah naga merah [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose] (Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose])

(Org: Eng)

JITV 29(4): 208-220

One of the advantages of broiler chickens is their short harvest period. Factors that influence their growth include the nutrient content of the feed. However, it will be difficult to get optimal growth only with conventional feed. Therefore, growth supplements are needed. Red dragon fruit water-extract contains flavonoids and vitamins that act as antioxidants. This research aimed to study the effect of red dragon fruit water-extract treatment in drinking water on the growth of broiler chickens. This research used 300 DOC male broiler chickens which were reared until they were 21 days old. This research was conducted using a completely randomized design, with 5 groups and 5 replications, each replication group consisting of 12 DOC. The five groups consisted of the control group (P0), P1 0.25%, P2 0.50%, P3 1%, and P5 2%. Parameters observed included the histological structure of the small intestine, pectoralis muscle, and spleen, as well as growth performance. The results showed that red dragon fruit water extract could increase villi length and area, ratio of villi length to crypt depth, number and area of goblet cells, fasciculus area, muscle fiber area and white pulp area, splenic organ index, as well as improving growth performance including chicken body weight and feed efficiency. Therefore, it can be concluded that red dragon fruit water extract can improve the histological structure of the small intestine, pectoralis muscle, and spleen, as well as increase the growth performance of broiler chickens, especially in red dragon fruit extract with a concentration of 2%.

(Author)

Key Words: Broiler Chicken, Growth Performance, Histological Structure, Red Dragon Fruit Water-Extracted, Small Intestine

UDC: 633.3

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Respon pertumbuhan dan produksi karkas puyuh Jepang jantan yang diberi pakan tepung biji karet (*Hevea brasiliensis*) Fermentasi [Growth response and carcass yield of male Japanese quail-fed diets contained fermented rubber (*Hevea brasiliensis*) seed meal]

(Org: Eng)

JITV 29(4): 221-226

This paper discusses the previous research on the use of fermented rubber seed meal using tempeh yeast (Rhizopus oligosporus) (FRSM) given to male quail from 1 to 5 weeks

of age on the growth performance and carcass production. The research used a Completely Randomized Design (CRD) experimental method using about 260 male quail aged 0-5 weeks divided into 4 treatment groups with the level of giving fermented rubber seed meal (FRSM) using different fermented yeast, namely R0= feeding without FRSM (control), R1= feeding of 4% FRSM, R2 = feeding of 8% FRSM, and R3= feeding of 12% FRSM. Each treatment was repeated 5 times, making 20 units of 13 male quails. The parameters observed were feed consumption, body weight gain, feed conversion, and carcass production. Rubber seed fermentation in this research showed that rubber seeds' cyanide acid (HCN) content was reduced from 158.64 ppm to 17.84 ppm. The experimental results showed that adding fermented rubber seed meal to the diets did not decrease feed consumption or prevent weight gain, feed conversion, final body weight, carcass weight, and carcass percentage. This research concluded that adding fermented rubber seed meal up to 12% did not harm male quail's growth performance and carcass yield.

(Author)

Key Words: Cyanide Acid, Fermentation, Rubber Seed, Tempeh Yeast, Quail Cascass

UDC: 633.2

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Suplementasi tepung larva lalat tentara hitam (*Hermetia illucens*) yang diperkaya selenium terhadap performa pertumbuhan, parameter darah, dan fungsi imun pada itik pedaging (Supplementation of selenium-enriched black soldier fly (*Hermetia illucens*) larvae meal on growth performance, blood parameters, and immune function in broiler ducks)

(Org: Eng)

JITV 29(4): 227-235

This study aimed to evaluate the effects of Se-enriched Hermetia illucens larvae meal (Se-BSF) on production performance, blood biochemistry, and immune status of broiler ducks. Two hundred one-day-old hybrid broiler ducks without sex were randomly allocated into four groups, each with five replications of 10 ducklings. Broiler ducks were fed diets with a controlled diet, an experimental diet with 5% and 7.5% Se-BSF, and positive control with 10 mg/kg Se-Yeast, respectively, daily for 49 days with drinking water. The results showed that the performance production on the carcass traits and visceral organs of broiler ducks was significantly affected (P<0.001) by Se-BSF supplementation. Dietary 7.5% Se-BSF showed trends in decreasing the feed intake, final body weight, average daily gain, and feed efficiency ratio. The effect of dietary Se-BSF was significant differences (P<0.001) between all treatments in live body weight, carcass weight, dressing, breast meat, thigh meat, liver, and spleen. The addition of Se-BSF to broiler ducks' diet showed no significant differences between all heart, gizzard, abdominal fat, and bursa treatments. The effects of dietary 5% to 7.5% Se-BSF had significantly affected (P<0.001) HDL, LDL, Cholesterol, Triglyceride, Ig-A, SOD, and IL-6 levels in serum compared with control and 10 mg/kg yeast-Se. These results indicate that dietary Se-BSF improves broiler ducks' blood biochemistry and immune function, suggesting potential benefits from using Se-BSF as a feed additive for poultry.

(Author)

Key Words: BSF Larvae Meal, Growth Performance, Immunity, Organic Selenium

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Sifat kualitatif kelinci lokal Bambu Apus (Qualitative traits of local Bambu Apus rabbits. qualitative traits of local Bambu Apus rabbits)

(Org: Eng)

JITV 29(4): 236-250

The local Bambu Apus rabbit is crossbred with diverse qualitative traits, many resembling Rex and New Zealand White rabbits. Hence, it is necessary to compare the qualitative traits of local Bambu Apus rabbits with Rex and New Zealand White rabbits. The total samples observed were 94 local Bambu Apus rabbits, 89 Rex rabbits, and 89 New Zealand White rabbits. This study aims to evaluate the qualitative traits of local Bambu Apus rabbits as rabbits that have adapted to the environment of DKI Jakarta, so they are expected to become broiler rabbit strains that support urban farming in DKI Jakarta. Variables observed included qualitative traits such as head type, ear type, eye color, predominant body color, body color pattern, stripe color, stripe distribution, fur characteristics, body type, and body size. Qualitative traits observed included head type, ear type, eye color, body color, color pattern, stripe characteristics, fur type, body type, and size. Data were analyzed using SAS 9.4 with PROC FREQ for frequency and percentage of each variable and Multiple Correspondence Analysis (MCA) using PROC CORESP. Results showed that Bambu Apus rabbits exhibit distinct traits, including the presence of blue and heterochromia eye color, predominantly light brown color and harlequin color pattern that is more prevalent in Bambu Apus rabbits, variations in compact body type, lion fur characteristics, and lop ear type. With a commercial body type and medium size, these traits highlight their genetic potential as tropical climate-adaptive broiler rabbits supporting urban farming in DKI Jakarta.

(Author)

Key Words: Adaptability, Bambu Apus Rabbit, Broiler Rabbit, Qualitative Traits

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Book:

a. Alshelmani M, Abdalla E, Kaka U, Basit M. 2021. Advances in poultry nutrition research. In: Kumar Patra A, editor. Adv Poult Nutr Res. London (UK): IntechOpen; p. 19–32. DOI: 10.5772/intechopen.91547.
b. Reece W. 2015. Respiration in mammals. New Jersey (USA): Willey-Blackwell.
c. Van Soest P. 2018. Nutritional ecology of the ruminant. 2nd ed. New York (USA): Cornell University Press.

Proceeding:

Damayanti R, Wiyono A, Dharmayanti N. 2021. Pathogenicity study of ducks infected with a local isolate of highly pathogenic avian influenza-H5N1-clade 2.3. . In: Inounu I, Priyanti A, Burrow H, Morris S, Min R, Suhubdy, Sutaryono Y, editors. Proc 4th Int Semin Livest Prod Vet Technol. Bogor (Indones): Indonesian Center for Animal Research and Development; p. 277–288.

Thesis:

Mwasame DB. 2020. Analysis of the socio-

economic contribution of donkey ownership and use to houshold livelihoods in Kiambu country, Kenya (Thesis). Nairobi (KE). University of Nairobi

Electronic magazines:

Maranga B, Kagali R, Omolo K, Sagwe P. 2022. Effect of growth substrates on water quality, catfish (*Clarias gariepinus*) culture, and spinach (*Spinacia oleracea*) propagation under the aquaponic system. Livest Res Rural Dev.:82. http://www.lrrd.org/ lrrd34/9/3482mara.html.

Institution:

a. [PSA] Philippine Statistics Authority. 2016. Dairy Industry Performance Report, January – December 2015. Quezon City (Philiphine): Philippine Statistics Authority. P. 1-11
b. [FAO] Food and Agriculture Organization. 2021. Gateway to dairy production and products. Food Agric Organ United Nations. [accessed August 10, 2021]. https://www.fao.org/dairy-production-products/production/feed-resources/en/.

Patent:

Raab RM, Lazar G, Shen B. 2022. AGRIVIDA Inc, assignee. Engineered phytases in animal feed. 2022 Feb 8.

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