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Online J. Anim. Feed Res., 12 (6): 324-397; November 25, 2022

Editorial Team

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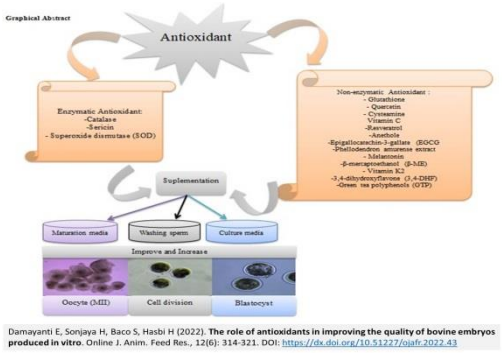
DOI: https://dx.doi.org/10.51227/ojafr.2022.43



Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). **The role of antioxidants in improving the quality of bovine embryos produced in vitro**. Online J. Anim. Feed Res., 12(6): 314-321. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Abstract
Antioxidants are molecular compounds that can give their electron structure to free radical molecules without disturbing them and can break the chain of free radical compounds. Antioxidants that can be used include enzymatic and non-enzymatic antioxidants. Supplementation of antioxidants into maturation mediums or cultures with the right concentration can efficiently improves oocyte maturation, cell division, and embryo quality in bovine. Enzymatic and non-enzymatic antioxidant supplementation of the maturation medium increase the number of oocytes that reach metaphase II (MII). Furthermore, the supplementation of both antioxidants in maturation and culture mediums are also able to increase cell division and embryo that reaches blastocyst. Non-enzymatic antioxidant supplementation is more effective than enzymatic antioxidants in improving the maturation and division of cells in the production of bovine embryos in vitro. In conclusion, non-enzymatic antioxidant supplementation is more effective in supporting embryonic development in vitro.
Keywords: Bovine, Embryo, Enzymatic antioxidants, Non-enzymatic antioxidants
[Full text-PDF]

Review

Quantitative and qualitative traits characterisation of indigenous chickens in Southern African countries

Hlokoe VR, and Tyasi TL.

Online J. Anim. Feed Res., 12(6): 333-340, 2022; pii: S222877012200044-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.44

Hlokoe VR, and Tyasi TL (2022). **Quantitative and qualitative traits characterisation of indigenous chickens in Southern African countries.** Online J. Anim. Feed Res., 12(6): 324-330. DOI: https://dx.doi.org/10.51227/ojafr.2022.44

Abstract
Indigenous poultry production is important to human lives as they alleviate poverty by providing an affordable source of animal proteins. Besides, it serves as the easiest source of income for rural farmers in developing countries. The current review was conducted to evaluate the research findings of indigenous chickens' quantitative and qualitative traits characterisations in Southern African countries. Quantitative and qualitative traits characterisation is the first step for genetic improvement in livestock. The documents used in the review were attained from different search engines, most of which were published lately (after 2010). Potchefstroom Koekoek, Venda, Naked Neck, Ovambo, Boschveld and Tswana indigenous chicken breeds in the Southern African countries were characterised. The studies suggest that there are variations in quantitative traits including live weight, egg weight, wing length, comb length and shank length and qualitative traits including feather color, comb type, comb color and eggshell color of indigenous chickens of Southern African countries. The single red comb types and red wattles were observed in most indigenous chicken breeds in Southern African countries. The present review concludes that the characterisation of quantitative and qualitative traits assists greatly in the differentiation and identification of indigenous chicken breeds.
Keywords: Breed; Characterisation; Chickens; Quantitative traits; Qualitative traits.
[Full text-PDF]

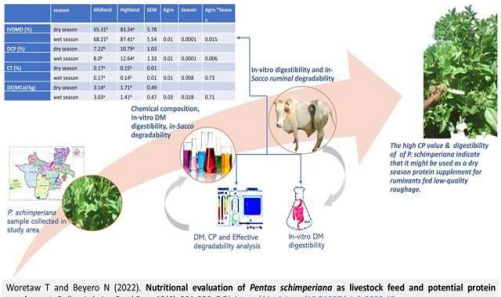
Research Paper

Nutritional evaluation of *Pentas schimperiana* as livestock feed and potential protein supplement

Woretaw T and Beyero N.

Online J. Anim. Feed Res., 12(6): 341-349, 2022; pii: S222877012200045-12

DOI: https://dx.doi.org/10.51227/ojafr.2022.45



Woretaw T and Beyero N (2022). **Nutritional evaluation of *Pentas schimperiana* as livestock feed and potential protein supplement.** Online J. Anim. Feed Res., 12(6): 331-339. DOI: https://dx.doi.org/10.51227/ojafr.2022.45

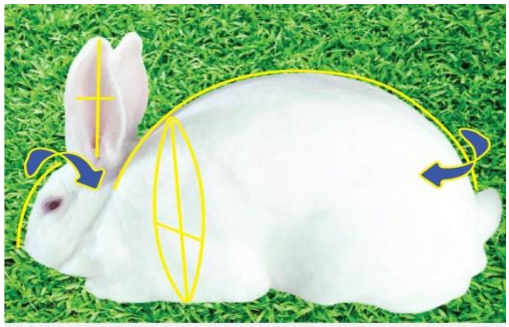
Abstract
Pentas schimperiana is locally available and dominantly used as a dairy cattle feed in the Dawuro zone's Maraka district, Ethiopia. There is some information about its utilization practice, however, data on its nutritional benefit is limited. This research aimed to examine the chemical content and digestibility of *P. schimperiana* for use as cattle feed during the dry season. For this study, a 2x2 factorial design with two agro-ecologies (midland and highland) and two seasons (wet and dry) was used for this study. *P. schimperiana* leaves, twigs and stems were gathered and processed for laboratory analysis from two agro-ecologies and two seasons. The chemical composition, in-vitro digestibility, and in-Sacco degradability of the samples were determined after incubation at 0, 6, 12, 24, 48, 72, and 96 hours. Effective degradability (ED) and Potential degradation (PD) were computed. The collected data were subjected to an Analysis of Variance and the means that had significant deference, the mean separation was performed by Tukey with alpha level of 0.05. *In-vitro* dry matter digestibility (IVDMD) (87.41%) and crude protein (CP) (17.39%) were significantly higher in highland. Digestible crude protein (DCP) was significantly larger in dry than in the wet season with the content of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) decreased. During the wet season, potential degradability (PD) and effective degradability (ED) of DM were highest in the highland with a decrease in leg time, which was related to the low content of ADF, ADL, NDF, tannin, and high CP in vegetative stages of *P. schimperiana.* The result indicates that *P. schimperiana* might be used as supplementary feeds to enhance utilization of low-quality feed resources and improve the performance of ruminants during the dry season when feed is scarce.
Keywords: Chemical composition, Degradability, Digestibility, *Pentas schimperiana,* Tannin.
[Full text-PDF]

Research Paper

Morphometric characterization of New Zealand white rabbit raised at different areas

Setiaji A, Sutopo S, Lestari DA, Kurnianto E, Novianti ME.

Online J. Anim. Feed Res., 12(6): 350-355, 2022; pii: S222877012200046-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.46


Setiaji A, Sutopo S, Lestari DA, Kurnianto E, Novianti ME (2022). **Multivariate analysis for morphometric characterization of New Zealand white rabbit raised at different areas.** *Online J. Anim. Feed Res.*, 12(6): 340-345. DOI: https://dx.doi.org/10.51227/ojafr.2022.46

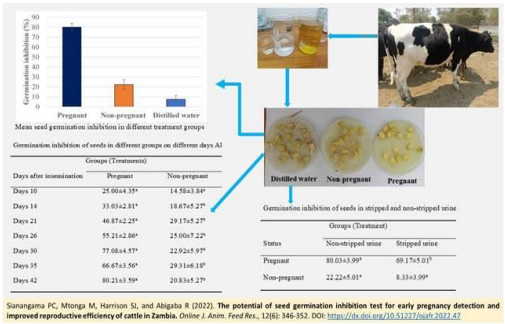
Abstract
The study aimed to morphometric characterization the New Zealand White (NZW) doe at three different areas. The materials used were 295 heads of NZW doe rabbits from 29 farms located at different areas. Twelve morphometric characteristics consist of body weight, eight body measurements, and three-body indices. Data analysis was performed by Mixed model, Pearson's correlation, Principal component, and Canonical discriminant procedures. The most of parameters showed significant differences among areas. The heaviest body weight (4.71 kg) was observed in low-land and the lightest in medium land (3.54 kg). Most of the morphometric characters showed positive correlations with each other. Results of principal component show that the body indices of NZW doe raised in three different areas were similar. Canonical discriminant analysis showed that low-land was more favorable than high-land and medium land. In conclusion, The variation in size difference for morphometric characters of female New Zealand white rabbit could be explained by body index and thoracic index. The morphometric characteristics of New Zealand white doe raised in low-land area were superior to those raised in high-land and medium land areas.
Keywords: Body index, Eigenvalues, Least-square means, Phenotypic correlation, Thoracic index.
[Full text-PDF]

Research Paper

The potential of seed germination inhibition test for early pregnancy detection and improved reproductive efficiency of cattle in Zambia

Sianangama PC, Mtonga M, Harrison SJ, and Abigaba R.

Online J. Anim. Feed Res., 12(6): 356-362, 2022; pii: S222877012200047-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.47



Sianangama PC, Mtonga M, Harrison SJ, and Abigaba R (2022). **The potential of seed germination inhibition test for early pregnancy detection and improved reproductive efficiency of cattle in Zambia.** *Online J. Anim. Feed Res.*, 12(6): 346-352. DOI: https://dx.doi.org/10.51227/ojafr.2022.47

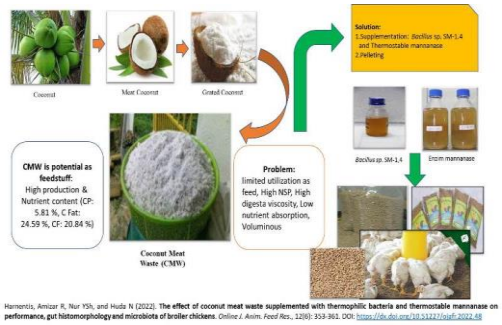
Abstract
Early pregnancy diagnosis is an important management practice for reducing calving interval, increasing cattle reproductive efficiency, and the overall herd productivity. This study was undertaken to assess the viability of seed germination inhibition technique (Punyakoti test) for early pregnancy detection in cattle under the tropical rearing conditions. Twenty-four randomly selected cows were used for the experiment. Urine samples were collected and subjected to Punyakoti test, using maize seeds, within 6 hours of collection. Descriptive statistics employing means and standard error were used to analyse data, also, inferential statistics including analysis of variance and t-tests were employed to ascertain differences between the variables under study. Urine from pregnant cows had the highest (80.03±3.99) inhibitory effect while the distilled water group had the least (7.50±3.81) mean seed germination inhibition. The means of germinated maize seeds in pregnant and non-pregnant cow urine treatment groups were significantly different. The means of germinated maize seeds in unstripped and stripped pregnant cow urine were significantly different, while the mean shoot length values for

the same treatments were not significantly different. Urine from 42 days' pregnant cows had the highest germination inhibition (80.21±3.59) while the least (25.00±4.35) was observed on day 10. The Punyakoti test reliably detected pregnancy starting from 26 days after insemination. In conclusion, this technique can be used for detecting pregnancy as early 26 days after insemination. The potential role of steroids and involvement of abscisic acid (ABA) in seed germination inhibition requires further investigation.
Keywords: Abscisic acid, Cattle, Maize, Pregnancy detection, Punyakoti test.
[Full text-PDF]

Research Paper

The effect of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase on performance, gut histomorphology and microbiota of broiler chickens

Harnentis, Amizar R, Nur YSh, and Huda N.



Harnentis, Amizar R, Nur YSh, and Huda N (2022). The effect of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase on performance, gut histomorphology and microbiota of broiler chickens. *Online J. Anim. Feed Res.*, 12(6): 353-361. DOI: https://dx.doi.org/10.51227/ojafr.2022.48

Online J. Anim. Feed Res., 12(6): 363-371, 2022; pii: S222877012200048-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.48

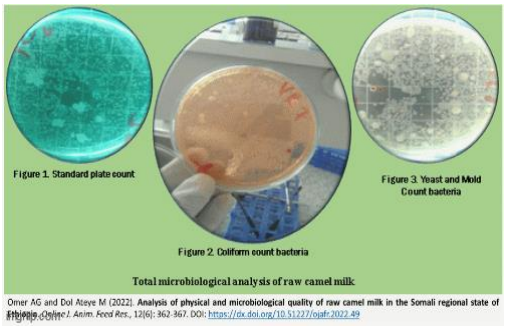
Abstract
An experiment was conducted on Arbor Acress broiler chicks to evaluate the effects of coconut meat waste (CMW) supplemented with 10^{10} CFU/kg CMW of thermophilic bacteria (*Bacillus* sp. SM-1.4) and 800 U/kg diets of thermostable mannanase on performance and gut histomorphology of broilers. One hundred and fifty of day-old chicks (unsexed) were used in this study. The birds were fed five diets containing coconut meat waste supplemented with bacteria and mannanases (CBM); 0% CBM, 10% CBM, 20% CBM, 30% CBM, and 40% CBM. Feed and water were available at all times. A completely randomized design was applied in this experiment with five diets and five replicate cages. The parameters determined are performance (feed consumption, body weight gain, and feed conversion ratio), gut histomorphology, and microbiota of broilers. Data were analyzed by analysis of variance according to a completely randomized design and Duncan's Multiple Range Test. Data indicated that feed consumption, body weight gain, and gut histomorphology of birds fed the CMW supplemented with thermophilic bacteria and thermostable mannanase were up to 30% higher than those of birds fed the non-supplemented (0% CBM) diet. The birds fed a 20% coconut meat waste diet supplemented with 10^{10} CFU/kg diet thermophilic bacteria, and 800 U/kg CMW thermostable mannanase had higher body weight gain than others and significantly ($P<0.05$) affected on microbiota of broiler. Adding coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase did not affect the feed conversion ratio. In conclusion, up to 20%, CBM level in the diet could improving performance, gut histomorphology, and composition microbiota of broiler.
Keywords: Broiler, Coconut meat waste, Histomorphology, Microbiota, Thermophilic bacteria, Thermostable mannanase.

[Full text-PDF]

Research Paper

Analysis of physical and microbiological quality of raw camel milk in the Somali regional state of Ethiopia

Omer AG and Dol Ateye M.



Omer AG and Dol Ateye M (2022). Analysis of physical and microbiological quality of raw camel milk in the Somali regional state of Ethiopia. *Online J. Anim. Feed Res.*, 12(6): 362-367. DOI: https://dx.doi.org/10.51227/ojafr.2022.49

Online J. Anim. Feed Res., 12(6): 372-378, 2022; pii: S222877012200049-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.49

Abstract
The objective of this study was to determine the physical and microbial quality of raw camel milk along the milk market chain a total of forty-two raw milk samples were taken from milk producers (21 samples) and milk collection centers (21 samples). Each sample was analyzed for physical and microbial quality including temperature, pH, titratable acidity, specific gravity, and clot on boiling, the overall mean and standard deviation values were 27.93 °C, 6.29, 1.030 g/cm3, 0.95%, and 88.1% respectively. Microbial quality and safety attributes that include total bacteria count, coliform count, and yeast and mold counts were analyzed. The overall mean $\log_{10}$ counts per ml and standard deviation values for each total bacterial count, coliform count, and microbial analysis were 7.48 $\log_{10}$ CFU/ml, 5.85 $\log_{10}$ CFU/ml, and 4.78 $\log_{10}$ cfu/ml, respectively. The total bacterial count, coliform count, yeast, and mold counts were calculated and show that the milk collection center samples were significantly higher than milk samples obtained from household producers. This study indicated that the quality of camel milk in the study area had low quality and this could cause public risks through the consumption of raw camel milk produced and sold under the present production and handling conditions along the chain. Thus, these calls for strict hygienic measures to improve the quality and safety of camel milk produced and marketed in the study area.
Keywords: Camel milk, Hygiene, Microbial quality, Raw milk, Somali Regional State.

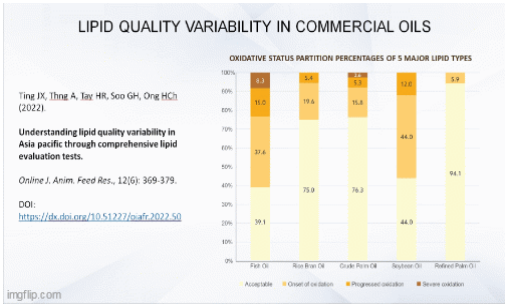
[Full text-PDF]

Research Paper

Understanding lipid quality variability in Asia Pacific through comprehensive lipid evaluation tests

Ting JX, Thng A, Tay HR, Soo GH, and Ong HCh.

Online J. Anim. Feed Res., 12(6): 379-389, 2022; pii: S222877012200050-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.50



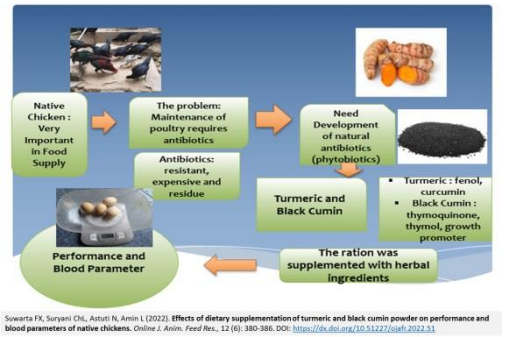
Abstract
The objective of this study was to investigate the variability in the oxidative quality and nutritional values of different lipid samples collected across Asia-Pacific region. The oxidative quality was evaluated through the peroxide value (PV) and malondialdehyde (MDA) content, while the free fatty acid (FFA) content and degree of fatty acid saturation (U/S ratio) were two essential parameters used to understand the nutritional values or metabolizable energy (ME) values of lipid samples. A total of 1221 lipid samples were collected and analyzed over a period of 10 years. The study showed high variability in oxidative quality between the lipid samples. Due to higher unsaturated fatty acid composition, the oxidative quality for most of the fish oil and soybean oil was at a less favorable range compared to rice bran oil, crude palm oil, and refined palm oil. The standard deviation of free fatty acids (FFA) content of soybean oil and refined palm oil was smaller compared to tallow, rice bran oil, crude palm oil, and fish oil. Fish oil and soybean oil had a higher standard deviation in U/S ratio. Variations in the FFA content and U/S ratio contributed to fluctuation in ME values.
Keywords: Fatty acid composition, Hydrolysis, Lipid quality, Metabolizable energy, Oxidation.
[Full text-PDF]

Research Paper

Effects of dietary supplementation of turmeric and black cumin powder on performance and blood parameters of native chickens

Suwarta FX, Suryani ChL, Astuti N, and Amin L.

Online J. Anim. Feed Res., 12(6): 390-397, 2022; pii: S222877012200051-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.51



Abstract
This study was conducted to determine the effect of supplementation of a mixture of turmeric and black cumin powder on the production performance, blood parameters, and quality of native chicken eggs. Ninety-six female native chickens aged 17 weeks were randomly allocated in a completely randomized design into four treatments. The four treatments were differentiated based on the level of supplementation of a mixture of turmeric (TP) and black cumin powder (BCP), namely T0: Control (without a mixture of TP and BCP); T1: (2.5 g TP + 2.5 g BCP)/kg ration; T2: (5 g TP + 5 g BCP)/kg ration and T3: (7.5 g TP + 7.5 g BCP)/kg ration. Each treatment was repeated 3 times, using 8 native chickens. The variables measured were blood parameters including Hemoglobin (Hb), hematocrit, total red blood cells (RBC), white blood cells (WBC), blood cholesterol and triglycerides; and also, the performance variables of feed consumption, egg production (HDA), body weight at 34 weeks, egg weight, egg mass, feed conversion, yolk weight, shell weight, shell thickness, yolk color index, cholesterol, LDL and HDL of yolks. The Data were collected for 16 weeks and analyzed by variance analysis. The results showed that supplementation with a mixture of TP and BCP increased feed consumption; HDA; body weight; egg mass; shell weight, thickness, and yolk color ($P<0.05$). Egg weight and yolk weight were not significantly different. TP + BCP supplementation significantly reduced feed conversion, cholesterol level, HDL, and LDL. Increased TBCP supplementation increased Hb, hematocrit, red blood cells and white blood cells and significantly decreased cholesterol, lipoproteins, and triglycerides in the blood ($P<0.05$). It was concluded that TBCP mixture supplementation could improve production performance, some of blood parameters, and quality of native chicken eggs.
Keywords: Black cumin, Native chicken, Performance, Turmeric, Yolk
[Full text-PDF]

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Online Journal of Animal and Feed Research

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THE ROLE OF ANTIOXIDANTS IN IMPROVING THE QUALITY OF BOVINE EMBRYOS PRODUCED IN VITRO

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Supporting Information

REVIEW ARTICLE
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ABSTRACT: Antioxidants are molecular compounds that can give their electron structure to free radical molecules without disturbing them and can break the chain of free radical compounds. Antioxidants that can be used include enzymatic and non-enzymatic antioxidants. Supplementation of antioxidants into maturation mediums or cultures with the right concentration can efficiently improves oocyte maturation, cell division, and embryo quality in bovine. Enzymatic and non-enzymatic antioxidant supplementation of the maturation medium increase the number of oocytes that reach metaphase II (MII). Furthermore, the supplementation of both antioxidants in maturation and culture mediums are also able to increase cell division and embryo that reaches blastocyst. Non-enzymatic antioxidant supplementation is more effective than enzymatic antioxidants in improving the maturation and division of cells in the production of bovine embryos in vitro. In conclusion, non-enzymatic antioxidant supplementation is more effective in supporting embryonic development in vitro.

Keywords: Bovine, Embryo, Enzymatic antioxidants, Non-enzymatic antioxidants

INTRODUCTION

Researches on in vitro embryo production (IVEP) in bovine continue to develop with improvements to support their success. Factors that often lead to unsuccessful embryo production include the quality of oocytes, sperm, and suboptimal culture conditions, which lead to oxidative stress. Cellular oxidative stress can cause a variety of cell damage, including lipid peroxidases on the membrane, oxidation of amino acids, death, and cell necrosis, thereby reducing embryo viability (Kitagawa et al., 2004; Su et al., 2019). Reactive oxidative species (ROS) at normal limits play a role in aiding in the process of proliferation and differentiation (Valko et al., 2007; Sharifi-Rad et al., 2020). A deeper number of cells, however, can result in cell damage and death (Agarwal et al., 2005; Pizzino et al., 2017). Oocytes or embryos cultured in vitro undergo manipulation of environmental conditions which may lead to the increase of ROS level, causing a decrease in cell regulation (Yu et al., 2014; Agarwal et al., 2022). Most ROS is formed from the process of degradation of long-chain fatty acids resulting from the process of respiration of mitochondria and peroxisomes (Ganguli et al., 2019). To prevent excessive ROS formation during the period of oocyte or embryo culture, several studies have been carried out including the addition of antioxidants to the medium used. Antioxidants are compounds with a molecular structure that can give their electron structure to free radical molecules without being disturbed and can break the chain of free radical compounds (Budani and Tiboni, 2020). Antioxidants play a vital role in reducing the activity of hydrogen peroxide (H_2O_2) levels and reactive oxygen species (ROS) (Kurutas, 2016). Antioxidant substances in cells present at low concentrations and significantly reduce or prevent oxidation of oxidizable substrates (Kurutas, 2016). The antioxidants used are divided into two, namely enzymatic and non-enzymatic antioxidants. Antioxidant enzymes include superoxide dismutase (SOD) (Lee et al., 2011), katalase (Ali et al., 2003) and glutathione ethyl ester (GSH-OEt) (García-Martínez et al., 2020), while non-enzyme antioxidants are sericin (Satrio et al., 2022), vitamin K2 (Baldoceda-Baldeon et al., 2014) and vitamin C (Sovernigo et al., 2017).

Enzymatic and non-enzymatic antioxidants work synergistically to support each other in protecting cells and organ systems of the body against free radical damage (Walczak-Jedrzejowska et al., 2013; Kurutas, 2016). Researches on the addition of media antioxidant, such as catalase are still regarded as controversial (Lane et al., 2001; Ali et al., 2003). Some reports, however, stated that the addition of catalase to embryo produced in vitro can reduce intracellular ROS levels and the rate of apoptosis in bovine embryos (Rocha-Frigoni et al., 2016). Cells naturally have physiological mechanisms to inhibit the formation of excessive free radicals including specific enzymes that control their intracellular levels (Liu et al., 2016). Several studies have been conducted regarding the use of antioxidants in maturation media or cultures to improve the quality of oocytes and embryos produced. The primary thing to know about the use of antioxidants is the right concentration of supplements so that it can effectively support embryonic development. This paper will outline the use of some enzymatic and non-enzymatic antioxidants in maturation media and bovine cultured in vitro.

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Antioxidant supplementation to the maturation medium towards the degree of maturity of bovine oocytes

The first stage in the in vitro production of embryos is the maturation of oocytes (Strączyńska et al., 2022). Oocyte maturation is very important as an early stage and it is crucial to obtain quality oocytes before fermentation (Sirait et al., 2021). Mature oocytes are oocytes that reach the stage of metaphase II (MII) (De Vos et al., 1999; Parrella et al., 2019). Proper use of antioxidants can improve oocytes maturation and embryonic development (Budani and Tiboni, 2020; Rodríguez-Varela and Labarta, 2020). One of the important things to know is the proper concentration of antioxidants in the in vitro maturation medium to increase the number of oocytes that reach MII. Some studies that have been conducted regarding antioxidant supplementation on in vitro maturation media of bovine are presented in Table 1.

Table 1 - Antioxidant supplementation towards the degree of in vitro maturation of bovine oocytes

Name of antioxidant	Concentration	Number of Oocytes	Maturation Rate (MII) (%)	References
Non-antioxidant	-	154	75.3±0.8	(Satrio et al., 2022)
	-	120	76.7±1.7	(Rocha-Frigoni et al., 2016)
	-	59	86.4±2.7	(Sovernigo et al., 2017)
	-	101	84.2±2.3	(Wang et al., 2007)
	-	165	76.04±1.22	(Huang et al., 2018)
Average		119.8±38.11	79.73±4.62	
Enzymatic antioxidants				
Catalase	100 UI	104	80.6±5.2	(Rocha-Frigoni et al., 2016)
Serisin	0.1%	120	87.0±3.1	(Satrio et al., 2022)
Average		112±8	83.8±3.2	
Non-Enzymatic antioxidants				
Quercetin	2 µM	60	90.01±5.0	(Sovernigo et al., 2017)
Cysteamine	100 µM	62	91.9±2.9	(Sovernigo et al., 2017)
Carnitine	0.5 mg/mL	61	88.5±2.5	(Sovernigo et al., 2017)
Vitamin C	50 mg/mL	61	90.2±0.7	(Sovernigo et al., 2017)
Resveratrol	2 µM	61	91.8±3.0	(Sovernigo et al., 2017)
Green tea polyphenols (GTP)	10 µM	107	87.5 ±2.1	(Wang et al., 2007)
Epigallocatechin-3-gallate (EGCG)	50 µM	180	88.04±2.49	(Huang et al., 2018)
Average		84.57±42.07	89.71±1.63	

Table 1 shows that antioxidant supplementation of both enzymatic and non-enzymatic antioxidants increased the number of oocytes that reach MII. The average oocyte that reached the MII stage without antioxidant supplementation was 79%, while the enzymatic and non-enzymatic antioxidants were 83% and 89% respectively. The addition of enzymatic antioxidants increased the MII stage by 4%, while the non-enzymatic antioxidants could increase the MII stage by 10%. This suggests that non-enzymatic antioxidants have more potential to preserve oocytes during maturation than enzymatic antioxidants. Based on Table 1, the addition of antioxidants of both enzymatic and non-enzymatic could inhibit the formation of free radicals. Antioxidants are formed as a defense mechanism against toxic reactive oxygen species (ROS). These pathways include photorespiration pathways, enzymatic and non-enzymatic pathways, regulation of corresponding response genes, and anatomical pathways (Kumar et al., 2014). Antioxidant system to ward off free radicals is naturally formed by the body itself (Vona et al., 2021). If the amount of ROS exceeds the amount of antioxidants in the cell, the excess will attack the lipid, protein, and DNA components, resulting in a damage called oxidative stress (Pham-Huy et al., 2008).

Some previous studies have reported enzymatic antioxidant supplementation such as catalase (Rocha-Frigoni et al., 2016) and sericin (Satrio et al., 2022). Catalase plays a role in reducing the production of H_2O_2 produced in extracellular environments (Nandi et al., 2019). The use of catalase becomes a strategy to prevent the formation of intracellular ROS at the end of maturation (Circu and Aw, 2010). Espinosa-Diez et al. (2015) reported an evidence that ROS affects cellular function by controlling the production and activation of biologically active substances, while Isobe et al. (2012) stated that sericin has a high hydroxyl amino acid (serine) that possess a potential as an antioxidant. Furthermore, Dash et al. (2008) reported that sericin can lower ROS levels in cultures by preventing H_2O_2 induced oxidative stress.

Non-enzymatic antioxidant supplementation such as quercetin, cysteamine, carnitine, vitamin C and resveratrol has been reported by Sovernigo et al. (2017). In addition, there are also green tea polyphenols (GTP) (Wang et al., 2007) and epigallocatechin-3-gallate (EGCG) (Huang et al., 2018). Quercetin is one of flavonoids which has antioxidant capacity in reducing ROS levels, protects mitochondrial functions and regulates the defense system of enzymatic antioxidants (Sameni et al., 2018). Cysteamine is an amino thiol ($HSCH_2CH_2NH_2$) formed in the body from the degradation of coenzyme A during the formation of pantetheine, which is eventually hydrolyzed into cysteine and pantothenic acid

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(Sameni et al., 2018). Cysteamine is able to increase glutathione (GSH) production during the maturation process by reducing cystine to cysteine (Gasparrini et al., 2003). Cysteamine may increase intracellular GSH levels in oocytes and embryos in the absence of cumulus cell monolayers (de Matos et al., 2002). Carnitine is a quaternary ammonium synthesized from the amino lysine and methionine acids and acts as an antioxidant that neutralizes ROS and protects cell organelles (Ye et al., 2010; Mishra et al., 2016). L-carnitine supplementation during maturation improves the development of bovine embryos from less competent meiotic oocytes and accelerates the formation of blastocysts of more competent oocytes (Knitlova et al., 2017). 50 mg/mL vitamin C in maturation media could increase oocytes to reach metaphase II up to 90.2% and the addition of 2 µM resveratrol could increase oocytes to reach metaphase II in bovine up to 91.8% (Sovernigo et al., 2017). Likewise, Kere et al., (2013) reported that the addition of 50 mg/mL vitamin C to swine maturation media can increase the maturation of oocytes to reach metaphase II up to 79.8%. Vitamin C is no longer only an enzymatic cofactor, antioxidant, and extracellular promoter of matrix formation by stabilizing collagen structures, but it also has the potential to modulate gene expression (Ivanyuk et al., 2015; Duran et al., 2019). Another study carried out on goats stated that the addition of 1 µM of resveratrol in maturation media could increase oocytes to reach metaphase II up to 93% (Piras et al., 2019). Wang et al. (2014) found that resveratrol triggers the maturation of oocyte nuclei due to its antioxidant properties and can induce progesterone secretion. Meanwhile, GTP supplementation on maturation media also improves the quality of oocytes at the maturation level (Wang et al., 2007). The antioxidant effects of tea polyphenols are thought to be related to their ability to stimulate antioxidant defense metabolism through cell cycle regulation that relies on redox-regulated transcription factors and mitogen-activated protein kinase (MAPK) (Jiao et al., 2003; Williams et al., 2004). Epigallocatechin-3-gallate (EGCG) also has the potential to increase MII in in vitro cultured bovine oocytes (Huang et al., 2018). EGCG is the main component of polyphenol catechins in green tea and is considered one of the components with the most bioactive compounds due to its powerful antioxidant properties (Roychoudhury et al., 2017). Reduction of intracellular ROS levels after EGCG supplementation can contribute to direct radical clearance activity, or increase EGCG-induced antioxidant enzyme activity (Huang et al., 2018).

Research by Sovernigo et al. (2017) revealed that cysteamine and resveratrol are non-enzymatic antioxidants that are best at increasing oocyte maturation in cattle, which is around 91%. This proves that antioxidant supplementation on maturation media is effective in reducing ROS and in increasing GSH in bovine oocytes in vitro. The right amount of antioxidant supplementation can increase cytoplasmic maturation during oocyte maturation and can contribute further to protect embryos from oxidative attacks in their early development.

Antioxidant supplementation in the medium on embryos ability to reach the blastocyst stage

The ability of embryo development after fertilization is influenced by various factors including oocyte competence, spermatozoa and the ability to divide to blastocyst (Colaco and Sakkas, 2018). The occurrence of deviations at the beginning of embryogenesis can cause disruptions in embryonic development and survival (Feugang et al., 2009). Improving the quality of embryos in the production of bovine embryos in vitro is very important (Demetrio et al., 2020). To prevent metabolic disorders that can occur during the culture process, it is necessary to add antioxidants to the media (Kurutas, 2016). Different types of antioxidants that can be used in embryo production can be seen in Table 2.

Table 2 shows that the addition of antioxidants, both enzymatic and non-enzymatic, was able to increase the number of splitting embryos and blastocyst in bovine cultured in vitro. Enzymatic antioxidant supplementation increased about 5% of splitting embryos and 4% of blastocyst of control. Likewise, the use of non-enzymatic antioxidants could increase about 10% of the control splitting embryo and about 16% of embryos reached blastocyst of control (without antioxidant suplementation.

Antioxidants can be added to maturation media, sperm washing and culture, and have been shown to have an effect on improving embryo quality. The use of antioxidant enzymes had an average of about 30% of embryos reaching blastocyst from splitting embryos, while the use of non-enzymatic antioxidants had an average of about 42% of embryo reaching blastocyst. The use of enzymatic antioxidants, namely superoxide dismutase (SOD), in bovine culture media (Lee et al., 2011) and the use of enzymatic antioxidant catalase (CAT) in maturation media (Ali et al., 2003) have been done. Superoxide dismutase (SOD) is responsible for converting oxygen free radicals into H_2O_2, which is highly toxic and immediately discarded (Kürüm et al., 2019). In the in vivo culture, sperm and oocyte spasms in the oviduct ducts are involved in the regulation of SOD (Yan et al., 2014). Supplementation of 300 U/mL SOD in the culture medium increased blastocyst production by 33.6% (Lee et al., 2011). Superoxide dismutase (SOD) catalyzes the distillation of superoxide into oxygen and hydrogen peroxide. Antioxidant is important for every cell exposed to oxygen as an antioxidant chelator which is effective in transferring metal ions and can reduce free radicals such as oxygen and nitrogen and inhibit the generation of primary oxygen radicals and oxidation (Kostyuk et al., 2004). Catalase (CAT) can be the dominant antioxidant in the early stages of bovine folliculogenesis (Gupta et al., 2011).

Non-enzymatic antioxidants used in an effort to improve embryonic development in bovine are glutathione (Itahashi, 2022), quercetin, cysteamine, carnitine, vitamin C, resveratrol (Sovernigo et al., 2017), anethole (Anjos et al., 2019), *Phellodendron amurense* extract and melatonin (Do et al., 2017), β-mercaptoethanol (β-ME) (Hosseini et al., 2009), vitamin K2 (Baldoceda-Baldeon et al., 2014), flavonoid 3,4-dihydroxy flavone (3,4-DHF) (Lee et al., 2011), and green tea polyphenols (GTP) (Wang et al., 2007). The use of such antioxidants has been shown to be able to increase embryos to reach blastocyst by 42% of the dividing cells.

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Table 2 - Antioxidant supplementation on the development of bovine embryos in vitro

Name of antioxidant	Concentration	Media	Number of oocytes	Cleavage rate (%)	Blastocyst (%)	References
	-		114	47.4±4.0	15.8±1.3	(Itahashi, 2022)
	-		142	85.9±4.1	47.2±2.7	(Sovernigo et al., 2017)
	-		130	-	31.5	(Lee et al., 2011)
Non-antioxidant	-		-	69	20	(Ali et al., 2003)
	-		-	87.4±1.9	25.4±2.8	(Anjos et al., 2019)
	-		36	89.4±6.2	25.0±0.0	(Do et al., 2017)
	-				23	(Baldoceda-Baldeon et al., 2014)
Average				75.82	26.84	
Enzymatic antioxidants						
Superoxide dismutase (SOD)	300 U/mL	Culture	124	-	33.6	(Lee et al., 2011)
Catalase	127 U/mL	Maturation	-	81	28	(Ali et al., 2003)
Average			-	81	30.8	
Non-enzymatic antioxidants						
Glutathione	1 µM	Sperm washing	119	82.4 ±3.7	39.5±2.3	(Itahashi, 2022)
Quercetin	2 µM	Maturation	142	85.9±4.5	53.5±3.9	(Sovernigo et al., 2017)
Cysteamine	100 µM	Maturation	143	86.7±7.2	52.4±2.7	(Sovernigo et al., 2017)
Carnitine	0.5 mg/mL	Maturation	144	84.7±6.5	54.2±3.1	(Sovernigo et al., 2017)
Vitamin C	50 mg/mL	Maturation	142	85.9±7.1	52.1±3.1	(Sovernigo et al., 2017)
Resveratrol	2 µM	Maturation	142	88.7±8.4	54.2±4.0	(Sovernigo et al., 2017)
Anethole	30 µg/ml	Maturation	-	86.2±5.8	32.1±4.7	(Anjos et al., 2019)
Phellodendron amurense extract	0.01 µL	Culture	36	85.6±6.2	30.6±0.9	(Do et al., 2017)
Melatonin	0.01 µL	Culture	114	84.2±7.4	35.7±5.1	(Do et al., 2017)
β-mercaptoethanol (β-ME)	100 µL	Culture	1250	92.0 ± 3.1	40.1±1.1	(Hosseini et al., 2009)
Vitamin K2	0.50 mM	Culture	-	-	31	(Baldoceda-Baldeon et al., 2014)
3,4-dihydroxyflavone (3,4-DHF)	10 µM	Culture	129	-	44.2	(Lee et al., 2011)
Green tea polyphenols (GTP)	15 µM	Maturation	-	76	38.1	(Wang et al., 2007)
Average				85.3	42.9	

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1 µM GSH supplementation on sperm washing media caused the number of the dividing cell to reach about 89% and was able to reach blastocyst of about 39% of the dividing cells. GSH not only reduces the formation of ROS, but also reduces the formation of disulfide bonds (Oikawa et al., 2018). The stimulating effect of GSH on embryonic development is mainly on the reduction of effective disulfide bonds in spermatozoa (Mayor et al., 2001). Itahashi et al. (2022) explained that GSH is important for decondensation of sperm chromatin after sperm penetration in oocytes, destabilization and replacement of protamines by oocyte-derived histones (Caglar et al., 2005), and the development of sperm nuclei into the male pronucleus (Canel et al., 2017).

Resveratrol and quercetin are secondary plant metabolites found in vegetables, fruits, flowers and seeds, and are flavonoid compounds produced by the interaction of plants and microorganisms to protect themselves against fungal and bacterial infections (Kwak et al., 2012; Kang et al., 2013). Cysteamine (CYS) supplementation in maturation media increases intracellular levels of GSH in mature oocytes and embryonic development rate (de Matos et al., 2002; Alsalim, 2020). Cysteamine (CYS) is one of the thiol compounds which has been successfully used in the maturation of oocytes in vitro by acting as a ROS collector and maintaining cellular redox balance for proper conditions in embryonic development (Gasparrini et al., 2000).

Vitamin C supplementation in maturation medium has a positive impact on embryo division by about 85% and also increases blastocyst by about 54% of the dividing cells (Sovernigo et al., 2017). Vitamin C (ascorbic acid) by virtue of its chemical structure is an electron donor and therefore a reducing agent with two different biochemical roles, namely as antioxidant and enzymatic cofactor (Belin et al., 2009; Khazaei and Aghaz, 2017). Due to its antioxidant properties, vitamin C is able to protect cells from ROS (Padayatty et al., 2003).

The rate of cell division with the use of anethole antioxidants was quite high at around 86.2%. It becomes an alternative because anethole has the opportunity to be used as a fairly good antioxidant in supporting embryonic development. Furthermore, supplementation of 30 µg/mL anethole in maturation medium increased blastocyst and decreased ROS concentration (Anjos et al., 2019). Anethone compounds can support glutathione (GSH) synthesis to fight or suppress oxidative and can also simultaneously increase GSH which is considered to be a homocysteine-lowering agent (Giustarini et al., 2014). Do et al. (2017) reported that the utilization of plants extraction containing antioxidants can be done to improve the quality of blastocyst. 0.01 µg/mL of *Phellodendron amurense* extract in the culture medium improved the quality of embryos produced, cell division and blastocyst as well as allows the repair of poor quality embryo (Do et al., 2017).

Melatonin 0.01 µL supplementation in culture media was able to achieve cleavage by 84% and blastocyst by 35% (Do et al., 2017). Exogenous melatonin increases the speed of blastocysts in fresh embryos and vitrification by various mechanisms improves embryonic development (Wang et al., 2014). Other mechanisms may use melatonin to induce changes in the production of antioxidant enzymes and oxidative substrates, as well as regulate gene expression associated with oxidative stress responses. The use of melatonin can be a supporting tool in embryo production in vitro to reduce negative influences during culture (Mehaisen et al., 2015; Truong and Gardner, 2020). Melatonin is a unique antioxidant compared to other antioxidants not only because of its prevalence and the various mechanisms involved in the transmission of signals it carries, but also because of its anti-oxidant activity (Chrustek and Olszewska-Słonina, 2021).

β-mercaptoethanol (β-ME) is an antioxidant that can be added to the culture medium to increase the embryo to reach blastocyst on the eighth day of the culture. Hosseini et al (2009) stated that β-mercaptoethanol (βME) has a low molecular weight thiol compound, and is often used to increase antioxidant capacity in embryos through increased levels of intracellular reactive such as glutathione (GSH) (Takahashi et al., 2002).

Vitamin K2 is known to have antioxidant and anti-inflammatory properties that can act as a powerful protective molecule (Shandilya et al., 2021). Baldoceda-Baldeon et al. (2014) reported that the addition of 0.5 mM of vitamin K2 into the culture medium could increase the production of blastocyst percentage by 8.6% in cattle. Vitamin K2 plays a role of an electron carrier in the mitochondrial electron transport chain complex, resulting in more efficient use of oxygen and ATP production (Vos et al., 2012). Other studies have also reported that vitamin K2 supplementation after geonomics geonomic activation of day 3 and 7 may improve embryo development competence (Sefid et al., 2017).

3,4-dihydroxyflavone (3,4-DHF) is a group of flavonoids reported to have anti-apoptotic and anti-oxidant activity (Hossain et al., 2014). 3,4-DHF 10 µM supplementation in culture media could increase the number of blastocyst about 44% of the dividing cells (Lee et al., 2011). Another study also revealed that 3,4-DHF significantly reduced ROS content and apoptotic cell count as well as increased expression of antioxidant and anti-apoptotic genes to increase yak's in vitro development capacity (Xiong et al., 2014).

Green tea polyphenol (GTP) is a source of polyphenols from green tea that acts as a source of antioxidants and has a molocular mechanism that plays a role in preventing various diseases (Ding et al., 2017). The addition of 15 µM of green tea polyphenol (GTP) in maturation medium efficiently improves the fertilization competence and development of bovine embryos, and this increase correlates with an increase in intracellular GSH concentrations of embryos (Wang et al., 2007). The antioxidant effects of tea polyphenols are also thought to be related to their ability to stimulate antioxidant defense metabolism through redox-regulated transcription factors and protein kinase activated by mitogen cellular cycle regulation (Yan et al., 2020).

Some studies have revealed that non-enzymatic antioxidant supplementation in embryo production in vitro is more effective than enzymatic antioxidants. Enzymatic antioxidants increased the embryo to reach blastocyst by about 50% of the dividing cells while the enzymatic antioxidant was about 30%.

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Antioxidant mechanisms in cells

High level of ROS which exceed the physological range causes cell imbalances and can decrease the survival rate, triggering apoptosis in oocytes (Khazaei and Aghaz, 2017). Antioxidants are defensive factor against Oxidative Species (OS) induced by ROS (Bhattacharyya et al., 2014). During the embryo culture process, the addition of antioxidants to the culture medium can reduce ROS so that the quality of oocytes increases and the apoptotic factor decreases (Khazaei and Aghaz, 2017).

Antioxidants in cells are distinguished into 2 groups, namely enzymatic (primary) and non-enzymatic (secondary) antioxidants. Enzymatic antioxidants are also referred to as antioxidants that work by preventing the formation of new free radical compounds (Zulaikhah, 2017). Enzymatic antioxidants include the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). The mechanism of action of enzymatic antioxidants is to catalyze the extermination of free radicals in cells (Azat Aziz et al., 2019). Chain-breaking antioxidants are small molecules that can receive or give electrons to or from free radical so as to form stable compounds (Lü et al., 2010).

Non-enzymatic antioxidants are also called preventive defense antioxidants that work by cutting or capturing chain oxidation reactions from free radicals so that they will not react (Nimse and Pal, 2015). Non-enzymatic mechanisms consist of: 1) glutathione which is a very important antioxidant and is abundant in the cytoplasm (Lushchak, 2012); 2) bilirubin which is an antioxidant found in the blood (Sedlak et al., 2009); 3) melatonin which is a type of powerful antioxidant (Tarocco et al., 2019); and 4) coenzyme Q which acts as an antioxidant that dissolves inside the fat membrane (Littarru and Tiano, 2007). In addition, vitamin C, β-carotene, flavonoids and albumin are found in plants (Kumar and Pandey, 2013). One of the flavonoid components of plants that can function as antioxidants is a natural coloring agent called anthocyanins (He and Monica Giusti, 2010). Non-enzymatic antioxidants have great value as additives for pharmaceuticals and others (Kumar et al., 2014).

CONCLUSION

Enzymatic and non-enzymatic antioxidant supplementation on the medium increases the number of oocytes that reach metaphase II (MII), cell division and blastocyst. Non-enzymatic antioxidant supplementation is more effective than enzymatic antioxidants in supporting the development of bovine embryos produced in vitro. As a suggestion, antioxidant supplementation in maturation media or culture media with the use of non-enzymatic antioxidants

DECLARATIONS

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Author's contribution

E. Damayanti collected and drafted the manuscript, formatted it, and approved the final manuscript. H. Sonjaya, S. Baco and H. Hasbi approved the final manuscript.

Conflict of interests

The authors declared that they have no conflict of interest.

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QUANTITATIVE AND QUALITATIVE TRAITS CHARACTERISATION OF INDIGENOUS CHICKENS IN SOUTHERN AFRICAN COUNTRIES

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REVIEW ARTICLE

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ABSTRACT: Indigenous poultry production is important to human lives as they alleviate poverty by providing an affordable source of animal proteins. Besides, it serves as the easiest source of income for rural farmers in developing countries. The current review was conducted to evaluate the research findings of indigenous chickens' quantitative and qualitative traits characterisations in Southern African countries. Quantitative and qualitative traits characterisation is the first step for genetic improvement in livestock. The documents used in the review were attained from different search engines, most of which were published lately (after 2010). Potchefstroom Koekoek, Venda, Naked Neck, Ovambo, Boschveld and Tswana indigenous chicken breeds in the Southern African countries were characterised. The studies suggest that there are variations in quantitative traits including live weight, egg weight, wing length, comb length and shank length and qualitative traits including feather color, comb type, comb color and eggshell color of indigenous chickens of Southern African countries. The single red comb types and red wattles were observed in most indigenous chicken breeds in Southern African countries. The present review concludes that the characterisation of quantitative and qualitative traits assists greatly in the differentiation and identification of indigenous chicken breeds.

Keywords: Breed; Characterisation; Chickens; Quantitative traits; Qualitative traits.

INTRODUCTION

Poultry farming is the raising of poultry birds for the production of protein sources such as eggs and meat and it remains one of the vital enterprises among the underprivileged smallholder households in Africa (Ndegwa et al., 2014; Kamau et al., 2018). Indigenous chickens have special significance to the local community (Machete et al., 2021). These chickens have varied morphological characteristics and possess genes with adaptive values to their environment and local diseases (Aklilu et al., 2013). Often, the populations of indigenous chickens are differentiated and classified based on the geographical place or phenotypic features, whereas their categorization into genotypes is limited (Manyelo et al., 2020). In sub-Saharan countries, indigenous chickens generate about 80% of poultry production, with Nigeria being recognized as the country with the highest number of indigenous chickens (Desha et al., 2016).

According to Manyelo et al. (2020), indigenous chickens in Africa are hardy, well adapted to rural areas under scavenging conditions, can sustain themselves and survive with minor or no input of feeds and are disease resistant. According to Liswaniso et al. (2020), indigenous chickens contribute abundantly to the socio-economic status of people in rural communities as a source of good animal proteins for human nutrition and income for the households as well as for cultural practices. Indigenous chickens' meat is highly preferred because of its nutritious pleasant taste over exotic chicken genotypes (Mengesha, 2012).

Poultry products, namely eggs and meat, have been recognized as the finest source of affordable and quality protein, particularly to those people experiencing malnutrition difficulties in Sub-Saharan Africa (Kamau et al., 2018). Mengesha (2012) indicated that shortages in the obtainability of protein are the main concern in Africa. Indigenous chickens have poor production performance in meat and eggs because of their reduced genetic potential (Mengesha and Tsega, 2014). Also, loss of local genetic resources of chickens is experienced because of poor management of the indigenous chicken breeds, which leads to mortalities (Siyaya and Masuku, 2013). Lack of access to the nearby market, extension services, high cost of feeds and lack of education and knowledge of local chicken farmers are issues that might negatively influence the productivity of local chicken production (Natukunda et al., 2011). Numerous studies were done previously to characterize the indigenous chickens based on their phenotypic characteristics (Aklilu et al., 2013; Mengesha and Tsega, 2014; Singh et al., 2014; Reddy et al., 2015; Agarwal et al., 2020; Machete et al., 2021) to improve their production performances.

However, based to our knowledge, there is no documented study combining the information on the quantitative and qualitative traits characterisation of indigenous chickens in Africa.

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Hence, the objective of this review was to document the research findings of indigenous chickens' characterisations, thus, provide information to farmers for breeding to improve indigenous chicken production strategies. Therefore, reviewing the research outcomes will also allow the provision of useful information that will assist in the future planning to advance native chicken production projects of Africa.

METHODS

The current review was conducted in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009). Several research outcomes that have a relation with indigenous chickens in South African countries were reviewed, shown and referenced. Reports of studies that focused on the characterisation of indigenous chickens were reviewed as well. The search was done using search engines such as Google, Yahoo and Baidu and databases such as Google Scholar, Semantic Scholar, Science Direct and PubMed. During the search process, the keywords used were indigenous chickens, chicken characteristics, quantitative and qualitative traits. Studies were considered for inclusion in the review provided, they dealt with indigenous chickens and their phenotypic characterisation. Research outcomes on the genetics of indigenous chickens were also considered for inclusion in some sections of the review.

RESULTS

Origin of Southern African indigenous chickens

Africa comprises numerous indigenous chicken breeds, which have a less productivity of egg and meat (Sil et al., 2002). Indigenous chickens are recognised as a gene pool for significant traits including adaptability, hardiness and disease resistance (Alabi et al., 2012). According to Grobbelaar et al. (2010), their different carcass color, the toughness of the muscle meat, shell color and yolk color are preferred by most consumers. The indigenous chicken breeds common in the Southern African region include Potchefstroom koekoek, Venda, Ovambo, Naked neck and Boschveld (Mtileni et al., 2012; Idowu et al., 2018). Another African local breed includes Tswana chicken.

Potchefstroom Koekoek chicken breed

Potchefstroom Koekoek (Figure 1) is an indigenous chicken breed that was produced in the 1950s in Potchefstroom Agricultural College by a researcher, Marais, and it is a crossbred of White Leghorn, Black Australorp and barred Plymouth (Grobbelaar et al., 2010). The Koekoek name refers to the barred color pattern of the chicken. Potchefstroom Koekoek is a dual-purpose chicken breed reared for meat and egg production; it is highly adapted to free-range conditions (Dessie et al., 2011). It performs better than all the other indigenous chickens in South Africa (Heit, 2017).

Figure 1 - Potchefstroom Koekoek chicken breed

Figure 2 - Venda chicken breed

Venda chicken breed

According to Norris and Ng'ambi (2006), the Venda chicken breed was discovered in Venda in the Limpopo province, South Africa, by the veterinarian, Dr Naas Coetzee, in 1979. The breed was named after its origin. Venda chicken breed (Figure 2) is multicolored with predominating colors as black, white and red. The breed is used for egg and meat production; the hens have good mothering ability and broodiness. They can survive well in harsh environments with minimum additional feed and are highly disease resistant (Ng'ambi et al., 2013).

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Naked Neck chicken breed

The Naked Neck chicken (Figure 3) is an African breed believed to have originated in Malaysia (Mtileni et al., 2011). Naked Neck chickens are divided into two types namely, purebred which has a fully naked neck and the one with a tassel on the front part of the neck, which is not purebred (Kingori et al., 2014). The chicken has a diversity of color designs, which help camouflage to protect them from ground predators (Amao, 2017). Naked Neck chickens can sustain themselves through scavenging for food and are well adapted to adverse conditions (Manyelo et al., 2020).

Ovambo chicken breed

Ovambo is a small chicken breed that originated in the northern part of Namibia and Ovamboland in Africa. It is a dual-purpose breed reared for meat and egg production (Grobbelaar and Fourie, 2006). Ovambo breed (Figure 4) is considered as being aggressive because of its ability to capture and feed on young rats and mice. They can fly and climb on top of the trees to run away from predators at night and they can also survive in harsh environments and sustain themselves (Grobbelaar et al., 2010).

Figure 3 - Naked Neck chicken breed

Figure 4 - Ovambo chicken breed

Boschveld chicken breed

According to Bosch (2011), the Boschveld chicken breed (Figure 5) is a crossbred of Venda, Ovambo and Matebele breeds. It was developed in Mantsole ranch in Limpopo province, South Africa, in 1998. The breed can perform well under free-range system and in scavenging situations for survival; it also possesses disease-resistant traits. The hens are good brooders and have good mothering ability. It is reported that 50% of the traits were inherited from the Venda breed by Boschveld chicken, 25% from the Ovambo breed and 25% from the Matebele breed (Manyelo et al., 2020). Boschveld chicken is a dual-purpose breed mainly bred for meat and egg production (Okoro et al., 2017).

Tswana chicken breed

Tswana chicken (Figure 6) is an indigenous breed that originated in Botswana and is the most widely spread domestic animal, which almost every rural family owns in Botswana. The Tswana chickens contribute more to rural households by providing meat and eggs for human consumption (Machete et al., 2021). They are categorised into five strains namely dwarf, naked neck, frizzled, rumples and normal, with the most common strain being the normal (Machete et al., 2017). Tswana chickens have good self-sustainment, and they are usually reared under an extensive farming system where they scavenge for feed and feed on insects with little or no feed supplementation (Badubi et al., 2006). These indigenous chickens can produce meat and eggs with minimal management (Magpantay et al., 2016).

Figure 5 - Boschveld chicken breed

Figure 6 - Tswana chicken breed

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Table 1 - Quantitative traits of Southern African indigenous chicken breeds

Chicken breed	LW (kg)	EW (g)	WL (cm)	CL (cm)	BL (cm)	SL (cm)	References
Potchefstroom Koekoek	4.50	55.80	5.50	5.90	43.33	29.33	(Alabi et al., 2012; Makhafola et al., 2012; Dessie and Gatachew, 2016)
Venda	3.6	53.00	21.00	5.80	45.50	30.00	(Alabi et al., 2012; Ng'ambi et al., 2013)
Naked Neck	3.50	55.50	21.50	5.40	45.00	32.50	(Alabi et al., 2012; Makhafola et al., 2012)
Ovambo	2.16	52.50	14.50	5.80	24.50	8.30	(Bett et al., 2013; Makhafola et al., 2012; Mngonyama, 2012)
Boschveld	2.60	53.40	20.00	5.32	23.70	9.79	(Dessie et al., 2011; Petrus et al., 2019)
Tswana	2.56	49.95	20.61	6.30	22.40	9.94	(Kgwatalala et al., 2012; Kgwatalala et al., 2013; Machete et al., 2017)

LW: Live weight, AEW: Egg weight, WL: Wing length, CL: Comb length, BL: Body length and SL: Shank length

Table 2 - Qualitative traits of Southern African indigenous chicken breeds

Chicken breed	Feather color	Comb type	Comb color	Egg shell color	References
Potchefstroom Koekoek	Black and white	Single	Red	Brown	(Magothe et al., 2012; Heit, 2017)
Venda	Black, white and black	Single	Red	Tinted	(Ng'ambi et al., 2013)
Naked neck	Red, blue, buff, white and black	Single	Red	Light brown	(Grobbelaar et al., 2010; Amao, 2017)
Ovambo	Black, white and orange	Single	Red	Brown	(Grobbelaar et al., 2010)
Boschveld	Light red-brown and white	Single	Red	Brown	(Bosch, 2011)
Tswana	Black, brown, grey and white	Single	Red	Cream white-light brown	(Machete et al., 2021; Kgwatalala et al., 2013)

Quantitative characterisation of Southern African indigenous chickens

The quantitative characterisation of the selected Southern African indigenous chickens is presented in Table 1.

Potchefstroom Koekoek chicken breed

Potchefstroom Koekoek breed is heavy, the roosters weigh about 1.84 kg at 16 weeks of age, 2.4 kg at 20 weeks and about 3.5 kg to 4.5 kg at maturity while hens can weigh around 1.4 kg at 16 weeks, 1.7 kg at 20 weeks and 3.5 kg at maturity (Dessie and Gatachew, 2016). The sexual maturity of Potchefstroom Koekoek is at 130 days, with the hens being able to lay an average of 198 eggs per annum and the eggs are large, with an average weight of 55.78 grams (Mtileni et al., 2012). Tyasi et al. (2020) reported that the average body measurements of Potchefstroom Koekoek chicken hens at 22 weeks for wing length, back length, beak length, shank length, shank circumference, chest circumference, keel length, body girth, toe length and body length were noted as 4.70, 0.49, 0.22, 8.17, 4.69, 26.56, 11.22, 36.83, 6.28 and 33.15 cm, respectively. Alabi et al. (2012) reported that male chickens grow faster than females. The average body measurements of male Potchefstroom Koekoek chickens at 22 weeks of age for body length, body girth, wing length and shank length were noted as 43.33, 19.67, 5.50 and 29.33 cm, respectively. The comb length and wattle length measurements were found to be 5.9 and 5.6 cm, respectively (Makhafola et al., 2012).

Venda chicken breed

Venda chicken is a fairly large breed, at 16 weeks of age, the roosters are weighing about 1.57 kg, 2.01 kg at 20 weeks and when they reach maturity, they weigh about 2.9 kg to 3.6 kg. At 16 weeks of age, the hens are weighing about 1.24 kg, 1.4 kg at 20 weeks and when they mature, they weigh about 2.4 kg to 3 kg (Ng'ambi et al., 2013). The Venda chicken hen reaches maturity at 143 days and can lay large eggs of roughly 129 eggs per annum and average egg weight of 53 g (Grobbelaar et al., 2010). The average body measurements of male Venda chickens at 22 weeks of age for body length, body girth, wing length, comb length, shank length and shank thickness were found to be 45.50, 42.50, 21.00, 5.8, 30.00 and 4.75 cm, respectively. The females were found to measure 38.40, 35.20, 18.00, 25.60 and 4.05 for body length, body girth, wing length, shank length and shank thickness, respectively (Alabi et al., 2012).

Naked Neck chicken breed

Naked Neck chicken is categorized as a massive breed. At 16 weeks of age, the roosters weigh about 1.5 kg, 1.95 kg at 20 weeks and at maturity, they weigh about 3.5 kg. At 16 weeks of age, the hens weigh around 1.1 kg, 1.4 kg at 20 weeks and 3 kg at maturity. The chicken breed reaches sexual maturity at 155 days and yields eggs with an average weight of 55.5 g. Alabi et al. (2012) reported that the average body measurements of male Naked neck chickens at 22 weeks of age for body length, body girth, wing length, shank length and shank thickness were found to be 45.00, 41.00,

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21.50, 32.50 and 5.50 cm, respectively. The female chicken's body measurements were found to be 38.90, 35.30, 17.20, 25.70 and 4.00 cm for body length, body girth, wing length, shank length and shank thickness, respectively. The comb length and wattle length measurements were found to be 5.4 and 5.6 cm, respectively (Makhafola et al., 2012).

Ovambo chicken breed

Ovambo chicken is a lightweight breed, at 16 weeks of age, the roosters weigh about 1.74 kg and 2.16 kg at 20 weeks. At 16 weeks, the hens weigh about 1.32 kg and 1.54 kg at 20 weeks. The birds reach sexual maturity 143 days old. The hens yield 129 eggs per annum, with an average weight of 52.5 g per egg (Bett et al., 2013). Makhafola et al. (2012) found that the shank length, comb length and wattle length average measurements at maturity were 8.3, 5.8 and 6.0 cm, respectively. The measurements of head length, head width, comb height, beak length, body length, neck length, wing length and keel length at maturity were noted as 5.08, 3.51, 3.82, 1.75, 24.5, 1.79, 14.5 and 14.4 cm, respectively (Mngonyama, 2012).

Boschveld chicken breed

According to Dessie et al. (2011), the Boschveld chicken breed is large. At 12 weeks of age, the roosters weigh about 1.6 kg and 2.6 kg at 20 weeks while at 12 weeks, the hens weigh 1.5 kg and 1.7 kg at 20 weeks. Sexual maturity is reached at 136 days of age by the birds. Each hen produces about 4 eggs in a week and an average of 200 eggs in a year, with an average egg weight of 53.4 g. According to Petrus et al. (2019), Boschveld chicken hens average body measurements at 18 weeks of age for shank length, wing length, body length, chest girth, beak length, comb length, neck length and keel length were found to be 9.79, 20.00, 23.70, 30.40, 3.78, 5.32, 18.70 and 11.64 cm, respectively.

Tswana chicken breed

Tswana indigenous chicken is a large breed with males weighing an average live weight of 2.56 kg and females weighing 2.02 kg at maturity (Machete et al., 2017). The average body measurements of male Tswana chickens are higher than of females, which indicate that males grow faster than females (Kgwatalala et al., 2012). In males, the shank length, shank circumference, spur length, wingspan, wing length, body length, comb length and wattle length measures 9.94, 0.99, 1.19, 41.22, 20.61, 22.40, 6.30 and 3.44cm, respectively. In females, the shank length, shank circumference, spur length, wingspan, wing length, body length, comb length and wattle length measures 8.35, 0.84, 0.35, 37.19, 18.60, 20.91, 3.48 and 2.40 cm, respectively (Machete et al., 2017). The hens can lay between 180 and 200 eggs in a year with an average egg weight of 49.95 g, egg length of 5.68 cm and egg width of 4.23 g (Kgwatalala et al., 2013).

Qualitative characterisation of Southern African indigenous chickens

The qualitative characterisation of the selected Southern African indigenous chickens is presented in Table 2.

Potchefstroom Koekoek chicken breed

The Potchefstroom Koekoek is characterised by its black-and-white striped soft feathers and yellow legs as shown in Figure 1 and it is also having yellow attractive skin color (Magothe et al., 2012). The wattle is red colored, large in cocks and small in hens. This chicken breed lays brown shelled eggs with rich yellow to orange yolk colors and it is also having a single comb type, which is red (Heit, 2017).

Venda chicken breed

Venda chicken breed has multi-colored soft feathers with black, white and red as predominating colors. The chicken breed is having a single rose-colored comb type, which is red and five-toed feet, beards or crests are common. Venda chickens lay large and tinted eggs (Ng'ambi et al., 2013). The cocks have a large red wattle when compared to hens with small-sized red wattle (Grobbelaar and Fourie, 2006).

Naked Neck chicken breed

The Naked neck chicken breed common feather colors are known as red, blue, buff, white and black. The chicken breed has about 30% fewer feathers than other chicken breeds and they lay a respectable number of eggs with light brown shells (Grobbelaar et al., 2010). Naked neck chickens carry a large single comb and the skinned neck, earlobes and the heads are dazzling red. The neck of this chicken breed is featherless to the upper breast; the wattle is large with a red coloration and these chickens have large, four-toed feet and reddish to bay-colored eyes (Amao, 2017). This chicken breed has a plain head, highly developed wattles in males and medium-size wattles in females (Mammo et al., 2008).

Ovambo chicken breed

The Ovambo chicken breed has feathers of a dark to black color, with stripes of white and or orange. The varying color patterns help them to camouflage for protection from their predators (Grobbelaar et al., 2010). The chicken breed has a single red colored comb, which is large in cocks when compared to hens as shown in Figure 4 above. The cocks have highly developed hanging red wattle while the hens are having a small-sized red wattle (Van Marle-Köster and Casey, 2001).

Boschveld chicken breed

The Boschveld chicken has a light red-brown color with white feathers in-between that assists to camouflage and defend them from predators. The hens can lay medium brown shelled eggs and they have a single comb type, which is red colored (Bosch, 2011).

Tswana chicken breed

Tswana chickens have varying plumage colors with normal chicken strains being most common with varying plumage color frequencies. The body has black, brown, grey and white colors with the tail having 60.5% black, 23.3%

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brown, 11.5% grey and 4.7% white plumage colors. The breast plumage has 44.2% brown, 41.9% black, 7.0% grey and 4.7% white colors, the back plumage has 46.5% brown, 37.2% black, 9.3% grey and 4.7 white and neck has 22% brown, 15% black, 4% white and 3.1% grey plumage colors (Figure 6). The normal Tswana chicken has a single comb type, plain head shape and red earlobes color. The breed has 67.4% featherless shank and 36.4% feathered shank and 55.8% blue, 34.9% khaki, 7.0% yellow and 2.3% grey shank color (Machete et al., 2021). The hens can lay cream white to light brown eggs (Kgwatalala et al., 2013).

CONCLUSION

This review has provided information on the characterisation and importance of local chickens in African livelihoods. The review focused on local chicken breeds in the Southern African region. Only fewer indigenous chickens have been classified into breeds. Often, they are differentiated and classified based on the geographical area or phenotypic features. The discoveries showed that there is variation in the feather colors, live weights and morphological traits measurements of the local chickens. The characterisation of quantitative and qualitative traits plays an important part in the differentiation and identification of local chicken breeds. The quantitative traits also assist in the understanding of growth patterns and egg production performance of the chickens. However, not much work was done on the qualitative traits characterisation of local chickens in the Southern African countries, hence, more studies need to be done on the characterisation of local chickens based on their qualitative traits.

DECLARATIONS

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Authors' contribution

V.R. Hlokoe performed conceptualization, writing, original draft preparation, review and editing. T.L. Tyasi performed conceptualization and evaluation of the manuscript before submission.

Conflict of interests

The authors declare that there is no conflict of interest.

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NUTRITIONAL EVALUATION OF *Pentas schimperiana* AS LIVESTOCK FEED AND POTENTIAL PROTEIN SUPPLEMENT

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ABSTRACT: *Pentas schimperiana* is locally available and dominantly used as a dairy cattle feed in the Dawuro zone's Maraka district, Ethiopia. There is some information about its utilization practice, however, data on its nutritional benefit is limited. This research aimed to examine the chemical content and digestibility of *P. schimperiana* for use as cattle feed during the dry season. For this study, a 2×2 factorial design with two agro-ecologies (midland and highland) and two seasons (wet and dry) was used for this study. *P. schimperiana* leaves, twigs and stems were gathered and processed for laboratory analysis from two agro-ecologies and two seasons. The chemical composition, in vitro digestibility, and in sacco degradability of the samples were determined after incubation at 0, 6, 12, 24, 48, 72, and 96 hours. Effective degradability (ED) and Potential degradation (PD) were computed. The collected data were subjected to an Analysis of Variance and the means that had significant deference, the mean separation was performed by Tukey with alpha level of 0.05. In vitro dry matter digestibility (IVDMD) (87.41%) and crude protein (CP) (17.39%) were significantly higher in highland. Digestible crude protein (DCP) was significantly larger in dry than in the wet season with the content of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) decreased. During the wet season, potential degradability (PD) and effective degradability (ED) of DM were highest in the highland with a decrease in leg time, which was related to the low content of ADF, ADL, NDF, tannin, and high CP in vegetative stages of *P. schimperiana.* The result indicates that *P. schimperiana* might be used as supplementary feeds to enhance utilization of low-quality feed resources and improve the performance of ruminants during the dry season when feed is scarce.

Keywords: Chemical composition, Degradability, Digestibility, *Pentas schimperiana,* Tannin.

INTRODUCTION

Ethiopia is well-known for its abundant and diverse livestock resources with 70.29 million cattle, 42.91 million sheep, 52.46 million goats (CSA, 2021). However, because livestock contributes more to rural households' livelihoods, the benefit we derive from the resource is negligible and not compromised by the livestock's potential (Asfaw et al., 2011; Kebebe, 2019). The major ruminant livestock feed supplies in Ethiopia are green fodder from natural pasture (grazing) and crop residue with low nutritional value (Alemayehu et al., 2017; CSA, 2020). Feed scarcity, particularly during the dry season, severely limits animal production in most of Ethiopia. In many parts of the country, available feed resources are insufficient to meet the nutritional needs of animals throughout the year, either due to a lack of supply or due to poor feed quality (Alemayehu et al., 2017). Under Ethiopian conditions, the use of indigenous forages as a feed resource is appealing in order to increase livestock production and productivity (Shapiro et al., 2015). Indigenous fodder trees could be considered as reliable feed resource to develop sustainable livestock feeding system due to their ability of remaining green for a longer period (Aganga and Tshwenyane 2003). Using locally available indigenous fodder species, on the other hand, increases livestock production and productivity while also increasing the livestock producer's income.

Indigenous fodder species in the study area are potentially cheap feed resources for livestock and are highly valued by farmers, especially during the dry season of the year when feed quantity and quality are limited. *Pentas schimperiana*, named after Georg Heinrich Wilhelm Schimper, who collected primarily in Ethiopia and is known locally as *Dawuro-daama*, is one of the locally available livestock feeds in the Maraka districts of the Dawuro zone. It is a woody herb up to 2.5 m tall, with mostly oblong twinkle broad leaf. It stayed green throughout the year. *Pentas schimperiana* is locally known indigenous fodder, which provides valuable benefits such as a source of traditional medicine, and as livestock feeds especially during the dry season (Woretaw et al., 2022). Local people provide the leaf and stem chopped and mixed with salt and water and given to drink mainly for milking cows and fattening cattle. The leaves and twigs of *P. schimperiana* were preferred by the various cattle groups in terms of palatability, according to Woretaw et al. (2022). According to the same authors, *P. schimperiana* was applied to cows during milking to boost milk yield and both the quality and amount of butter. As a result, it was given the name "milk feed," which improved the milk's quality and flavor. The leaf and stem were chopped, ground, and mixed with salt and water to make a drink primarily for milking cows and fattening cattle (Andualem et al., 2015).

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However, limited researches on the nutritional benefits of this locally accessible cattle feed has been undertaken. Therefore, the purpose of this study was to determine the chemical composition, degradability and digestibility of *P. schimperiana.*

MATERIAL AND METHODS

The study area

The study was carried out in Maraka districts of Dawuro zone, Southern Nation Nationalities and People Region (SNNPR), Ethiopia. The area is located 544 kilometers southwest of Addis Ababa and 17 kilometers from the zonal city of Tarcha. It is located between 6°56'00" to 7°04'00"N and 37°02'00" to 37°16'00"E, at an elevation of 1000-2400 m above sea level. The predominant macroclimatic conditions included temperatures ranging from 15.10°C to 27.50°C and rainfall ranging from 1314 to 1516 mm. It is classified into three agro-ecological zones based on the agro-ecological categorization criteria: highland (>2300 m.a.s.l), midland (1500 – 2300 m.a.s.l), and lowland (500-1500 m.a.s.l), with total coverage of 41.77, 50 and 8.23 percent, respectively (MoA, 2000). Since *P. schimperiana* is growing in high- and midland altitudes the lowland was not considered in this study (Woretaw et al., 2022).

Ethical consideration

The experimental animals were treated or managed according to an animal experimental protocol approved by the Ethics Commission of the Wolaita Sodo University reference number WSU/41/14/1023.

Sample collection and preparation

Pentas schimperiana samples were collected, labeled, and dried in each of the two major seasons, wet (May to September) and dry season (November to March) from two (midland and highland) agroecological zones of the study area. The samples were partly dried at 60°C for 72 hours and ground by Thomas Willy mill (model 4) to pass through 1 mm sieve for chemical analysis and in vitro digestibility and 2 mm sieve size for in sacco degradability study. Ground samples were kept in sealed plastic containers for further chemical and digestibility testing.

Chemical analysis

To estimate dry matter (DM), ash, and crude protein (CP), the AOAC (1990) proximate analytical methodologies were employed. Using the Kjeldahl method, the nitrogen (N) levels were measured, and the CP value was computed by multiplying the N concentration in the feed by 6.25. The AOAC (2000) technique was used to determine ether extract (EE). The neutral detergent fiber (NDF) contents were determined using Van Soest et al. (1991), while the acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed by Van Soest and Robertson (1985).

In vitro dry matter digestibility

To determine the in vitro dry matter digestibility (IVDMD), the Tilley and Terry (1963) technique, as modified by Van Soest et al. (1991), was employed. A 0.5 g of feed was incubated at 39°C in 125 ml flasks containing rumen fluid-medium, which was collected from rumen-fistulated sheep. The sheep were fed Rhodes grass hay at *ad libitum* and 400 g concentrate mix daily in two equal parts.

In sacco degradability

About 2.5 g of feed sample was put in nylon bags and incubated in the rumens of three rumen-fistulated sheep to investigate the in sacco DM degradability characteristics. The sheep were fed as indicated above. The feed samples were incubated for 0, 6, 12, 24, 48, 72, and 96 hours in three ruminal fistulated steers fed a maintenance diet. Following the incubation period, the nylon bags were removed, rinsed with running tap water, and oven-dried for 72 hours at 60°C. The protocols for zero-hour incubation losses were obtained by washing and drying duplicate bags with samples which were not incubated in the rumen. The following formula was used to calculate the percentage disappearance of DM and CP:

$$\text{Dry matter degradability (DMD)} = \frac{(\text{DM in sample} - \text{DM in residues})}{\text{DM in sample}} * 100$$

$$\text{Crude Protein degradability (CPD)} = \frac{\text{CP in sample} - \text{CP in residues}}{\text{CP in sample}} * 100$$

Effective degradability (ED) of DM was calculated following the method of Ørskov and McDonald (1979). Estimation of protein degradability in the rumen based on incubation measurements weighted by passage rates. $Y = a+b\ (1-e^{-ct})$, where: P = the potential disappearance of DM at time t, a = rapidly degradable fraction, b = the potentially, but a slowly degradable fraction, c = the rate of degradation of b, e = the natural logarithm, t = time. Effective degradability (ED) of DM was computed in accordance with Ørskov et al (1988) using the formula ED = A+ [Bc/(c+k)] at rumen outflow rates (k) of 0.04/h. Potential degradation (PD) was calculated as (A+B).

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Tannin determination

The condensed tannin was determined by using 70% aqueous acetone and Butanol-HCl procedures and expressed as leucocyanidin equivalent (% of DM) (Makkar et al., 2007). Absorbance was measured spectrophotometrically at 550 nm. The concentrations of condensed tannins were calculated from tannic standard curve by the formula:

$$\frac{\text{Absorbance at 550 nm} \times 78.26 \times \text{Dilution factor}}{\%\ \text{DM}}$$

The dilution factor was equal to 1 if no 70% acetone was added or 0.5 ml per volume of the extract was taken (Porter et al., 1986). Digestible crude protein was estimated from crude protein (Church, 1982): DCP = 0.929*CP (g) - 3.48.

Statistical analysis

All chemical composition, degradability and digestibility data were subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS version 9.4 (2013) in a 2×2 factorial design with two agro-ecologies (midland and highland) and two seasons (wet and dry season). For the means had a significant difference, mean separation was performed by Tukey with an alpha level of 0.05.

The statistical model used was:

$Y_{ijk} = \mu + S_i + A_j + SA_{(ij)} + e_{ijk}$

Where: Y_{ijk} = the response of the parameter under investigation, DM, OM, Ash, CP, CF, EE, NDF, ADF and ADL, IVDMD, DCP, DE and tannin

μ = Overall mean

S_i = the effect of i^{th} agro-ecology (I = Midland, Highland)

A_j = the effect of j^{th} study season (j = Wet, Dry)

SA_{ij} = the interaction effect of agroecology and season.

e_{ijk} = random error

RESULTS AND DISCUSSIONS

Chemical composition

Table 1 shows the influence of season and agroecology on the chemical compositions of *P. schimperiana*. The dry season had a greater (P<0.05) DM content. The findings of Gaiballa (2012) who studied on 43 tree and shrub species which useful browse source for livestock were comparable to our result. However, Babayemi and Bamikole (2006) reported that advancing in age or higher temperatures in the dry season resulted in nutrient depletion in the soil due to weather and continual usage by season. The ash content was lower (P<0.05) during the dry season, which might be attributed to the poor translocation of the root system and the lignification process during the dry season. The ash concentration (1.29-5.79%) was lower than that of previously reported on fodder browse species (6-20.5%) and (4.62% and 16.22%) by Abdullah et al. (2014) and Geng et al. (2020), respectively.

Table 1 - Chemical composition (as DM basis) of *P. schimperiana*

Items	Season	Midland	Highland	SEM	Agro.	Season.	Agro.*Season.
DM	Dry	51.01[a]	42.44[b]	2.56			
	Wet	47.36[a]	40.27[b]	2.05	0.001	0.026	0.43
OM	Dry	98.71[a]	94.27[b]	1.28			
	Wet	97.33[a]	94.27[b]	0.96	0.0001	0.002	0.003
Ash	Dry	1.29[b]	5.73[a]	1.28			
	Wet	2.47[b]	5.79[a]	0.96	0.0001	0.002	0.003
CP	Dry	11.56[b]	15.40[a]	1.11			
	Wet	12.4[b]	17.39[a]	1.44	0.001	0.0001	0.006
EE	Dry	3.08	3.12	0.06			
	Wet	3.34	3.19	0.58	0.574	0.132	0.329
ADF	Dry	16.60[a]	9.14[b]	2.16			
	Wet	13.89[a]	8.20[b]	11.64	0.0001	0.005	0.52
ADL	Dry	11.56[a]	7.35[b]	1.22			
	Wet	10.06[a]	6.10[b]	1.14	0.0001	0.003	0.607
NDF	Dry	25.35[a]	17.06[b]	2.44			
	Wet	22.23[a]	14.94[b]	2.13	0.0001	0.011	0.479

[a, b,] rows means with different superscripts are significantly different (P<0.05); DM=Dry matter; OM=Organic matter; CP=Crude protein; NDF is neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; SEM=Standard error of the mean; Agro. = Agroecology effect (P<0.05), Season. = Season effect (P<0.05); Agro.×season. = Agroecology×Season effect (P<0.05).

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Significant interaction (P<0.05) was recorded in CP values between agroecology and seasons. The observed CP values of *P. schimperina* were ranging from 11.56% to 17.39% in midland-dry season and highland-wet season, respectively. The values were within the range reported by Shenkute et al. (2012) for browse species, which ranged from 8.9 to 20.9 percent. Similarly, Liman et al. (2016) reported in the range of 6.57% and 28.13% for *Boswellia dalzelia* and *Newbouldia laevis,* respectively in Ardo-Kola, Jalingo and Lau local government of Taraba State, Nigeria. The crude protein content (CP) of the browse species was higher in the wet season except *Acacia seyal* which had higher value in the dry season (210 g/kg DM). The lower CP content of browse species harvested during the dry season as compared to the wet season may be caused by the plant's advancing age, which is associated with leaf maturity, the soil's lower moisture content and availability of less nitrogen, and the higher proportion of the fiber fraction (Belachew et al 2013). The present value varied from 11.6 percent to 17.4 percent, which was higher than the ruminant's maintenance protein requirement as the minimum average CP for a rumen microbe's requirement is 7% (NRC, 2001). The high CP in *P. schimperiana* implies its potential for a dry season protein supplement to ruminants fed on low-quality hay and crop residues.

In the current study NDF concentrations were consistent with Singh and Oosting (1992) finding who stated that roughage diets with less than 45 percent NDF content were classified as high-quality feed. During the dry season, the midland had considerably (P<0.05) greater NDF levels than the highland. The moderate temperatures and high precipitation tend to decrease the cell wall fraction of the plants (Boufennara et al., 2012). The midland (16.60 percent) had a greater (P<0.05) ADF content during the dry season than the highland (8.20 percent) during the rainy season. ADF values were lower than those reported by Liman et al. (2013) for browse species which had a range of (19.25-28.98%). ADF content plays a significant role in voluntary feed intake and digestibility. ADL contents in the current study were comparable with the results of Mtui et al. (2009) for browse species, which ranged from 3.3 to 11.0%. However, Babayemi and Bamikole (2006) reported that advancing in age or higher temperatures in the dry season resulted in nutrient depletion in the soil due to weather and continual usage by season. The observed disparities in fiber fractions of NDF, ADF, and ADL in dry and wet seasons might be due to increased solar radiation intensity and less rainfall during the dry season, resulting in quicker maturation and higher cell wall contents (Evitayani et al., 2004). The influence of genotype on nutrient intake, soil qualities that give nutrients to plants, growth stage, plant maturity, and the proportion of different browsed components in harvested samples (Upreti and Shrestha, 2006).

In vitro dry matter and crude protein digestibility

During the wet season, the in vitro dry matter digestibility (IVDMD) value was greater (P<0.05; Table 2). The low ADF, ADL, NDF, and high CP might explain the high IVDMD values during the wet season. The current variations of IVDMD may be related to the effect of growth stage, plant maturity, and the fraction of tree components chosen for chemical composition analysis (Mtengeti and Mhelela, 2006). Significant (P<0.05) interaction effect was recorded in DCP and IVDMD values between agroecology and seasons. In the wet season, the DCP values ranged from 8.0 to 12.64%, which was significantly higher (P<0.05) in highland with the highest value in wet season. The values in the current study were slightly lower than those reported by Ahamefule et al. (2006) among browse plants; the highest DCP value recorded in *Napoleona vogelii* was 13.65%. Ahamefule et al. (2006) reported feedstuffs containing greater than 7% CP to have good digestibility. Therefore, *P. schimperiana* has better digestibility, as DCP ranged from 3.24 to 14.98% reported by Liman et al. (2016) for 20 browse fodder species. DCP was significantly (P<0.05) higher in the highland-wet season as compared to the midland-dry season as the ADF, ADL, and NDF contents decreased. The moderate temperatures and high precipitation in the study area tend to decrease cell wall fraction (Boufennara et al., 2012). The significant (P<0.05) interaction result of IVDMD was observed between agroecology and season. The value was ranged from 65.31 to 87.41% in midland-dry season and highland-wet season, respectively. The observed results are consistent with those reported by Nasrullah et al. (2003) and Evitayani et al. (2004) and lower than the results reported by Aster et al. (2012), the IVDMD values among the 10 browse species in Borana rangeland, Ethiopia varied from 96.57 to 71.89% and 97.47 to 67.63% in the dry and rainy seasons, respectively. The differences in IVDMD might be attributed to the seasonal variation during harvesting and selection of plant parts that improved the nutrient contents and its rumen degradability (Kassahun et al., 2016). Lignification interferes with the digestion of cell-wall polysaccharides by acting as a physical barrier to microbial enzymes (Moore and Jung, 2001).

Digestible energy and tannin

In the wet season, DE ranged from 1.41 to 3.14 MCal/kg. A higher DE (P<0.05) was recorded in midland (Table 2). DE was significantly (P<0.05) lower in the wet season and this might contribute to the decrement of the energy source of fiber content (Gaiballa, 2012). Lignification controls the amount of fiber that can be digested and, hence has a direct and frequently significant influence on the forage's digestible energy (DE) value of the forage (Jung and Allen 1995; Moore and Jubg, 2001). Worknesh and Getachew (2018) showed that feeds containing DM more than 2.87 and DE less than 2.15 MCal/kg are categorized as high and low energy feeds, respectively.

The condensed tannin (CT) value in the current study (0.14 – 0.17%) was in the lower range of the value reported by Aster et al. (2012) ranged from 0.12 to 33.2% DM in *Balanites aegyptiaca* and *Acacia tortilis*, respectively in Borana rangeland, Ethiopia. However, the results indicated that the tannin contents (0.14 - 0.17%) were lower than the level of tannin that adversely affects digestibility in ruminants (2 and 5%) (Gidado et al., 2013). However, the combined effect of both agroecology and season didn't bring a significant difference (P<0.05) in tannin content.

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Table 2 - In vitro dry matter and crude protein digestibility, digestible energy and condensed tannin

Items	Season	Midland	Highland	SEM	Agro.	Season.	Agro.*Season.
IVDMD (%)	Dry	65.31[b]	83.34[a]	5.78			
	Wet	68.21[b]	87.41[a]	5.54	0.01	0.0001	0.015
DCP (%)	Dry	7.22[b]	10.79[a]	1.03			
	Wet	8.0[b]	12.64[a]	1.33	0.01	0.0001	0.006
CT (%)	Dry	0.17[a]	0.15[b]	0.01			
	Wet	0.17[a]	0.14[b]	0.01	0.01	0.008	0.73
DE (MCal/kg)	Dry	3.14[a]	1.71[b]	0.49			
	Wet	3.03[a]	1.41[b]	0.47	0.03	0.028	0.71

[a, b,] rows means with different superscripts are significantly different (P<0.05); IVDMD= in vitro dry matter digestibility; DCP=Digestible crude protein; ME=Metabolic energy; CT=condensed tannin; SEM=Standard error of the mean; Agro.=Agroecology effect (P<0.05); Season.=Season effect (P<0.05); Agro.×season.= Agroecology×Season effect (P<0.05).

In sacco dry matter degradability of *P. schimperiana*

The dry matter degradability was higher (P<0.05) in highland than in midland during all incubation hours (Table 3). There were significant differences (P<0.05) at 6, 12, 24, and 48 hours incubation periods during both the dry and wet seasons in midland and highland agroecology. The differences in dry matter degradability between dry and wet seasons suggested that the high intensity of solar radiation and lower amount of rainfall during the dry season caused faster maturation, resulting in higher cell wall contents and lower cell contents than those of the rainy season, which was consistent with the findings of Evitayani et al. (2004) on the nutritive value of tropical forages. The degradability percentage of *P. schimperiana* was above 40% at 24 h of incubation implying its potential utilization for feeding ruminants based on the recommendations of FAO (1986) which suggested a minimum of 40 to 50% degradation at 48 hours in the rumen Malgwi and Mohammed (2015). The DMD values of browse species were higher in wet season than in the dry seasons and increased with increasing incubation periods in all browse species in the semi-arid Rift Valley areas of Ethiopia (Ahmed et al., 2017). Njidda (2011) reported that dry matter disappearance of browse plants increased with increased incubation time.

The DM disappearance rate of *P. schimperiana* was higher (P<0.05) during the wet season might be attributed to the lower in fiber content that the dry season. Aster et al. (2012) reported that the DM disappearance rates of browse species were higher in the wet season due to seasonal differences in accumulation of structural component differences in the stage of maturity and possibly wider climate and edaphic differences. Dry matter degradability is influenced by the nutrient contents of the plant in turn nutrient contents are influenced by the harvesting season, soil fertility, the amount of rainfall, and temperature (Kassahun et al., 2016). Aster et al. (2012) found that the DM disappearance rates of browsing species were greater during the rainy season due to seasonal changes in structural component accumulation, differences in the maturity stage, and perhaps larger climatic and edaphic differences. The nutrient content of the plant influences dry matter degradability, and nutrient content is determined by the harvesting season, soil fertility, quantity of rainfall, and temperature (Kassahun et al., 2016).

A rapidly degradable fraction of *P. schimperiana* was recorded in the highland (24.75%) compared to that of the midland (20.27%) during the wet and dry seasons, respectively (Table 4). A rapidly degradable fraction in highland is related to the low level of lignification in browse species (Liman et al., 2016). The slow degradable fraction in the midland might be the higher content of cell wall components of *P. schimperiana* than in the highland. Significantly lower (P<0.05) rates of degradation were observed in highland (0.03 fraction/h) and higher (P<0.05) in midland (0.044 fraction/h) in the wet and dry seasons, respectively. This study indicated that *P. schimperiana* had higher in sacco dry matter degradability across all the degradability parameters which made it a good nutritional contribution to supplement poor quality roughages. The result is in agreement with the study of Kassahun et al. (2016) on *Ficus thonningii* and *Terminalia schimperiana* in Chilega District, North Gondar. The recorded lag time indicated that degradation did not occur similarly in the rumen. The effective degradation in the rumen is determined by the length of time the feed remains in the rumen, which is also determined by the quality of the feed supplied to the animals (Liman et al., 2016). During the dry and wet seasons, effective degradability (ED) of *P. schimperina* was in line with Liman et al. (2016) on browse species (40.90-66.10%) which were conveniently used in the formulation of high-quality ruminant feed for fattening and maintenance feeding. The potential degradability (PD) varied significantly (P<0.05) from 66.52 to 76.5% and 68.08 to 77.60% in the dry and wet seasons of midland and highland respectively. The PD value from the current study was higher than reported values of browse species ranged from 61.6 – 67.8 % in wet season and from 55.2 – 65.2% during the dry season in our study (Ahmed et al., 2017). Previous research revealed that variations in the degrading parameters of browse species might be attributed to chemical composition (Kamalak, 2006; Belachew et al., 2013; Gusha et al., 2013), differences in the stage of maturity and possibly climate and edaphic differences (Ahmed et al., 2017). These variations in PD in the rumen have been reported as a result of variations in fibre content and tannins levels (Gusha et al., 2013). These values were in the range obtained by Liman et al. (2016) who reported a potential degradability ranging from 40.40 to 93.40% in browse species.

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Table 3 - Percentage Dry matter degradability of *Pentas schimperina* (%)

Time	Season	Midland	Highland	SEM	Agro.	Season	Agro.×Season
0	Dry	21.52[b]	24.42[a]	0.85			
	Wet	24.33[b]	27.62[a]	0.95	0.0001	0.0001	0.29
6	Dry	27.76[b]	31.38[a]	1.09			
	Wet	30.79[b]	31.70[a]	0.27	0.004	0.013	0.03
12	Dry	35.65[b]	37.79[a]	0.63			
	Wet	41.64[a]	41.61[b]	0.045	0.001	0.0001	0.001
24	Dry	48.72[b]	50.34[a]	0.48			
	Wet	53.36[b]	58.44[a]	1.47	0.0001	0.0001	0.0001
48	Dry	61.52[b]	66.36[a]	1.40			
	Wet	52.83[b]	68.71[a]	1.69	0.0001	0.0001	0.03
72	Dry	61.63[b]	70.63[a]	2.31			
	Wet	65.56[b]	73.45[a]	2.28	0.0001	0.0001	0.74
96	Dry	64.63[b]	72.64[a]	2.32			
	Wet	67.48[b]	75.08[a]	2.19	0.0001	0.0001	0.16

[a,b,]rows means with different superscripts are significantly different (P<0.05); SEM is Standard error of the mean, Agro.= Agroecology effect (P<0.05), Season.= Season effect (P<0.05); Agro.×season.= Agroecology × Season effect (P<0.05).

Table 4 - Dry matter degradability parameters (%)

Parameters	Season	Midland	Highland	SEM	Agro.	Season	Agro.×Season
a	Dry	20.27[b]	23.06[a]	0.81			
	Wet	23.01[b]	24.75[a]	0.5	0.001	0.001	0.001
b	Dry	46.25[b]	53.47[a]	2.09			
	Wet	45.06[b]	52.65[a]	2.19	0.001	0.001	0.073
c	Dry	0.04[a]	0.03[b]	0.003			
	Wet	0.044[a]	0.04[b]	0.11	0.001	0.001	0.002
PD	Dry	66.52[b]	76.53[a]	2.89			
	Wet	68.08[b]	77.40[a]	2.68	0.006	0.045	0.509
ED	Dry	62.43[b]	64.08[a]	0.52			
	Wet	63.40[b]	64.66[a]	0.37	0.001	0.001	0.001
Leg.T	Dry	0.64[a]	0.44[b]	0.57			
	Wet	0.48[a]	0.34[b]	0.40	0.001	0.001	0.001

[a, b,] rows means with different superscripts are significantly different (P<0.05SEM is Standard error of the mean, a=rapidly degradable fraction; b=slowly degradable fraction; c=rate of degradation; PD=potential degradability; ED=effective degradability (P<0.05), Agro.= Agroecology effect (P<0.05), Season = Season effect (P<0.05); Agro.×season = Agroecology × Season effect (P<0.05).

Crude protein degradability of *P. schimperiana*

The percentage of crude protein disappearance at 0, 12, 24, 48, 72, and 96 hours incubation time was significantly (P<0.05) affected by agroecology and season (Table 5). The significant difference (P<0.05) of CPD was related to lesser crude protein and higher fiber fractions (NDF, ADF, and ADL) in midland during both the dry and wet seasons for the macroclimatic factors variations in harmony with Lebopa et al. (2011) on crude protein degradation of woody plant species. The disappearance of CP increases with increased incubation time. The CP disappearance of *P. schimperiana* was greater in the highland (P<0.05) than in midland except at 48 and 72 hours incubation time in the dry season. CP disappearance at 96 h had values above 80%, which was in line with Njidda (2011) on the tree and shrub. Similarly, Larbi et al. (1998) stated that the crude protein disappearance of multi-purpose trees and shrubs was higher in the wet season. The recorded differences in midland and highland agro-ecology during the dry and wet seasons contributed to the effect of genotype on nutrient absorption, properties of soil to supply nutrients to plants, growth stage, and maturity of the plant (Njidda, 2011).

The CP degradability values (Table 6) revealed a high rate of CP degradation constant in high effective degradability, which was consistent with the findings of Njidda (2011) who experimented on multiple trees and shrub leaves which were served as a high-protein value feed source for ruminants. During the dry and wet seasons, *P. schimperiana* had a short lag time due to a high rapidly degradable fraction (a) and a low slowly degradable fraction (b). The short lag time in this study indicated that *P. schimperiana* had a higher amount of CP and low ADF, ADL, and NDF in the wet season and this result was in line with Liman et al. (2016) on browse species in Taraba, Nigeria.

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Table 5 - Crude protein degradability (%)

Time	Season	Midland	Highland	SEM	Agro.	Season	Agro.×Season
0	Dry	46.75[b]	48.56[a]	0.54			
	Wet	50.53[b]	52.67[a]	0.63	0.01	0.0001	0.46
6	Dry	55.43	59.53	1.19			
	Wet	59.51	56.31	0.93	0.068	0.07	0.0001
12	Dry	62.57[b]	63.53[a]	0.28			
	Wet	65.67	63.34	0.68	0.07	0.0001	0.0001
24	Dry	67.38[b]	67.58[a]	0.11			
	Wet	69.74[b]	70.41[a]	0.22	0.057	0.0001	0.22
48	Dry	72.42[a]	70.39[b]	0.59			
	Wet	74.55[b]	79.67[a]	1.48	0.0001	0.0001	0.0001
72	Dry	75.43[a]	75.33[b]	0.09			
	Wet	76.73[b]	85.48[a]	2.53	0.0001	0.0001	0.0001
96	Dry	80.28[b]	85.40[a]	1..49			
	Wet	82.55[b]	88.12[a]	1.61	0.0001	0.0001	0.37

[a, b,] rows means with different superscripts are significantly different (P<0.05); SEM is Standard error of the mean, Agro.= Agroecology effect (P<0.05), Season.= Season effect (P<0.05); Agro.×season.= Agroecology × Season effect (P<0.05).

Table 6 - Crude protein degradability (%)

Parameters	Season	Midland	Highland	SEM	Agro.	Season	Agro.×Season
a	Dry	47.36[b]	53.07[a]	1.64			
	Wet	52.14[a]	52.11[b]	0.05	0.0001	0.0001	0.0001
b	Dry	30.33[b]	34.28[a]	1.14			
	Wet	27.63[b]	39.27[a]	3.36	0.0001	0.0001	0.0001
c	Dry	0.05[a]	0.02[b]	0.009			
	Wet	0.051[a]	0.03[b]	0.006	0.001	0.31	0.22
ED	Dry	67.12[a]	67.09[b]	2.05			
	Wet	68.25[b]	69.54[a]	0.37	0.0001	0.0001	0.001
PD	Dry	77.69[b]	87.35[a]	2.80			
	Wet	77.77[b]	91.38[a]	3.81	0.0001	0.0001	0.001
Leg.T	Dry	0.15[b]	0.17[a]	0.52			
	Wet	0.11[b]	0.13[a]	0.51	0.0001	0.0001	0.94

[a, b,] rows means with different superscripts are significantly different (P<0.05SEM is Standard error of the mean, a=rapidly degradable fraction; b=slowly degradable fraction; c=rate of degradation; PD=potential degradability; ED=effective degradability (P<0.05), Agro.= Agroecology effect (P<0.05), Season = Season effect (P<0.05); Agro.×season = Agroecology × Season effect (P<0.05).

CONCLUSION

The findings of this study indicated that the chemical compositions of *P. schimperiana* in the study area were influenced by agroecology and season. This study showed that *P. schimperiana* has relatively high crude protein and lower fiber fraction which leads to a better digestibility. Therefore, it has the potential to use as a dry season protein supplement for ruminants fed on low-quality hay and crop residues and it can correct the nutrient deficiency that exists in low-quality feeds. However, it needs a biomass yield study and also to know its effect on animal performance, the researchers recommend supporting this result with animal experiments.

DECLARATIONS

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Authors' contribution

T. Woretaw performed conceptualization, data curation, formal analysis, investigation, methodology, software, validation, writing original draft; N. Beyero performed conceptualization, methodology, supervision, writing – original draft, writing, review & editing of the manuscript for important academic contents.

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Conflict of interests

The authors have not declared any conflict of interests.

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MORPHOMETRIC CHARACTERIZATION OF NEW ZEALAND WHITE RABBIT RAISED AT DIFFERENT AREAS

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ABSTRACT: The study aimed to morphometric characterization the New Zealand White (NZW) doe at three different areas. The materials used were 295 heads of NZW doe rabbits from 29 farms located at different areas. Twelve morphometric characteristics consist of body weight, eight body measurements, and three-body indices. Data analysis was performed by Mixed model, Pearson's correlation, Principal component, and Canonical discriminant procedures. The most of parameters showed significant differences among areas. The heaviest body weight (4.71 kg) was observed in low-land and the lightest in medium land (3.54 kg). Most of the morphometric characters showed positive correlations with each other. Results of principal component show that the body indices of NZW doe raised in three different areas were similar. Canonical discriminant analysis showed that low-land was more favorable than high-land and medium land. In conclusion, The variation in size difference for morphometric characters of female New Zealand white rabbit could be explained by body index and thoracic index. The morphometric characteristics of New Zealand white doe raised in low-land area were superior to those raised in high-land and medium land areas.

Keywords: Body index, Eigenvalues, Least-square means, Phenotypic correlation, Thoracic index.

INTRODUCTION

New Zealand white (NZW) rabbit, the commercial breed has been imported from the American rabbit breeding association (Setiaji et al., 2022). The rabbits are raised by smallholders with the purpose of meat production, pet, and show. The important traits for meat production are average daily gain (ADG), slaughter weight (SW), carcass weight (CW) of bucks, and litter size (LS), liveability (LA), kidding interval (KI) of Doe. The average of ADG, SW, CW, LS, LA and KI reported in NZW rabbit are 20.40 g, 1.900 kg, 1.499 kg, 6.23, 90%, 109 days (Marai et al., 2008; Ghosh et al., 2008). Most buck is slaughtered, while the doe is used for breeding purposes. The population of doe is 80% of the total population of rabbits. Most of the breeding farm for New Zealand white rabbits in Indonesia is located in Central Java Province, and covers low-land to high-land areas (Blasco et al., 2017; Ume et al., 2018).

Morphometric characterizes are at times favored in light of the fact that measurements of body weight can be biased due to gut fullness (Obike et al., 2010). The quantitative characters have been used to perform morphometric were: chest circumference, chest depth, chest width, body length, head length, head width, tibia length, femoral length, humerus length, radius-ulna length, hip width, ear length, ear width. Multivariate analysis generally used for these trait to estimate phylogeny three (Brahmantiyo et al., 2006).

Theoretically, rabbits are well adapted and grew optimum in the high-land areas due to low air temperature and humidity. Rabbits are sensitive to high temperatures (>26°C) and relative humidity (>70%) (Silva et al., 2021). High temperature can significantly decrease growth and reproductive performance (Szendrö et al., 2018). This condition represents a problem in rabbit farming in the low-land areas. Closed-house system that can control temperature and humidity might solve the problem. No characterization of NZW rabbit at different areas have been published recently. The study can be carried out based on morphometric characteristics. The morphometric characteristics of the same breed from different populations have been reported in goats (Ouchene-Kelifi et al., 2018; Depison et al., 2020) and sheep (Dekhili, 2014; Markovic et al., 2019).

The characterization based on morphometric of rabbits is essential for any purpose, such as strategies of breeding or conservation, genetic improvement, and sustainable utilization of a breed. The aim of this study was to characterize the NZW doe at three different areas.

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MATERIALS AND METHODS

Ethical approval

The protocol was based on the standard rule of animal treating as appointed in the Republic of Indonesia's law, that is, number 41, 2014. This research was funded by Hibah Dana Penelitian Universitas Diponegoro Semarang with Contract No. 41/UN7.5.5.2/HK/2022.

Material

The number of NZW doe used in the study was 295 heads. The age of doe was grouped to be: 6 - <12 months (n=146); 12 - <18 months (n=79), and ≥18 months (n=70) for preliminary analysis. The doe was obtained from 29 farms located at different areas. The doe measured were not pregnant or lactating. The location of farm was classified based on the areas above sea level (a.s.l.) as follows: low-land, medium land, and high-land. The detailed information of the data is presented in Table 1. The body measurement consist of body weight (BW); head length (HL); head width (HW); ear length (EL); thoracic circumference (TC); thoracic depth (TD); thoracic width (CW); hip-width (HP); and body length (BL). Body indices were calculated from body weight and body measurements as follow: index of body weight (IBW) = [BW/TD] x 100; body index (BI) = [BL/TC] x 100; and thoracic index (TI) = [TW/TD] x 100.

Table 1 - General Information of study areas.

Description	Low-land	Medium-land	High-land
Altitude (m.a.s.l)	<250	250-750	≥750
Number of farms	1	18	20
Number of doe	52	172	71
Type of house	Closed house	Open house	Open house
Type of cage	Individual cage	Individual cage	Individual cage
Feed	Complete feed	Complete feed + forage	Complete feed + forage

m.a.s.l: meters above the sea level.

Data analysis

All of the data analysis was performed using Statistical Analysis System (SAS) University Edition V.6 p.2. software (SAS, 2014). One-Way ANOVA procedure was used to estimate least-square means and analyze the effect of age on body measurement and body indices. A mixed procedure was used for analyzing the effect of areas on morphometric characteristics with the farm as a random effect. The Tukey-Kramer multiple comparisons was tested at 5% of probability. Pearson's correlation was used to estimate the phenotypic correlation among morphometric characteristics. Multivariate analysis was performed to determine principal components, canonical structure, and distribution mapping by using Principal component and Canonical discriminant methods.

RESULTS AND DISCUSSION

The least square means of morphometric characteristics are presented in Table 2. Preliminary analysis showed that age class only affected significantly BW, HL, and BL whereas; most morphometric characteristics were not significant differences among age classes. This result indicated that after six months of age, bones were growing slower or almost stopped. Masoud et al. (1986) reported that whole bone longitudinal growth of NZW doe was inclining after six months. According to the results, further analysis for morphometric characteristics was not separated into different classes of age.

Table 2 - Least-square means for morphometric characteristics of NZW doe

Parameters \ Age of doe		6 - <12 months		12 - <18 months		≥18 months		Pr > F
		Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	
BW (kg)		3.64 ± 0.06	21.23	3.91 ± 0.08	18.82	4.00 ± 0.09	18.98	0.0178
Body measurement (cm)	HL	10.28 ± 0.11	13.09	10.84 ± 0.18	14.83	10.75 ± 0.13	10.21	0.0052
	HW	4.94 ± 0.05	11.13	4.93 ± 0.05	8.73	5.00 ± 0.06	9.59	0.7839
	EL	11.14 ± 0.08	8.68	11.39 ± 0.12	9.13	11.29 ± 0.11	7.99	0.3157
	TC	35.84 ± 0.30	10.04	36.27 ± 0.36	8.76	36.15 ± 0.36	8.38	0.1879
	TD	9.30 ± 0.10	13.64	9.40 ± 0.12	11.65	9.61 ± 0.11	9.94	0.7287
	TW	8.91 ± 0.09	12.18	8.95 ± 0.19	18.65	9.13 ± 0.15	13.85	0.2854
	HP	10.29 ± 0.10	11.42	10.34 ± 0.15	12.48	10.33 ± 0.14	11.44	0.0644
	BL	35.75 ± 0.24	8.03	37.00 ± 0.28	6.80	37.35 ± 0.25	5.71	0.0379
Body indices	IBW	39.51 ± 0.73	22.38	41.80 ± 0.90	19.04	41.81 ± 0.93	18.78	0.3323
	BI	100.47 ± 0.90	10.80	102.61 ± 1.09	9.44	103.89 ± 1.11	8.94	0.2617
	TI	97.41 ± 1.63	20.20	96.07 ± 2.25	20.77	95.51 ± 1.57	13.78	0.6848

BW: Body weight; HL: Head Length; HW: Head Width; EL: Ear Length; TC: Thoracic Circumference; TD: Thoracic Depth; TW: Thoracic width; HP: Hip Width; BL: Body Length; IBW: Index of body weight; BI: Body index; TI: Thoracic index; SE: Standard error; CV: Coefficient of variation.

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Table 3 presents the morphometric characteristics of NZW doe from different areas. All parameters showed significant different among areas, except HL, BL, and BI. The heaviest BW observed in NZW doe raised in low-land and the lightest one by NZW doe raised in medium land. Body measurement of NZW doe (TW and HP) raised in low-land showed superiority over that for NZW doe raised in medium and high-land. Whereas, HW, TC, and TD were similar between low-land and high-land. BI was not different between areas, while IBW and TI of low-land were similar to that of high-land but higher than medium land. These results indicated that environment manipulation could increase the performance of NZW doe. Agreed with Elamin et al. (2012) and Arandas et al. (2017) that morphometric characteristics are affected by management techniques environment, and feed quality.

A total of 66 phenotypic correlations were computed among morphometric characteristics, of which 39, 8, 3, 2 were positively significant (P<0.01); negatively significant (P<0.01); positively significant (P<0.05); and negatively significant (p<0.05), respectively as presented in Table 4. Meanwhile, a high phenotypic correlation (>0.60) was shown between BW and TC (0.69), TW (0.66), IBW (0.72); between TW and TC (0.65), HP (0.63), and between IBW and TI (0.68). High and positive correlations among morphometric characteristics can be indicated that they are pleiotropic (Luo et al., 2017).

Table 3 - Morphometric characteristics of NZW doe from different areas

Parameters \ Areas		Low-land	Medium-land	High-land
BW (kg)		4.71 ± 0.34^{a}	3.54 ± 0.08^{c}	3.91 ± 0.11^{b}
Body measurement (cm)	HL	10.81 ± 0.55	10.58 ± 0.16	10.72 ± 0.23
	HW	5.34 ± 0.22^{a}	4.86 ± 0.06^{b}	4.97 ± 0.08^{ab}
	EL	11.07 ± 0.61	11.32 ± 0.14	11.05 ± 0.19
	TC	38.92 ± 1.73^{a}	35.19 ± 0.42^{b}	36.17 ± 0.56^{ab}
	TD	10.26 ± 0.53^{a}	9.15 ± 0.14^{b}	9.53 ± 0.19^{ab}
	TW	10.21 ± 0.43^{a}	8.48 ± 0.12^{c}	9.26 ± 0.18^{b}
	HP	11.96 ± 0.21^{a}	9.75 ± 0.08^{c}	10.39 ± 0.12^{b}
	BL	37.66 ± 1.57	36.22 ± 0.38	36.74 ± 0.50
Body indices	IBW	46.18 ± 3.25^{a}	38.69 ± 0.90^{b}	42.06 ± 1.29^{a}
	BI	97.02 ± 4.91	103.60 ± 1.27	102.15 ± 1.77
	TI	99.95 ± 5.78^{a}	93.15 ± 1.84^{b}	99.67 ± 2.76^{a}

BW: Body weight; HL: Head Length; HW: Head Width; EL: Ear Length; TC: Thoracic Circumference; TD: Thoracic Depth; TW: Thoracic width; HP: Hip Width; BL: Body Length; IBW: Index of body weight; BI: Body index; TI: Thoracic index; SE: Standard error; CV: Coefficient of variation.

Table 4 - Phenotypic correlation among morphometric characteristics of NZW doe

	BW	HL	HW	EL	TC	TD	TW	HP	BL	IBW	BI
HL	0.19**										
HW	0.52**	0.10									
EL	0.01	0.14*	0.04								
TC	0.69**	0.19**	0.46**	0.04							
TD	0.42**	0.14*	0.32**	-0.14*	0.41**						
TW	0.66**	0.17**	0.55**	-0.02	0.65**	0.39**					
HP	0.58**	0.19**	0.55**	-0.08	0.54**	0.39**	0.63**				
BL	0.59**	0.17**	0.29**	0.13*	0.35**	0.31**	0.34**	0.28**			
IBW	0.72**	0.11	0.30**	0.07	0.44**	-0.27**	0.43**	0.34**	0.37**		
BI	-0.21**	-0.04	-0.22**	0.08	-0.69**	-0.17**	-0.36**	-0.29**	0.42**	-0.13*	
TI	0.21**	0.03	0.17**	0.05	0.21**	-0.49**	0.52**	0.23**	0.01	0.68**	-0.19**

BW: Body weight; HL: Head Length; HW: Head Width; EL: Ear Length; TC: Thoracic Circumference; TD: Thoracic Depth; TW: Thoracic width; HP: Hip Width; BL: Body Length; IBW: Index of body weight; BI: Body index; TI: Thoracic index; *: significant at 0.05 levels; **: significant at 0.01 levels.

Table 5 shows the summary of the principal component analysis including eigenvalues and percent of the variance. The results explained 62.22%, 52.20%, and 88.87% of the total phenotypic variance in low-land, medium-land, and high-land, respectively. Ajayi and Oseni (2012) extracted two principal components from twelve body measurements of Nigerian rabbits which explained 55.55% of the total phenotypic variance. PC1 loaded heavily on TI in NZW doe raised in all over areas, PC2 loaded heavily on BI in low-land and high-land, then BI and TI in medium-land. The characteristics strongly correlated with each PC same among three areas implying that their morphometric characteristics were not differed genetically. This study shows that the body indices of NZW doe raised in three different areas were similar.

Squared distances of canonical discriminant between areas and their probability are presented in Table 6. The result indicates that despite the doe belonging to the same breed, there are differences among areas. The distance between

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land-land and medium-land was longer than that between low-land and high-land. As shown in Figure 1, low-land was more favorable than high-land and medium-land. Meanwhile, medium-land was near to low-land. The result was not in accordance with Depison et al. (2020) studied Kacang goats with the same management but raised at two different locations (low-land and high-land). They reported that morphometric characteristics cannot characterize the goats from low-land and high-land. The results of a recent study could be due to intensive management done in the low-land areas. It leads morphometric characteristics of NZW doe raised in low-land similar even exceed to their raised in high-land.

Table 5 - Eigenvalues, total variences and cumulative of Principal component analysis

Parameters	Low-land		Medium-land		High-land	
	PC1	PC2	PC1	PC2	PC1	PC2
BW	0.0179	0.0072	0.0051	-0.0039	0.0074	0.0042
HL	0.0138	-0.0026	-0.0079	-0.0109	0.0061	-0.0063
HW	0.0111	-0.0092	0.0122	0.0007	-0.0002	0.0038
EL	0.0112	0.0385	0.0099	0.0109	-0.0005	0.0142
TC	0.0603	-0.2025	0.1131	-0.1517	0.0225	-0.1976
TD	-0.0458	-0.0256	-0.0314	-0.0531	-0.0271	-0.0183
TW	0.0473	-0.0254	0.0409	-0.0022	0.0254	-0.0219
HP	0.0087	-0.0495	0.0149	-0.0051	0.0074	-0.0119
BL	0.0291	0.1708	-0.0709	0.1033	0.0015	0.1437
IBW	0.3825	0.0052	0.1999	0.1805	0.3139	0.1582
BI	-0.0735	0.9429	-0.5483	0.8031	-0.0577	0.9560
TI	0.9158	0.0052	0.7989	0.5345	0.9466	0.0105
Eigenvalues	118.59		178.65		1005.08	
Variance (%)	62.22		52.20		88.57	

BW: Body weight; HL: Head Length; HW: Head Width; EL: Ear Length; TC: Thoracic Circumference; TD: Thoracic Depth; TW: Thoracic width; HP: Hip Width; BL: Body Length; IBW: Index of body weight; BI: Body index; TI: Thoracic index; PC1: first principal component; PC2: second principal component.

Table 6 - Result of canonical discriminant: squared distance to areas (above diagonal) probability for squared distance (below diagonal)

	Low-land	Medium-land	High-land
Low-land		9.2652	4.9586
Medium-land	<0.0001		1.3431
High-land	<0.0001	<0.0001	

BW: Body weight; HL: Head Length; HW: Head Width; EL: Ear Length; TC: Thoracic Circumference; TD: Thoracic Depth; TW: Thoracic width; HP: Hip Width; BL: Body Length; IBW: Index of body weight; BI: Body index; TI: Thoracic index; *: significant at 0.05 levels; **: significant at 0.01 levels.

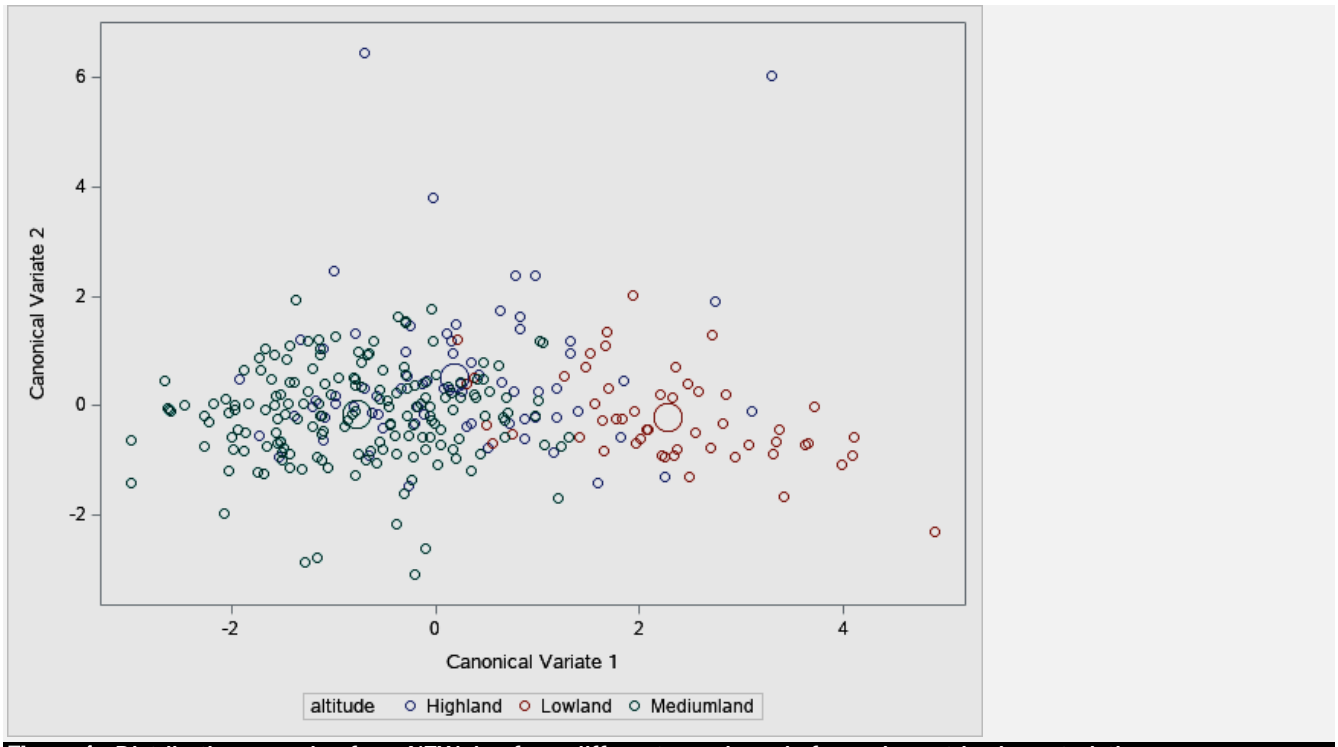

Figure 1 - Distribution mapping from NZW doe from different area based of morphometric characteristics.

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CONCLUSION

The variation in size difference for morphometric characters of female New Zealand white rabbit could be explained by BI and TI. The morphometric character of rabbits raised in low-land was dominant than others. The intensive management done in low-land areas might caused the morphometric characteristics of New Zealand white superior to those raised in high-land and medium-land.

DECLARATIONS

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Authors' contribution

E Kurnianto: Idea and research design; S Sutopo and ME NOVIANTI: Data collection; A Setiaji: Data analysis and Writing the manuscript; DA Lestari: Writing the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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THE POTENTIAL OF SEED GERMINATION INHIBITION TEST FOR EARLY PREGNANCY DETECTION AND IMPROVED REPRODUCTIVE EFFICIENCY OF CATTLE IN ZAMBIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Early pregnancy diagnosis is an important management practice for reducing calving interval, increasing cattle reproductive efficiency, and the overall herd productivity. This study was undertaken to assess the viability of seed germination inhibition technique (Punyakoti test) for early pregnancy detection in cattle under the tropical rearing conditions. Twenty-four randomly selected cows were used for the experiment. Urine samples were collected and subjected to Punyakoti test, using maize seeds, within 6 hours of collection. Descriptive statistics employing means and standard error were used to analyse data, also, inferential statistics including analysis of variance and t-tests were employed to ascertain differences between the variables under study. Urine from pregnant cows had the highest (80.03±3.99) inhibitory effect while the distilled water group had the least (7.50±3.81) mean seed germination inhibition. The means of germinated maize seeds in pregnant and non-pregnant cow urine treatment groups were significantly different. The means of germinated maize seeds in unstripped and stripped pregnant cow urine were significantly different, while the mean shoot length values for the same treatments were not significantly different. Urine from 42 days' pregnant cows had the highest germination inhibition (80.21±3.59) while the least (25.00±4.35) was observed on day 10. The Punyakoti test reliably detected pregnancy starting from 26 days after insemination. In conclusion, this technique can be used for detecting pregnancy as early 26 days after insemination. The potential role of steroids and involvement of abscisic acid (ABA) in seed germination inhibition requires further investigation.

Keywords: Abscisic acid, Cattle, Maize, Pregnancy detection, Punyakoti test.

INTRODUCTION

Livestock farming including cattle, as a source of meat and income, remains crucial considering that more than 40% of the population in Zambia lack adequate food (MFL, 2020). Due to the need to reduce existing animal protein deficit, programmes aimed to increase production have been developed, these include but not limited to stocking and re-restocking, promoting application of appropriate biotechnologies, and strengthened livestock extension (MFL, 2020). Among the crucial on-farm interventions for improved production is pregnancy detection; furthermore, establishing a pregnancy diagnostic tool allows for the detection of cows that are not pregnant, thus producers are able to make management decisions to increase reproductive efficiency. The decisions may be, culling of infertile females, or re-synchronizing the females that are open (Dilrukshi and Perera, 2009; Balhara et al., 2013; Fontes et al., 2022). When done early, calving interval is shortened thereby increasing the animal's life-time production (Balhara et al., 2013; Aswathnarayanappa et al., 2019).

There are a plethora of techniques which can be used to detect pregnancy in cattle, for example, rectal palpation, non-return to oestrus, radiography, hormonal based assays, and ultra-sound technique (Swamy et al., 2010; Abdullah et al., 2014). Despite their relevance, these techniques, on the one hand, are invasive, costly, laborious, and/or requires a high technical knowhow (Dilrukshi and Perera, 2009) which makes them unsuitable and prohibitive to resource-poor and unskilled rural farmers. On the other hand, Punyakoti test is a farmer friendly, simple, and non-invasive technique also used for pregnancy detection (Dilrukshi and Perera, 2009).

Although a number of studies, focusing on different livestock species with seeds from different cereal species, have recommended its application (Skálová et al., 2017; Aswathnarayanappa et al., 2019; Rahman and Saha, 2020), cattle farmers in Zambia have not yet applied it. Furthermore, there are scarce studies on Punyakoti test with maize seeds. Also, studies including those which used seeds of other cereal species reported variations in the test's reliability, which was attributed to different factors, namely moisture, temperature, daylight, nutrition, and seed storage (Bowden and Ferguson, 2008). Hence, a need exists to validate its applicability in Zambia, utilizing the main and readily available maize seeds, because of its unique tropical climate with unique weather conditions.

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There is also lack of clarity on the urine-containing factors which predispose the seeds to germination inhibition. Over the years, the presence of abscisic acid (ABA) in pregnant cow urine has been associated with reduced seed germination and shoot growth (Islam et al., 2014; Skálová et al., 2017; Rai et al., 2018); coincidentally, some studies have attributed the same to ovarian steroids (Erdal and Dumlupinar, 2010; Lázničková et al., 2020). In view of this controversy, a need exists for further studies to elucidate the potential role of steroids in seed germination. Thus, validation of the actual substance(s) influencing germination of seeds would be crucial in designing simple bioassay kits for use under field conditions. This study was carried out to 1) determine the level of germination and shoot length inhibition for maize seeds in different treatment groups; 2) evaluate the effects of steroids in bovine urine on germination and shoot growths of the maize seeds; and 3) determine the earliest time for detecting pregnancy and non-pregnancy, using maize seeds, in bovines under smallholder farming conditions in Zambia.

MATERIALS AND METHODS

Ethical regulations

Only physically healthy animals were used for this study. The procedures used were non-lethal with minimal to no distress to the animals. The animal handling and experimentation were done with strict supervision by the institutional committee on the animal research, and in compliance with the guide for the care and use of agricultural animals in research and teaching (ASAS, 2020).

Study location and animals

The experiment was conducted at the Field Station, Department of Animal Science, School of Agricultural Sciences, the University of Zambia, Zambia, during the period of June to October 2021. According to the GeoNames geographical database Google Earth-2022, the country is located at latitude S 14° 20' 0" and longitude E 28° 30' 0". Additionally, Zambia lies in the tropics, specifically in Southern Africa. The country's average annual precipitation ranges from 800-1400 mm. The temperatures during winter ranges from 10-20 ºC, and during the hot dry season it ranges from 20-30 ºC (RCCC, 2021). The current study was conducted using cattle that belonged to the department of Animal science. Noteworthy, cattle production is the most significant activity, especially among rural farmers in Zambia, compared to other livestock species. Moreover, the highest proportion (93.5%) of the national herd is owned by the smallholder farmers (Odubote, 2022).

Effect of pregnant cow urine on maize seed germination inhibition

A total of 15 pregnant, and 9 non-pregnant and clinically healthy cows were used for the experiment. This study was performed according to the previous study procedure (Swamy et al., 2010) with minor modification including the use of maize seeds. Good quality maize seeds were sourced from a reputable local supplier/research station to ensure better germination efficiency. From these cows, fresh urine samples were collected early in the morning, in clean dry containers, while they were naturally micturating. The samples were subjected to seed germination inhibition test within six hours after collection; each sample was diluted in the ratio of 1:4 from which 15 ml portions were introduced into clean petri dishes, in three replicates, each containing eight maize seeds. As a control, distilled water was also applied to additional petri dishes and nine samples were considered. After 4 days, data was collected for seed germination inhibition % and shoot length on each petri dish.

Assessing the steroids' effect on maize seed germination and growth

Urine samples from clinically healthy, 15 pregnant and 9 non-pregnant, cows were used for this study. The procedure was based on the previous study by Swamy et al. (2010), with modification including stripping the urine samples of steroids. Each urine sample was divided into two subsamples one of which was stripped. Steroid-stripped urine was produced by pouring a urine sample over activated charcoal contained in filter paper held in a funnel. Eight maize seeds were placed on each petri dish, in triplicate, to be grown in the stripped or non-stripped treatment groups. After 4 days, data was collected for seed germination (%) and shoot length (mm) from each petri dish.

Estimating earliest time for detecting pregnancy and non-pregnancy using maize germination inhibition test

The procedure for this experiment was based on the previous study (Rine et al., 2014) with some modification to suite the study objective. Eighteen cyclic Friesian dairy cows were exposed to synchronization using a double prostaglandin ($PGF_{2\alpha}$) injection and were used as either pregnant following insemination (12) or were used as non-inseminated (non-pregnant) hence open Friesian dairy cows (6). For both treatment groups, free-catch urine collection was done at day 10, 14, 21, 26, 30, 35, and 42, every morning and when cows are naturally micturating. Each collected sample was diluted (1:4) and 15 ml of this was introduced into the petri dish (in triplicates) containing eight seeds. The petri dishes were placed on an open area in a store room, and the results were collected after 4 days. Seed germination and shoot length among the treatment groups were determined. Shoot length of each seed was measured (in mm) using a ruler, and the seed germination inhibition (%) was calculated using a formula (Swamy et al., 2010);

$$\text{Germination inhibition } \% = \frac{number\ of\ seeds\ not\ germinated\ in\ petri\ dishes}{total\ number\ of\ seeds\ in\ petri\ dishes}\ x\ 100$$

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Data analysis

The data obtained, for various parameters, was analysed in SPSS IBM® (SPSS IBM 26 version, USA) using descriptive statistics to get the means and standard error of mean (SEM). Furthermore, the differences in the variables under study were determined using one-way ANOVA and the paired t-test statistics. Post hoc (Tukey's HSD) test was performed to obtain the pair(s) with significant mean differences. Significance level (alpha) was set at $P<0.05$. Microsoft excel version 2016 was used to generate the graphs.

RESULTS

Mean seed germination inhibition in distilled water, pregnant and non-pregnant cow urine.

The mean seed germination inhibition for each of the three treatment groups, namely distilled water, pregnant and non-pregnant cow urine are presented below (Figure 1). Urine from the pregnant cows had the highest germination inhibition percent (80.03±3.99) while the lowest (7.50±3.81) was observed for the seeds in distilled water. The analysis of variance revealed significant differences (P<0.05) between the three treatment groups. Additionally, the post-hoc (Tukey's HSD) tests revealed significant seed germination inhibition differences between the pregnant and non-pregnant cow urine groups (P<0.05), pregnant cow urine and distilled water (P<0.05), but not non-pregnant cow urine and distilled water (P >0.05).

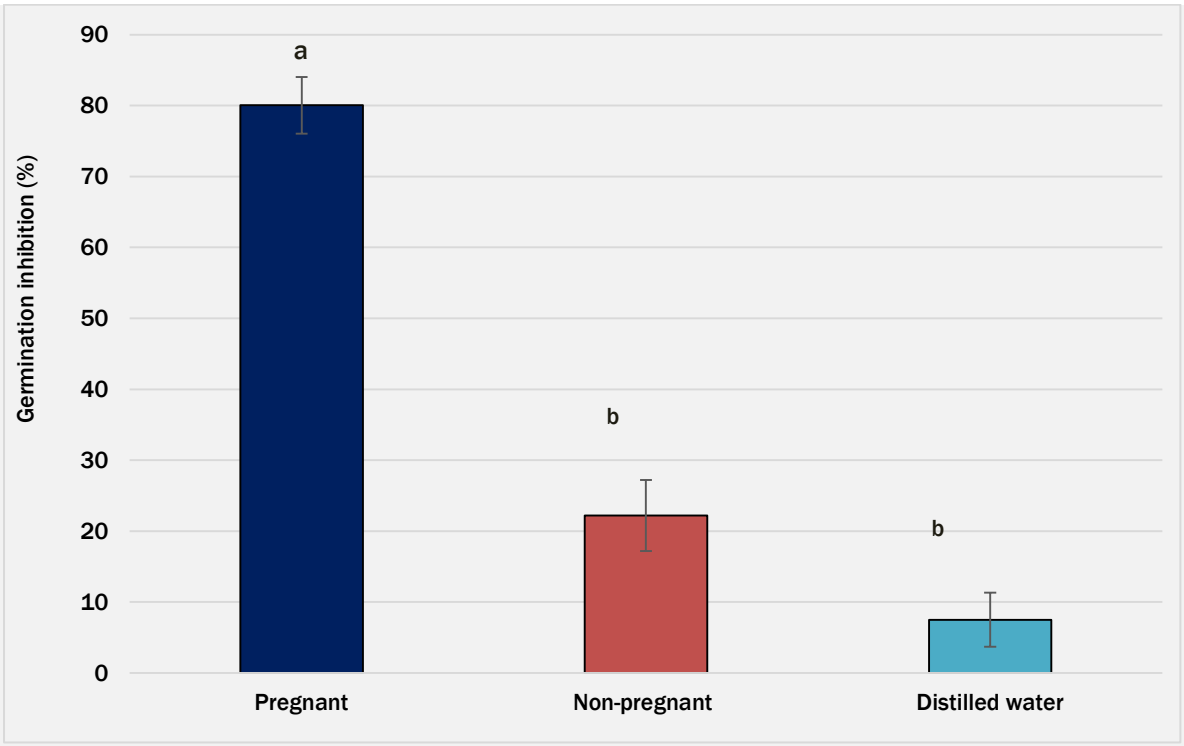

Figure 1 - Mean seed germination inhibition in distilled water, pregnant and non-pregnant cow urine. Different alphabetical letters (A, B) indicate significant difference at P<0.05; Error bars: standard error of mean; %: Percentages.

Mean seed germination inhibition percentage in stripped and non-stripped pregnant cow urine

The mean seed germination inhibition percentage in stripped and non-stripped urine, from pregnant and non-pregnant cows, are presented below (Table 1). The percentage germination inhibition in non-stripped cow urine was generally higher than that in stripped cow urine. The t-test analysis between the stripped and non-striped treatment groups (pregnant) revealed a significant difference (P<0.05) in their mean values; their observed correlation (r) was 0.450. Furthermore, there was no significant difference (P>0.05) in the mean germination inhibition between non-stripped and stripped groups for urine obtained from non-pregnant cows; also, no correlation (r=0.03) was found.

Table 1 - The mean germination inhibition of maize seeds in stripped and non-stripped pregnant and non-pregnant cow urine

Groups (Treatment) / Status	Non-stripped urine	Stripped urine
Pregnant	80.03±3.99^{a}	69.17±5.01^{b}
Non-pregnant	22.22±5.01^{a}	8.33±3.99^{a}

Data expressed as mean±SEM; SEM: standard error of mean; ^{a,b} means with difference superscripts in the same row are significantly different at $P<0.05$.

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Mean shoot length of germinated seeds in stripped and non-stripped pregnant cow urine

The mean shoot length (mm) of germinated seeds in non-stripped and stripped pregnant cow urine are presented in Figure 2. The mean shoot length of maize seeds in non-stripped urine was higher (5.49±0.84 mm) than that of the stripped urine group (3.35±0.83 mm). A t-test analysis revealed no significant difference (P>0.05) between the treatment groups. Furthermore, no significant (P>0.05) correlation (r=0.020) between the treatment groups was found.

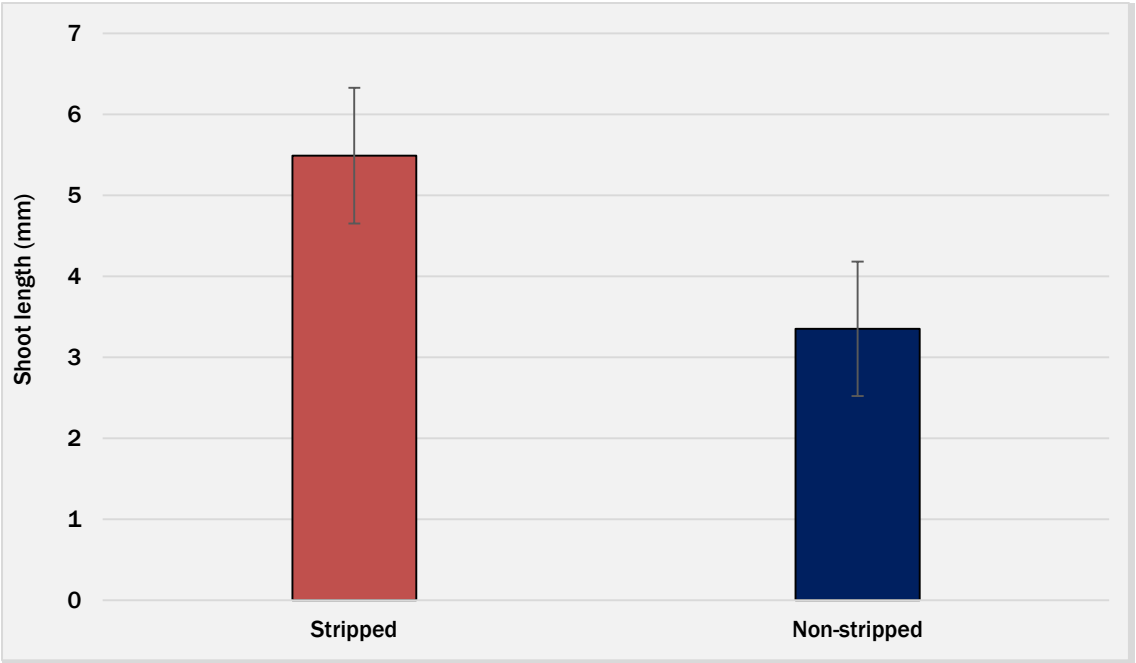

Figure 2 - Mean shoot length of germinated seeds in stripped and non-stripped pregnant cow urine. Error bars: standard error of mean; mm: millimeters.

Mean seed germination inhibition in different groups on different days after insemination

The mean seed germination inhibition in urine from pregnant (inseminated) and non-pregnant cows during days 10, 14, 21, 26, 30, 35, and 42 after insemination are presented below (Table 2). Urine from the 42 days' pregnant cows had the highest mean germination inhibition percentage (80.21±3.59), and that from 10 days' pregnant cows had the lowest inhibition (25.00±4.35). For non-pregnant cows, the highest mean germination inhibition was 29.31±6.18 on day 35, and the lowest mean value (14.58±3.84) was observed on day 10. The analysis of variance indicated a significant difference in mean germination inhibition of seeds in pregnant and non-pregnant cow urine at days 14 to day 42 (P<0.05), but not at day 10 (P>0.05). Generally, the mean germination inhibition, for maize seeds in pregnant cow urine, increased with advancing pregnancy.

Table 2 - Mean seed germination inhibition in different groups on different days after insemination.

Days after insemination \ Groups (Treatment)	Pregnant	Non-pregnant
Days 10	25.00±4.35	14.58±3.84
Days 14	33.03±2.81[a]	18.67±5.27[b]
Days 21	46.87±2.25[a]	29.17±5.27[b]
Days 26	55.21±2.86[a]	25.00±7.22[b]
Days 30	77.08±4.57[a]	22.92±5.97[b]
Days 35	66.67±3.56[a]	29.31±6.18[b]
Days 42	80.21±3.59[a]	20.83±5.27[b]

Data expressed as mean±SEM; SEM: standard error of mean; [a,b] means with difference superscripts in the same row are significantly different at P<0.05.

DISCUSSION

Pregnancy detection is an important part of good management which helps to maximise reproductive performance or productivity of cattle and the income of farmers (Dilrukshi and Perera, 2009; Okunlola et al., 2019). It allows for the detection of non-pregnant cows, thus enables farmers to make management decisions to increase reproductive efficiency, for example, culling of infertile females or re-synchronizing the females that are not pregnant (Balhara et al., 2013; Fontes et al., 2022). This shortens the calving interval which contributes to high life-time production and

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productivity in cattle; the costs of keeping non-pregnant cows on the farm without generating income are also avoided through early detection of pregnancy (Swamy et al., 2010; Balhara et al., 2013; Aswathnarayanappa et al., 2019; Okunlola et al., 2019; Fontes et al., 2022). Punyakoti test has been recognized as a door-step on-farm technology by farmers which can be performed at their homes using inexpensive materials with no special skills required (Swamy et al., 2010). Despite its relative advantage (Dilrukshi and Perera, 2009; Swamy et al., 2010), Zambia has not yet popularized this pregnancy detection technique perhaps due to the lack of awareness and/or uncertainty regarding its practicability. This is in view of the previous study which reported merely 8.7% of farmers who were aware of pregnancy detection methods, and that the level of biotechnology awareness influenced farmers' acceptance (Abigaba et al., 2022).

The current findings agreed with those of Okunlola et al. (2019) who reported a high (64.16±2.58) germination inhibition of maize seeds in pregnant cow urine. The significantly higher mean germination inhibition of maize seeds in urine from pregnant cows, for the current study, supports the narrative that Punyakoti test is a viable technique for cattle pregnancy detection in Zambia. It is in support of the previous studies, in other countries, although those studies employed seeds from various cereal species; furthermore, these studies recommended the use of this technique by farmers at local field stations to diagnose pregnancy (Rine et al., 2014; Rai et al., 2018). Of note, our results generally concurred with Okunlola et al. (2019) who reported a significant difference ($P<0.05$) in germination inhibition of maize seeds in urine from pregnant and non-pregnant cows. Various studies have reported similar findings in pigs (Kumar et al., 2017), buffaloes (Aswathnarayanappa et al., 2019), goats (Islam et al., 2014), Ewes (Constantin et al., 2021) and cattle (Skálová et al., 2017; Rai et al., 2018). Coincidentally, and similar to Okunlola et al. (2019), the current study didn't find any significant difference ($P>0.05$) in germination inhibition percentage for maize seeds grown in non-pregnant cow urine and distilled water. Scholars have attributed the higher inhibition effect of urine from pregnant cows to metabolites or other substances in urine, such as abscisic acid (ABA), auxins, and/or ovarian steroids (Hussain et al., 2016; Rai et al., 2018; Lázničková et al., 2020).

Although a plethora of studies have reported significant differences in germination inhibition of various seed species, few studies have focused on, and validated, the same for maize seeds. Moreover, maize is the most commonly grown and readily available cereal crop in Zambia; thus, their use to validate Punyakoti test for pregnancy detection among the most treasured livestock (cattle) in the country (Mumba et al., 2013; Namonje-Kapembwa and Chapoto, 2016) has been long overdue. Similar to the Zambian cattle farming mode, Swamy et al. (2010) reported that most of the times cows conceive by natural mating in their grazing fields and thus the occurrence of pregnancy goes unnoticed by the farmers. In contribution to the early pregnancy detection by cattle farmers, this study has confirmed that Punyakoti test using maize seeds can be relied on, to detect pregnancy in cattle above 26 days after insemination with inhibition of over 55.21%; although, significant inhibition difference ($P<0.05$) between pregnant and non-pregnant cow urine groups was observed from day 14. According to Oknunola et al. (2019), however, urine sample with over 60% germination inhibition effect gives an indication of pregnancy. The current findings also support Rine et al. (2014) who confirmed the test's ability to detect pregnancy by day 28 post-insemination. Considering the economic importance and improved reproductive efficiency of early pregnancy detection (Okunlola et al., 2019; Fontes et al., 2022), utilization of Punyakoti test will greatly benefit cattle farmers in Zambia and beyond.

The increasing germination inhibition with advancing pregnancy period was in agreement with a previous study (Rine et al., 2014). It is plausible that increasing levels of pregnanediol glucuronide (PdG) with advancing pregnancy was partly responsible for the increasing inhibition (Lázničková et al., 2020). Lázničková et al. (2020) previously confirmed that oestradiol-17β (E_2) and PdG were correlated with seed germination and growth. This notwithstanding, other metabolites and substances in urine, such as ABA, and plant auxins have also been associated with inhibition of seed germination and growth (Islam et al., 2014; Rine et al., 2014; Skálová et al., 2017; Rai et al., 2018), although, little is known about changes in their concentrations with advancing pregnancy. What is confirmed, however, is that pregnant cow urine has more levels of ABA than non-pregnant cow urine (Dilrukshi and Perera, 2009; Rai et al., 2018), in addition, a higher concentration of ABA leads to low germination of seed and shoot growth by way of embryo development arrest and inhibition of water uptake (Hussain et al., 2016). Still, there is a need for further studies to establish the changes in ABA levels with advancing pregnancy, and thereby justify the increasing inhibition as pregnancy advances.

As for steroids, some studies reported their influence on seed germination and growth (Erdal and Dumlupinar, 2010; Bowlin, 2014; Lázničková et al., 2020). With the controversy over ovarian steroids and plant growth factors like ABA, this study investigated the potential effect of ovarian steroids, on maize seed germination, by way of elimination approach. The current study found a significantly ($P<0.05$) higher germination inhibition percentage for seeds in stripped and non-stripped pregnant cow urine, which supports the previous reports by Lázničková et al. (2020) and Erdal and Dumlupinar (2010). However, there was no significant difference in the shoot length observed between the two treatment groups. Lázničková et al. (2020) and Bowlin (2014) reported that particular ovarian steroid(s), at different concentration(s), may or may not inhibit germination and/or shoot growth. It is plausible that the inefficiency of the stripping protocol (activated charcoal) used, in adsorbing steroids from urine, during the experiment contributed to the observed effects. On the other hand, the potential contribution, to germination inhibition, from multiple urine-containing factors could mean that only stripping urine of steroids was not enough to cause a significant effect on shoot growth. In light of this, further studies must focus on the proper stripping approach for steroid(s), as well as ABA, auxins and other potential factors to conclude on whether its auxins, ABA, steroids or specific combinations that underlay the Punyakoti test's mode of action.

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CONCLUSION

Punyakoti test is a viable technique for detecting pregnancy in cattle, with over 55% inhibition percentage beyond day 26 post-insemination. This technique relies on seed germination inhibition, caused by urine metabolites such as steroids, to detect pregnancy. Urine from pregnant cows presented higher germination inhibition than that obtained from non-pregnant cow urine or distilled water; the higher observed inhibition percentage levels was perhaps attributed to the ovarian steroids and/or other substances in the pregnant cow urine. To conclusively rule out the effect of steroids on the seed germination and growth rate, further studies are needed to check for the accuracy of the stripping procedure. Additionally, the search for more effective and reliable stripping protocols may prove beneficial. Also, a study on cattle farmers' awareness level of this biotechnology would benefit biotechnology policy formulation and implementation, and guide further research direction.

DECLARATIONS

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Authors' contribution

PC Sianangama conceived and designed the study, supervised, and reviewed manuscript; R Abigaba analysed data and wrote the manuscript; M Mtonga conceived the study, collected data, and reviewed manuscript; SJ Harrison supervised the study and reviewed the manuscript. All authors read and approved the final manuscript for publication.

Conflict of interest

Authors declare no conflict of interest regarding this publication.

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THE EFFECT OF COCONUT MEAT WASTE SUPPLEMENTED WITH THERMOPHILIC BACTERIA AND THERMOSTABLE MANNANASE ON PERFORMANCE, GUT HISTOMORPHOLOGY AND MICROBIOTA OF BROILER CHICKENS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: An experiment was conducted on Arbor Acress broiler chicks to evaluate the effects of coconut meat waste (CMW) supplemented with 10^{10} CFU/kg CMW of thermophilic bacteria (*Bacillus* sp. SM-1.4) and 800 U/kg diets of thermostable mannanase on performance and gut histomorphology of broilers. One hundred and fifty of day-old chicks (unsexed) were used in this study. The birds were fed five diets containing coconut meat waste supplemented with bacteria and mannanases (CBM); 0% CBM, 10% CBM, 20% CBM, 30% CBM, and 40% CBM. Feed and water were available at all times. A completely randomized design was applied in this experiment with five diets and five replicate cages. The parameters determined are performance (feed consumption, body weight gain, and feed conversion ratio), gut histomorphology, and microbiota of broilers. Data were analyzed by analysis of variance according to a completely randomized design and Duncan's Multiple Range Test. Data indicated that feed consumption, body weight gain, and gut histomorphology of birds fed the CMW supplemented with thermophilic bacteria and thermostable mannanase were up to 30% higher than those of birds fed the non-supplemented (0% CBM) diet. The birds fed a 20% coconut meat waste diet supplemented with 10^{10} CFU/kg diet thermophilic bacteria, and 800 U/kg CMW thermostable mannanase had higher body weight gain than others and significantly ($P<0.05$) affected on microbiota of broiler. Adding coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase did not affect the feed conversion ratio. In conclusion, up to 20%, CBM level in the diet could improving performance, gut histomorphology, and composition microbiota of broiler.

Keywords: Broiler, Coconut meat waste, Histomorphology, Microbiota, Thermophilic bacteria, Thermostable mannanase.

INTRODUCTION

Indonesia is the second biggest producer of coconut in the world, with a total production of 15.5 billion grains per year, resulting in 573.7 tons of coconut meat waste (CMW). Coconut meat waste is a residue after extracting coconut milk from coconut meat. CMW is qualitatively poor due to low concentrations of several limiting amino acids (lysine 0,081%, methionine 0,024%, tryptophan 0,002%, phenylalanine 0,065%), and high dietary fiber. Nutritionally, CMW dry matter contains crude protein 5.81%, crude fiber 20.84%, ether extract 24.59% (Harnentis and Syahruddin, 2015), carbohydrate 56.7%., lignin 1.88% (Ng et al., 2010). On a dry matter basis, CMW contains 61% galactomannan and 26% mannan (Balasubramaniam, 1976; Purawisastra, 2001).

Most feed ingredients contain some anti-nutritional factors, and the non-digested part obstructs feed utilization. Coconut meat waste (CMW) is an important alternative feed source in broiler diets because of its abundant production. Coconut meat waste (CMW) contains anti-nutritive such as mannans and galactomannans, which are non-starch polysaccharides (NSP). It can form highly viscous solutions, thus increasing the intestinal digesta viscosity. Increased gut digesta viscosity usually limits contact between digesta enzymes and substrates and between nutrients and absorption sites in the intestinal mucosa. The anti-nutritive effect is manifested by lowering nutrient utilization followed by poor growth (Nguyen et al., 2021; Johnson et al., 2022). The low use of coconut meat waste in poultry is due to the low value of its nutrition and voluminous. The low nutritional value of coconut meat waste is associated with a high content of crude fiber, crude lipid, low crude protein content, and high content of mannan and galactomannan (Rethinam and Krishnakum, 2022). At the same time, poultry does not produce the enzymes to break down fibers mannan and galactomannan. However, scarce information exists to improve the quality and use of coconut meat waste in poultry diets. Numerous studies have suggested that the results of the study showed that inclusion of mannanase enzyme and probiotics in diets containing high Non-Starch Polysaccharides (NSP) such as mannan and galactomannan could improve growth performance (Khanongnuch et al., 2006; Sundu et al., 2006; Harnentis and Syahruddin, 2015; Hsiao et al., 2022), gut histomorphology (Awad et al., 2009; Rahimi et al., 2009; Aliakbarpour et al., 2012; Mikulski et al., 2012) of broilers.

Citation: Harnentis, Amizar R, Nur YSh, and Huda N (2022). The effect of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase on performance, gut histomorphology and microbiota of broiler chickens. *Online J. Anim. Feed Res.*, 12(6): 363-371. DOI: https://dx.doi.org/10.51227/ojafr.2022.48

These show that there is an increase in the rate of hydrolysis fiber greater with the addition of the enzyme in the diet than without the enzyme (Lin and Olukosi, 2021; Lannuzel et al., 2022). Inclusion of thermostable mannanase in CMW improved digestibility of crude fiber, hemicellulose (Harnentis. et al., 2015), nitrogen retention, metabolizable energy, and gut histomorphology of broilers (Harnentis and Syahruddin, 2016).

No information on using coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase enzymes in poultry diets is available. This study was conducted to determine the effect of the use of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase enzyme in the diet on performance, gut histomorphology, and microbiota of the broilers.

MATERIALS AND METHODS

Bacteria preparation

Pure culture of *Bacillus* sp. SM-1.4 was isolated from hot springs in South Solok (Indonesian: Kabupaten Solok Selatan) is a landlocked regency (Kabupaten) of West Sumatra Province, Indonesia (Harnentis et al., 2013), which was then cultured in media nutrient broth (NB) and incubated at 60 ° C for 24 hours. Get the number of bacteria 10^{10} CFU/ml once the Standard Plate Count Agar has finished counting the bacteria. After that, the bacteria are kept in a bottle (Harnentis et al., 2013).

Production of enzymes

Mannanase thermostable enzyme produced by thermophilic bacteria (*Bacillus* sp. SM-1.4) according to the method (Harnentis et al., 2013). *Bacillus* sp. SM-1.4 were grown in a medium containing (g/L). 40.9 copra meal, NH_4NO_3 0.5, Na_2HPO_4 7.54, NaH_2PO_4 2.32, $MgSO_4.7H_2O$ 0.4, $FeSO_4.7H_2O$ 0.02, $CaCl_2.2H_2O$ 0.06, yeast extract (pH 7.0). *Bacillus* sp. SM-1.4 was utilized as the inoculum and was grown in NB medium at 60^0C on a shaker water bath (120 rpm) for 12 hours to produce the enzyme. The Minifors Benchtop Bioreactor, which has a 5 L capacity, is used to make enzymes. 10% inoculum (v/v) was added to the medium, which was then incubated at 60 ^{0}C for 18 hours before being centrifuged for 5 minutes at a speed of 5,000 rpm. After the initial enzyme activity test, the resulting supernatant is used as a crude enzyme in this study.

Composition diets

Before mixing the components of the diet with coconut meat waste, others added a mixture of crude enzyme mannanase with a dose of 800 U/kg of coconut meat waste and thermophilic bacteria (*Bacillus* sp. SM-1.4) 10^{10} CFU/kg diet (Harnentis and Syahruddin, 2015) is called the CBM (coconut meat waste supplemented with bacteria and mannanases). Diets were made using the ingredients and compositions listed in Table 1 and shaped into pellets using 3 mm die with a length of 0.5 cm.

Table 1 - Ration composition

Ingredients \ Treatments	D0	D1	D2	D3	D4
Corn	46.0	36.5	29.5	22.0	14.0
CBM[1]	0.0	10.0	20.0	30.0	40.0
Rice bran	12.0	10.0	6.5	3.0	0.0
Japfa BR I[2]	10.0	10.0	10.0	10.0	10.0
Soybean meal	11.5	13.0	13.5	14.5	15.5
Fish meal	20.0	20.0	20.0	20.0	20.0
Top mix[3]	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Calculated					
Crude protein (%)	22.28	22.40	22.16	22.0	22.0
ME (kcal/ kg)	3000	3000	3000	3000	3000
Crude fiber (%)	3.80	5.54	7.09	8.66	10.28
Lipid (%)	4.65	6.51	8.25	9.98	11.75
Calcium (%)	0.60	0.61	0.61	0.61	0.62
Phosporus - available (%)	0.35	0.35	0.34	0.34	0.33
Lysine (%)	1.29	1.28	1.28	1.27	1.26
Methionine (%)	0.49	0.48	0.48	0.47	0.46
Tryptophan (%)	0.24	0.23	0.23	0.22	0.21

[1] Coconut meat waste + Bacteria + Mannanase, [2] commercial feed from Japfa Comfeed Indonesia Tbk, [3] Top mix provided (in mg/kg): vit A 1200000 IU; vit D_3 200000 IU; vit E 800; vit K_3 200; vit B_1 200; vit B_2 500; vit B_6 50; vit B_{12} 1200µg; vit C 2500; Ca-D pantothenate 600; niacin 4000; choline chloride 1000; methionine 3000; lysine 3000; manganese 12000; iron 2000; iodine 20; zinc 10000; cobalt 20; copper 400; santoquin 1000; zinc bacitracin 2100

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Table 2 - Experimental diets	
Diets*	**Treatments**
D0 = 0% CBM	No *Bacillus* sp. SM-1.4 and mannanase
D1 = 10% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D2 = 20% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D3 = 30% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D4 = 40% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
*CBM = Coconut meat waste + Bacteria + Mannanase	

Experimental design

One hundred fifty of day-old chick (DOC) broilers (unsex) were placed on a floor pen from 1 to 6 days and given a control diet. After six days (the initial 6-day period), 125 chickens were weighed between 98 and 116 g (107.0 ± 0.65; mean ± SE) selected to minimize animal variations and transferred into 20 units box and lighted. Each box contains five chickens and is maintained until six weeks. The design was completely randomized with five levels of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase (0, 10, 20, 30 and 40%) with five replications. Diets were formulated in iso-nitrogenous (22% crude protein) and isocaloric (3000 kcal/kg), as presented in Table 1. Diets and water were given ad libitum. The five diets imposed are described in Table 2.

Histological preparations

Making prep diets for histological performed in chickens aged 42 days. Duodenal each chicken cut along the 3 cm and then inserted into 10% buffered formalin for 24 hours. After 24 hours, the sample was made histological preparations with Hematoxylin-eosin staining. Villi length and width measurements are done by shooting prepadiets with magnification four times and then measuring with a predetermined scale (Incharoen et al., 2010).

Viscosity

Digesta was collected from each chicken's duodenum, jejunum, and ileum for viscosity measurements. Digesta diluted (1.1) with distilled water and homogenized for 20 minutes at room temperature, then centrifuged at 3500 rpm for 15 minutes. The viscosity of the supernatant was measured at a temperature of 29°C and 60 rpm using a viscometer with coaxial cylinders (model NDJ-8S; Piel et al., 2005).

Microbial count

Digesta was collected from each bird's duodenum, ileum, caecum, and colon for the microbial count, stored in a sterile tube, and cooled at 4°C (Cowan, 2004). Digesta was mixed in a 10 ml pre-reduced salt medium and serially diluted according to the procedure described (Engberg et al., 2004) to examine the count of Lactobacilli (Rogosa, CM 0627, incubated anaerobically 48h) and coliforms (Mackonkey, CM 0115, incubated aerobically 24h). Gut tissue samples were serially diluted from 10-3 to 10-7, and 0.1 ml of each dilution was spread evenly on a medium for enumediet bacteria.

Organ weights

On day 42, one bird per replicate was sacrificed through cervical dislocation. They were subsequently opened, and the liver, heart, gizzard, and pancreas were Harvested and weighed.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) on a completely randomized design according to (Steel and Torrie, 1991). Duncans Multiple Range Test (DMRT) was used to determine differences between diets (Steel and Torrie, 1991).

Ethical regulations and considerations

The experimental procedures were approved by the Universitas Andalas Animal Care and Use Committee (Padang, Indonesia; NO. 1020/UN.16.2/KEP-FK/2022). This research was conducted in the poultry research enclosure of the Teaching Farm of the Faculty of Animal Science, Universitas Andalas.

RESULTS AND DISCUSSION

The effect of treatment on performance broiler

The effect of diets on feed consumption is presented in Table 3. Diets significantly affect feed consumption. Feed consumption increased with increasing levels of CBM up to 20%, and then decreased with increasing up to levels of 40% CBM. The highest feed consumption was obtained at the level of CBM 20%, while feed consumption to the level of CBM 40% is not different from control diet (0% CBM), as well as feed consumption in the D2 diet (10% CBM) had no significant ($P>0.05$) with D3 diet (30% CBM). The diet significantly affected the body weight gain of broilers ($P<0.05$). The body weight gain broiler increased with increasing levels of CBM to the level of 30% in the diet and significantly higher than the control and D4 diet (CBM 40%). Usage levels of CBM 10% have a significantly different effect ($P<0.05$) with the use of CBM 20%, but had no significant with the use of CBM 30%, while the use of CBM 40% in the diet had no significant ($P>0.05$) with a control diet 0% CBM. The feed conversion ratio is presented in Table 3. Feed conversion of broiler fed the different levels of CBM had no significant ($P>0.05$) on feed conversion of broiler chickens.

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Table 3 - Feed consumption, body weight gain, and feed conversion

Performance \ Treatments	D0	D1	D2	D3	D4
Feed consumption (g/week)	548.34[c]	589.0[b]	598.08[a]	589.68[b]	533.88[c]
Body weight gain (g/week)	296.4[c]	322.1[b]	337.9[a]	324.0[b]	296.6[c]
Feed conversion	1.85	1.83	1.77	1.82	1.80

[a,b,c] Means within a lines with different superscripts differ significantly (P<0.05)

Several studies have shown that including mannanase enzymes in the diets containing high NSP, such as mannan and galactomannan, could improve feed consumption and body weight gain of broilers (Pluske et al., 1997; Sundu et al., 2006). This shows that there is an increase in the rate of hydrolysis fiber more significant with the addition of the enzyme in the diet. In this study, feed consumption increased with increased levels of coconut meat waste supplemented with thermophilic bacteria as probiotic and thermostable mannanase up to 20% (20% CBM) and a decrease in the level of 30% CBM. With increasing levels of CBM in the diet, the amount of enzyme so that more coconut meat waste is hydrolyzed by mannanase, which ultimately accelerates the rate of digestion. This result is different from that reported Agboola et al. (2015) there is no effect of probiotic supplementation and carbohydrase enzyme in the diet based on wheat-soybean on feed consumption of broilers.

No information is available about the use of coconut meat waste supplemented with thermophilic bacteria and mannanase thermostable enzymes in the pellet form diet on the performance of broiler chickens. However, compared with studies on the diets containing feedstuff in high NSP such as coconut meal and palm kernel cake, coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase improved feed consumption and was higher than coconut meal and palm kernel cake. The results of this study, similarly to those reported Sundu et al (2006), showed an increase in the feed consumption of broiler 4-14 days old fed diets containing coconut meal supplemented with mannanase. Adrizal et al. (2011) reported an increase in feed consumption with increasing levels of palm kernel meal from 0% - 15% and 30% in laying hens. Higher feed consumption in foods fed chicken containing palm kernel flour also (Sundu et al., 2005; Ezieshi and Olomu, 2008). This is caused by its faster passage rate in the digestive tract (Onifade and Babatunde, 1998), high bulk density, and low water holding capacity (Sundu et al., 2006). The lower feed consumption in the D0 diet (0% CBM) is caused by pellet durability because of the composition of corn and soybean meal, which causes high amylose content, resulting in a high gelatinization degree in pelleting. This pellet's durability will affect the work gizzard, so the diet is longer in the gizzard. Consequently, the passage rate of feed will be lower. The lower feed consumption D0 (0% CBM) is due to the high viscosity of the corn and soybean meal-based diet, so the rate of feed passage is low. At the same time, the lower feed consumption in the D4 diet (40% CBM) results from the voluminous nature of coconut meat waste. Most of the probiotics in the diets had no significant effect on broiler feed consumption if compared without probiotics (Applegate et al., 2010; Mikulski et al., 2012; Agboola et al., 2015).

Feeding the CBM 10% and 20% in the diet increases the body weight gain of broilers. This is closely related to the supplementation of thermophilic bacteria (*Bacillus* sp. SM-1.4) as a probiotic and thermostable mannanase enzyme. Probiotic supplementation's beneficial effects on the broiler diet lead to an increase in body weight gain, and the feed conversion ratio is documented in a study of several research groups (Timmerman et al., 2006; Onderci et al., 2008; Bansal et al., 2011). According to Applegate et al. (2010), giving probiotics to birds fed at six weeks had a significantly higher body weight gain than the chickens fed a diet without probiotics. The body weight improvement at the end of the growth phase caused by probiotic supplementation will reduce the phatogen bacteria colonies, thereby reducing nutrient competition and lowering toxic compounds or immune system modulation. El-Nagmy et al. (2007) has also stated that the increase in body weight gain of broilers is associated with the effect of probiotics in improving nutrient absorption and reducing the harmful bacteria that cause suppression of growth. Several research results support the results of this study (Kabir, 2009; Rahman et al., 2013).

The effect of supplementation of poultry fed with mannanase enzyme has been shown by several studies (Daskiran et al., 2004; Sundu et al., 2005; Zou et al., 2006; Williams et al., 2014). Abdollahi et al. (2016), reported that supplementation of the exogenous enzyme (mannanase, xylanase, protease, amylase, cellulase, and β-glucanase) in a diet containing 24% Palm Kernel Meal (PKM) impaired weight gain and feed efficiency of broilers. In this study, broilers increased body weight gain with increasing levels of CBM in the diet up to the level of 30%. Increased levels of CBM cause it to produce greater mannose and mannooligosaccharide (MOS). This MOS is a result of mannan hydrolysis by mannanases, so that more energy contribution, and MOS as a prebiotic that can stimulate the growth of bacteria on the ceca, as reported Baurhoo et al. (2009), supplementation of MOS in broilers diet produce in higher Bifidobacteria concentration in their ceca in conjunction with in an increase in villus length and the goblet cells count in the segment of intestine. There was also an increase in the other nutrients, such as protein and intracellular lipids. Adding enzymes degrading mannan causes the digestive enzymes to digest the cell contents easily. The endosperm cell walls close the proteins and lipids that inhibit intra-cellular digestion and absorption (Knudsen, 1997). The digestibility of crude fiber, hemicellulose digestibility, nitrogen retention, and energy metabolism corrected by nitrogen (MEn) increases (Harnentis and Syahruddin, 2015). In turn, it affects the increase in broilers' weight gain. It is also supported by the supplementation

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of thermophilic bacteria (*Bacillus* sp. SM-1.4), as probiotics improve intestinal morphology and animal health to increase nutrient absorption. Improvement in weight gain of broilers by probiotics and these mannanases for improvement of growth caused by the two feed additives. Probiotics ultimately improve nutrient efficiency by reducing competition between host and intestinal microbial populations, thus providing a favorable effect on livestock health by improving intestinal microbial balance. Jackson et al. (2004), reported improved body weight and feed efficiency in broiler chickens fed corn-soybean-based diets supplemented with β-mannanase.

Effect of treatment on intestinal villi length and width of broilers (gut histomorphology)

The effect of diet on intestinal villi length and width of broilers are presented in Table 4. The diet affected the length and width of the villi duodenum significantly (P<0.05). Duodenal villi length and width increased with increasing levels of use of CBM in the diet. The length of the diet of duodenal villi D2 diet (20% CBM) had no significant (P>0.05) with the D3 diet (30% CBM) and was significantly higher (P<0.05) compared with other diets, while the lowest duodenal villi length obtained in the diet of D0 (0% CBM). Duodenal villi width at D2 diet (20% CBM) had no significant (P>0.05) with D3 diet (30% CBM) and was significantly higher (P<0.05) compared with other diets, while the width of the duodenal villi D1 diet (10% CBM) and D4 (40% CBM) had no significant (P>0.05) compared with controls.

Table 4 - Effect of treatment on intestinal villi length and width of broilers

Intestinal \ Treatments	D0	D1	D2	D3	D4
Villous length (µm)	608.5^{c}	1016.3^{b}	1044.8^{a}	1038.2^{a}	1019.6^{b}
Villous width (µm)	78.9^{b}	80.9^{b}	89.2^{a}	86.9^{a}	80.6^{b}
Digesta viscosity (dPas)	0.39	0.32	0.30	0.27	0.30

^{a,b,c} Means within a lines with different superscripts differ significantly (P<0.05)

Current studies suggest that adding thermophilic probiotics has improved intestinal villus length (Chichlowski et al., 2007; Awad et al., 2009; Rahimi et al., 2020). In this study, the intestinal villus in coconut meat waste treated with thermophilic bacteria and mannanase thermostable enzyme supplementation improved villus length and width more than without supplementation. It cannot be apart from two additives, namely thermophilic bacteria Bacillus sp. SM-1.4 as probiotic and mannanase thermostable. Improving intestinal villus length and width increases the digestive and absorption function of the intestine (Caspary, 1992) and can improve digestion by increasing the surface area for absorption and enhancing the nutrient transport system because it increases the production of digestive enzymes (Awad et al., 2009; Rahimi et al., 2009; Rahimi et al., 2020). In this study, coconut meat waste hydrolyzed by mannanase enzyme results from mannose and MOS. According to Sims et al. (2004), feeding MOS could improve intestinal villus length and width in Turkey poults. An increase in the length and width of the villus results because the fermentation of MOS by microflora in the cecum and colon produces short-chain fatty acids, especially butyric acid, which could increase intestinal proliferation villus (Ferket et al., 2002). An enhancement of intestinal villus length in the chicken by administering probiotics *Bacillus* subtilis is also reported by Samanya and Yamauchi (2002).

Broiler digesta viscosity was not affected by the level of use of CBM in the diet. Although statistically not significant, numerically digesta viscosity decreased with increasing levels of CBM in broiler diets. High digesta viscosity reduces the rate of substrate diffusion and digestive enzymes and inhibits their effective interaction with intestinal mucosa (Ikegami et al., 1990). The Inclusion of cereals rich in NSP increases the digesta viscosity, bacteria profile, and gut physiology (Aliakbarpour et al., 2012; Zduńczyk et al., 2020). In this research, coconut meat waste content is higher in insoluble NSP compared with soluble NSP, so not much impaired the viscosity, coupled with supplementation of mannanase, resulting in lower digesta viscosity.

Effect of treatment on microbiota digesta

The effect of diet on microbiota digesta showed that in Table 5. The diet significantly affected the broiler's total bacteria count in intestinal on the duodenum, ileum, caecum, and colon (P<0.05). The intestinal Total Plate Count (TPC) of the broiler decreased with increasing levels of CBM to 40% in the diet and significantly higher than in the control diet. The effect of diet on *E. coli* is presented in Table 5. *E. coli* of broilers significantly (P<0.05) affected by the diet. *E. coli* of broilers increased with increasing levels of use of CBM in the diet to the level of 40%. *E. coli* of broilers in the diet of D2 (20% CBM) had no significant (P>0.05) with D3 of diet (30% CBM) and significantly lower (P<0.05) compared with other diets. *E. coli* in the D1 diet (10% CBM) had no significance (P>0.05) with the D4 diet (40% CBM) and was significantly higher (P<0.05) than in the D2 and D3 diets.

The effect of diet on Lactic acid bacteria (LAB) is presented in Table 5. The diet significantly affected intestinal LAB intestines of broilers (P<0.05). Intestinal LAB of broilers increased with increasing levels of use of CBM in the diet to the level of 40%. Intestinal LAB of broilers in the D2 diet (20% CBM) had no significance (P>0.05) with the D3 diet (30% CBM) and was significantly higher (P<0.05) than in other diets. LAB on D1 diet (10% CBM) had no significant (P>0.05) with D4 diet (40% CBM) and significantly lower (P<0.05) than D2 and D3 diet.

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Table 5 - Microbiota digesta of broilers

Microbiota \ Treatments	D0	D1	D2	D3	D4
TPC (10^6 cfu/g)[1]	4.1[a]	3.3[b]	3.0[b]	2.9[b]	3.0[b]
E. coli (10^5 cfu/ g)	3.6[a]	3.1[b]	0.5[c]	1.3[c]	2.9[b]
LAB (10^8 cfu/g)[2]	0.4[c]	1.3[b]	2.8[a]	2.2[a]	1.4[b]

[a,b,c] Means within a lines with different superscripts differ significantly (P<0.05), [1] TPC = Total Plate Count, [2] LAB = Lactic Acid Bacteria

The TPC and *E. coli* count decreased with increasing levels of use of CBM in the diet, otherwise with LAB count. Several researchers reported the influence of probiotics (Higgins et al., 2008; Vilà et al., 2009) and enzymes (Bedford, 2000) on microbial populations in poultry. In this study, supplementation of thermophilic bacterium *Bacillus* sp. SM-1.4 and mannanase thermostable improved the health of the broiler, where lowering *E. coli* count. The *E. coli* count determines the presence and population of the pathogen in the intestine. The *E. coli* count was reduced in the ileum of broiler chicken supplemented with probiotics and mannanase enzymes, as reported by Agboola et al. (2014), which lowered the number of *E. coli* in the ileum of turkey given the probiotics and symbiotic diets. The result of this study, the decline *E. coli* count was followed by an increase in the LAB count in digesta of the broiler. According to Kizerwetter-Swida and Binek (2009), the Lactobacilli reduce coliform with competitive exclusion. The results of the number of E. coli in this study showed an effective modulation of intestinal microflora and significant obstruction of microflora pathogenic with the competition of nutrients, creating conditions and producing antimicrobial (free fatty acids, low pH, and bacteriocin), competition of site locations at ephitel intestines and immune system stimulation (Jin et al., 1996). The enzyme reduces the number of bacteria by increasing the rate of digestion and restricting the number of available substrates to the microflora in the ileum (Bedford, 2000). The mannanase supplemented with coconut meat waste hydrolyzes mannan and results from mannose and mannooligosaccharide (MOS). MOS can increase the use of nutrients by stimulating the population of certain bacteria in the digestive tract, such as Lactobacillus and Bifidobacterium (Jana et al., 2021). Addition of *Bacillus* sp. SM-1.4 can reduce *E. coli* colonization and TPC and improve the LAB. As well as, the addition of *Bacillus* subtilis in drinking water can reduce the colonization of *E. coli*, Salmonella enteritidis, and Clostridium perfringens in the chickens (Griggs and Jacob, 2005). Besides that, *Bacillus* subtilis can also increase the proliferation of Lactobacillus, which produces lactic acid so that it can control the bacterial pathogen.

Effect of treatment on physiological organs

The effect of diet on the percentage of broiler chicken's liver, heart, gizzard, and pancreas is shown in Table 6. The use of the different levels of CBM in the broiler diet had no significant effect (P>0.05) on the liver, heart, gizzard and chicken pancreas broiler percentage. Some of the study results found that the relative weight of the physiological organs of poultry is not affected by the feeding of probiotics (Konca et al., 2009; Agboola et al., 2014). In this study, liver, heart, gizzard, and pancreas weight were not affected by the CBM level in the diet, but numerically the relative weight of liver and heart tended to decline, and the relative weight of gizzard and pancreas tended to increase with increasing CBM levels in the diet. According to Iyayi and Yahaya (1999) relative weight of the liver, spleen, and heart are not affected by enzyme supplementation in broiler diets.

Table 6 - Percentage of physiological organs of broiler

Physiological organs \ Treatments [NS]	D0	D1	D2	D3	D4
Liver (%)	2.37	1.77	1.81	1.81	1.76
Heart (%)	0.52	0.48	0.45	0.42	0.43
Gizzard (%)	2.81	2.79	3.25	3.27	3.56
Pancreas (%)	0.20	0.16	0.18	0.20	0.23

NS = not significant

CONCLUSION

It was concluded that coconut meat waste supplemented with thermophilic and thermostable mannanase shows potential for improving the performance, gut histomorphology, and composition microbiota of broiler in the pellet form diet containing up to 20% CBM level.

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DECLARATIONS

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Authors' contribution

HARNENTIS, Robi AMIZAR, Yuliaty Shafan NUR, and Nurul HUDA contribute on experiment, data analysis and the write up of the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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ANALYSIS OF PHYSICAL AND MICROBIOLOGICAL QUALITY OF RAW CAMEL MILK IN THE SOMALI REGIONAL STATE OF ETHIOPIA

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ABSTRACT: The objective of this study was to determine the physical and microbial quality of raw camel milk along the milk market chain a total of forty-two raw milk samples were taken from milk producers (21 samples) and milk collection centers (21 samples). Each sample was analyzed for physical and microbial quality including temperature, pH, titratable acidity, specific gravity, and clot on boiling, the overall mean and standard deviation values were 27.93 ^{o}C, 6.29, 1.030 g/cm^{3}, 0.95%, and 88.1% respectively. Microbial quality and safety attributes that include total bacteria count, coliform count, and yeast and mold counts were analyzed. The overall mean $\log_{10}$ counts per ml and standard deviation values for each total bacterial count, coliform count, and microbial analysis were 7.48 $\log_{10}$ CFU/ml, 5.85 $\log_{10}$ CFU/ml, and 4.78 $\log_{10}$ cfu/ml, respectively. The total bacterial count, coliform count, yeast, and mold counts were calculated and show that the milk collection center samples were significantly higher than milk samples obtained from household producers. This study indicated that the quality of camel milk in the study area had low quality and this could cause public risks through the consumption of raw camel milk produced and sold under the present production and handling conditions along the chain. Thus, these calls for strict hygienic measures to improve the quality and safety of camel milk produced and marketed in the study area.

Keywords: Camel milk, Hygiene, Microbial quality, Raw milk, Somali Regional State.

INTRODUCTION

The global population of domesticated large camelids (*Camelus dromedaries* and *Camelus bactrianus*) is estimated to be about 28 million (Faye, 2015). More than 80% of the camel population inhabits Africa with 60% in the Eastern African countries which are important exporters of dromedary camels to the Arabian Peninsula and Egypt (Faye, 2015). The camel population in Ethiopia is estimated at 4.8 million (Behnke, 2010). Milk is susceptible to contamination with pathogenic microorganisms from the time it is milked until it reaches the consumer. The hygienic quality of milk and dairy products is dependent upon the quality of the raw milk and the conditions under which the milk is produced (Carloni et al., 2016; Kaskous, 2019; Ayuob et al., 2020).

Microbial contamination in milk may cause milk-borne diseases in humans (Berhe et al., 2020; Kakati et al., 2021), while others are known to cause milk spoilage (Fusco et al., 2020). Sources of microbial contamination in milk include primary microbial contamination that comes from the infection or mastitis in lactating animals. The secondary causes of microbial contamination occur along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment, and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation, and storage. Poor or improper handling of milk can exert both public health and economic constraints thus requiring hygienic vigilance throughout the milk value chain (Swai and Karimuribo, 2011).

In the Gursum district Somali region of the study areas, camel milk is produced in traditional ways and camels are milked by hand. Then the milk is handled under poor hygienic conditions and transported a long distance (up to Jigjiga town) where it is sold on the street (open market) or distributed to retailers. Thus, milk transported and handled under such conditions may have poor quality and contain pathogenic microorganisms of public health concern. Therefore, studying the quality and safety of raw camel milk along the milk marketing chain, i.e., from the production site until it reaches the milk collection centers site is very important in that the results generated will be used to devise appropriate intervention strategies aimed at improving the quality and safety of camel milk produced in the study area.

MATERIALS AND METHODS

Description of the study area

Somali Region is the second largest region in Ethiopia. It borders Djibouti in the north, Somalia in the east and northeast, and Kenya in the south. In the west, it borders Oromiya Region, and in the north-west Afar Region. The specific survey area of the study was Gursum Woreda, Fafan zone, Somali region.

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Methods of data collection

The study conducted among producers and milk collection centers involved experimental work on the physical and microbiological qualities of raw camel milk samples in the study area. All of procedures are in according to animal welfare rules and hygiene consideration according to ethical rules of Department of Animal and Range Science of Haramaya University and Food Science and Nutrition Research Directorate.

Milk sample collection

A total of three potential Kebeles, namely Bombass, Tikdam, and Kobijaro, were selected purposively from 18 Kebeles in the Gursum Somali district due to their high potential in camel milk production and the common practice of marketing camel milk. Using simple random sampling (lottery method), a total of 42 milk samples were collected for physical and microbial quality analysis (14 samples from each Kebele). In each Kebele, 7 samples from households and 7 samples from milk collection centers were collected. Approximately 15 ml of morning raw milk samples were collected using sterile bottles from each household and milk collection center for a period of three months. All the samples were then transported to the laboratory of dairy microbiology at Jigjiga University using an insulated icebox without delay.

Physical analysis of milk

Titratable acidity

The percentage acidity of the milk was measured by titrating ten milliliters of raw milk sample with 0.1 NNaOH to the phenolphthalein endpoint as described in (O'Connor, 1994). Ten ml of raw milk sample was pipetted into a beaker, and then 3 to 5 drops of 1% phenolphthalein indicator were added into the milk. The samples were titrated with 0.1 N NaOH until the faint pink color persisted. The titratable acidity was calculated using the following formula:

PH

The pH of milk was recorded using a digital pH meter. The instrument was at first calibrated using a buffer of pH 7.0 and 4.0 before measuring the pH of milk samples.

Temperature

The temperature of the milk samples was measured using an electronic thermometer.

Specific gravity

Measurement of milk density was done by using a lactometer. A fresh milk sample was first filled sufficiently into a glass cylinder (100 ml capacity) (O'Mahony, 1988). The following formula was used to calculate the milk-specific gravity.

Specific gravity = $\frac{L}{1000} + 1$

Where, L corrected the lactometer reading at a given temperature i.e. for every degree above 15.56 C^0, 0.2 degrees were added, but for every degree below 15.56 C^0, 0.2 degrees was subtracted from the lactometer reading.

Clot on boiling test

Five ml of milk was placed in a test tube and held in an alcohol flame for five minutes. Then the test tube was carefully removed from the flame and examined for precipitate (O'Mahony, 1988).

Microbiological analysis

For the microbial quality tests, Standard plate count, Coliform Count, and Yeast and Mould count were used. Serial dilutions of milk samples were plated on plate count agar, violate red bile agar (HIMEDIA), and potato dextrose agar (MICOGEN) for standard plate count, coliform count, and yeast and mold count, respectively. Media used for the determination of standard plate count, Yeast, and Mould count, and those used for serial dilution (peptone water) were sterilized by autoclaving at 121 ^oC for 15 minutes, while the media used for determination of CC (violet red bile agar) were sterilized by boiling on a hot plate. All plates used for the enumeration of total bacteria and coliform bacteria were incubated for 48 and 24 hrs at 30 ^oC and 37 ^oC, respectively. In contrast, those used for the enumeration of yeasts and molds were incubated at 22-25 C^o for 5 days (Richardson, 1985).

Standard plate count

The total bacterial count was done using the pour plate method. Standard Plate Count Agar was used to determine the extent of microbial contamination of milk before any processing was done. One milliliter (1 ml) of milk sample was serially diluted up to six dilutions using six test tubes each containing 9 ml of peptone water (ratio of 1:10). Sterile duplicate glass Petri dishes were labelled corresponding to each dilution. One ml of the dilutions was then aseptically withdrawn using a sterile 1 ml Pasteur pipette and delivered into a sterile Petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring about 15 ml of standard plate count agar which had been autoclaved at 121 °C for 15 min, cooled, and tempered in a water bath at 50 °C. The sample and the agar were gently swirled by an alternate clock and anti-clockwise rotations and left to solidify on the bench for about 30 min. The plates were inverted and incubated at 30°C for 48 h. After incubation, the plates inoculated with the dilution yielding between 30 and 300 colonies were counted. Colony counts were made using a colony counter (Richardson, 1985).

Estimation of the CFU/ml

The average colony count from the duplicate plates was used to estimate the total number of colony-forming units per ml (CFU/ml) using the following formula:

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$$N = \frac{\sum c}{[(1xn_1) + (0.1xn_2)]d}$$

Where, N = number of colonies per milliliter or gram; ΣC = sum of all colonies on all plates counted; n1 = number of plates in lower dilution counted; n2 = number of plates in the next highest dilution counted however, plates from two consecutive decimal dilutions yield colony counts of 30 to 300, the counts for each dilution was computed (APHA, 1992).

Total coliform counts

One ml of milk sample was added into a sterile test tube having 9 ml of peptone water. After mixing, the sample was serially diluted up to 10^{-5} and duplicate samples (0.1 ml) were spread plated on solidified Violet Red Bile Agar and evenly spread. The plates were then incubated at 32 ^{0}C for 24 hours. Finally, colony counts were made using a colony counter. Typical dark-red colonies were considered as Coli form colonies (Richardson, 1985). The total coliform counts were also expressed in CFUs/ml computed using the first formula shown above for total bacterial count.

Yeast and mold counts

For the enumeration of yeasts and molds potato dextrose agar spread plates were used. Here, the plates were, however, incubated at 22-25 C^o for 5 days. Either the first or the second formula was used for computing the CFU/ml of yeasts and molds as indicated for the determination of the TBC. The following models will be used to analyze the physical and microbiological quality of milk: 1, Model. $Y_{ij} = \mu + \beta_j + e_{ij}$

Where, μ =is the overall mean, Y_{ij} = refers to individual observation, β_i is the source effect i^{th} (i=1,2), e_{ij} is the error term.

Statistical analysis

Data recorded during sampling and laboratory findings were entered and stored in a separate Microsoft Excel spreadsheet. Mean and standard deviations were calculated for the physical quality of raw milk. Analyses of variance were performed. Microbial counts of raw milk samples were first transformed to logarithmic values (log_{10}) before statistical analysis by using general linear model procedures of SAS version 8.2 (SAS, 2001). For each experiment mean comparisons were done separately using the least significant difference for variables whose F-values showed significant differences at a 5 % significance level.

RESULTS AND DISCUSSION

Physical quality of raw camel milk

Specific gravity: the value of specific gravity of milk samples collected from the household and the milk collection centers were in the range of 1.028 g/cm^3 to 1.032 g/cm^3, respectively. The current results also show a significant difference (P<0.05) in density values between milk samples obtained from households (1.028g/cm^3) to milk collection centers (1.032 g/cm^3) (Table 1) the density of milk among others is commonly used for quality test mainly to check for the addition of water to milk or removal of cream addition of water to milk reduces milk density, while removal of cream increases it (O'Connor, 1994).

Table 1 - Physical properties (means ± SE) of camel milk produced and marketed (n=42)

Parameter	Household (n=21)	Milk collection center (n=21)	Overall mean (SE)
Specific gravity	1.028±0.00045^a	1.032±0.00045^b	1.03±0.00045^{ab}
Temperature	24.10±0.93^b	31.9±0.93^a	28±0.93^b
pH value	6.5±0.088^b	5.9±0.088^b	6.26±0.48^{ab}
TA (%LA)	0.83±0.043^a	1.07±0.043^a	0.95±0.043^a

Means are significantly (P<0.05) different; n=Number of samples; pH=, Hydrogen ion concentration; N=numbers; TA=titratable acidity; LA= lactic acid: $^{a, b, ab,}$ means in the same column with different letters show significant differences (p<0.05) among household and milk collection centers.

As indicated in Table 1, showing that the mean ± SE value of the physical quality of raw camel milk in the Gursum district. The temperature of milk samples collected from households and milk collection centers in the Gursum district were 24.10 and 31.9, respectively. The temperature of the milk samples collected at the household was significantly (P<0.05) lower than the temperature collected from milk collection centers in the Gursum district. The temperature difference of the milk along the chain might be because of the environment and exposure to sunlight. The pH of the milk samples progressively decreased from the time milk is collected from the household until it reaches the milk collection centers while the acidity follows the reverse trend. The pH of the milk samples collected from milk collection centers in the Gursum district was significantly (P<0.05) lower than the pH of milk samples collected from the household were 5.9, and 6.5 respectively with an average mean and standard division of 6.26±0.48. Milk pH indicates milk hygiene and it

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usually ranged between ≤6.6 and ≥6.8 when milk temperature is 20 °C because cooling of milk reduces the risk of growth of bacteria while high milk temperature is considered favorable for the growth of bacteria in milk (Walstra, 1999).

The decreased pH of the camel milk samples at the final market indicates increasing in acid production due to the multiplication of microorganisms in the milk during transportation and storage in milk collection centers. Tetra table acidity is a measure of freshness and bacterial activity in milk. Popescu and Ange (2009) reported that high-quality milk has to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one sample to another. (Baloch et al., 2018) reported the acidity of camel milk expressed interns of lactic acid contents varied between 0.12 to 2 g/100 with an average of (0.18 ±0.01 g per 100 g). The tetra table acidity value of milk samples from milk collection centers was significantly higher than the titratable values of milk from the household producers (P<0.05), indicating high bacterial activity in the former milk samples. According to a recent study lowest value was 0.83% for household milk producers and the highest value was 1.07% for milk collection centers indicating its relatively high acidity. The Clot-on-boiling test of milk samples collected from the Gursum district at household producers and milk collection centers showed negative results.

Microbiological qualities of raw camel milk samples

Bacteriological analysis

The bacteriological test considered for the determination of the bacterial load in raw camel milk samples was standard plate count, coliform count, and yeast and mold count.

Standard plate count

In this study, the magnitude of the microbial load and types of contaminant microbial groups in camel milk are presented (Table 2). The overall mean value of total bacterial counts of milk samples obtained from producers was significantly lower than (P<0.05) of the milk samples obtained from milk collection centers in the Gursum district. The total bacterial count determined from the milk samples was 7.43±0.019 $\log_{10}$ CFU/ml and 7.53±0.009 $\log_{10}$CFU/ml, for household and milk collection centers, respectively. The overall mean value of total bacterial count was 7.48±0.014 as seen from figure 1 $\log_{10}$ CFU/ml. This result generally disagrees with those reported by Younan and Abdurahman, (2004), El-Ziney and Al-turki, (2007) and Omer and Eltinay (2008).

This increase in total bacterial count might have occurred along the chain as the milk was transported from the households until it reached the terminal market of milk collection centers. These depended on several parameters such as the milk itself, contamination of the camels' udders, milking personnel, and other considerations such as transportation and the nature and hygienic conditions of containers. This indicates that the hygienic quality of raw milk sold by retailers was generally poor and this could most probably be due to improper handling of milk during transportation and storage. High total bacterial counts in raw milk mainly reflect the poor hygienic condition under which the milk was handled at storage temperature and time elapsed since milking and the poor health of the milking animal (Chambers, 2005). Moreover, factors that could contribute a lot to the contamination of milk in this study include lack of knowledge about clean milk production, absence of a separate place for milking, use of non-boiled water for cleaning milking equipment and storage containers, using plastic containers (since plastic containers scratch easily and provide hiding places for bacteria during cleaning) and milk residues on equipment surfaces provide nutrients for growth and multiplication of bacteria that could then contaminate the milk. In general, the higher bacterial counts might be an indication of a diseased udder, unsanitary handling of milk, or unfavourable storage temperatures (Fikrineh et al., 2012).

Table 2 - Microbial quality ($\log_{10}$ cfu/ml) (means ± SE) of camel milk produced and milk collection centers

Parameter	Household (n=21)	Milk collection center (n=21)	Overall mean (SE)	P-value
SPC	7.430.019^{b}	7.53±0.009^{b}	7.48±0.014^{a}	0.0003^{ab}
CC	5.6±0.042^{a}	6.07±0.043^{b}	5.85±0.042^{a}	0.0001^{ab}
YMC	4.20±0.199^{a}	5.36±0.142^{a}	4.78±0.170^{b}	0.0001^{a}

Means followed by different (P<0.05); SPC=Standard palate Count; CC=Coli form count; YMC=Yeast and mold Count; SE= standard error; N= number of respondents. $^{a, b, ab}$, means in the same column with different letters show significant differences (P<0.05) among household and milk collection centers: SPC=standard plate count; CC: coliform count bacteria and YMC=yeast and mold count bacteria.

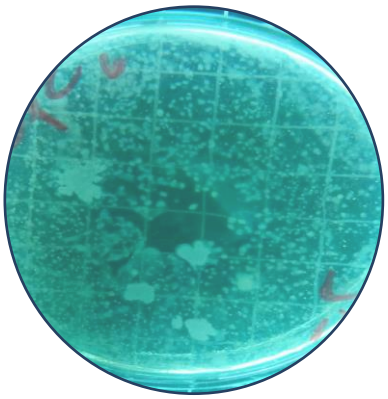

Figure 1 - Standard plate count.

Coli form count

The analysis of variance in Table 2 suggested that there was a significant difference (P<0.05) in coliform count between the two milk sources. The mean of household producers is 5.6±0.042 and milk collection centers are 6.07±0.043. As indicated figure 2 the overall mean of coliform counts of raw milk samples (5.85 $\log_{10}$ CFU/ml) observed in the current study is higher than the value of 2.83 $\log_{10}$ CFU/ml. However, this value was lower than the 6.85 $\log_{10}$ CFU/ml reported by Benkerroum et al. (2003) from Morocco and the 6.75 $\log_{10}$ CFU/ml. The difference could be

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attributed to differences in the levels of hygiene of milking equipment; animal milkers wash water and the environment. Moreover, it might be due to the differences in milk holding time and temperature during storage and transportation. The overall value of coliform counts observed in the current study was much higher when compared with recommended values given by the American Public Health Association and EU (100 cfu).

Yeast and mold count

Although an increase in yeast and mold count was observed along the chain as the milk was transported from the producer households to the milk collection center markets in the Gursum district, there was a significant difference ($P>0.05$) in yeast and microbial count between the milk samples collected from milk collection centers (5.3 $\log_{10}$ CFU/ml) and the household producer (4.2 $\log_{10}$ CFU/ml). As seen from figure 3 the overall mean yeast and microbial count (4.7 $\log_{10}$ CFU/ml) observed in this study was greater than the yeast and microbial count (1.9 $\log_{10}$ CFU/ml) reported by (El-Ziney and Al-Turki, 2007) for camel milk. The presence of yeasts and molds in milk samples collected from the household and milk collection centers is higher than the acceptance levels of yeast and molds. This might be due to improper sanitary conditions in the milking area, as well as poor personal hygiene of milkers and milk sellers.

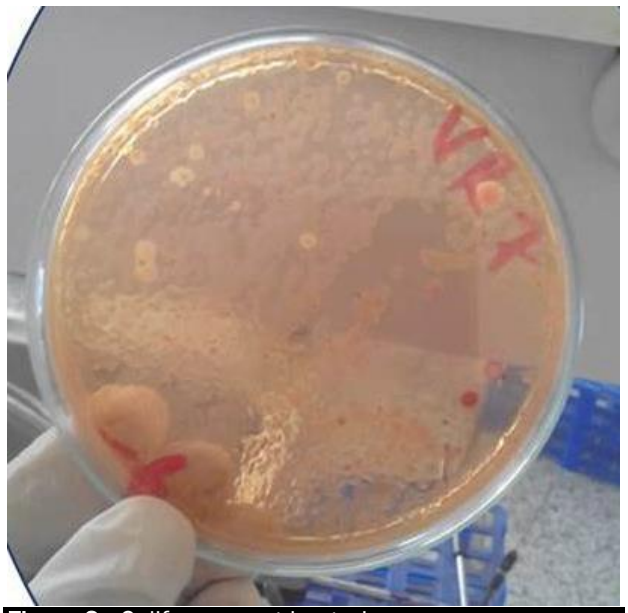

Figure 2 - Coliform count bacteria.

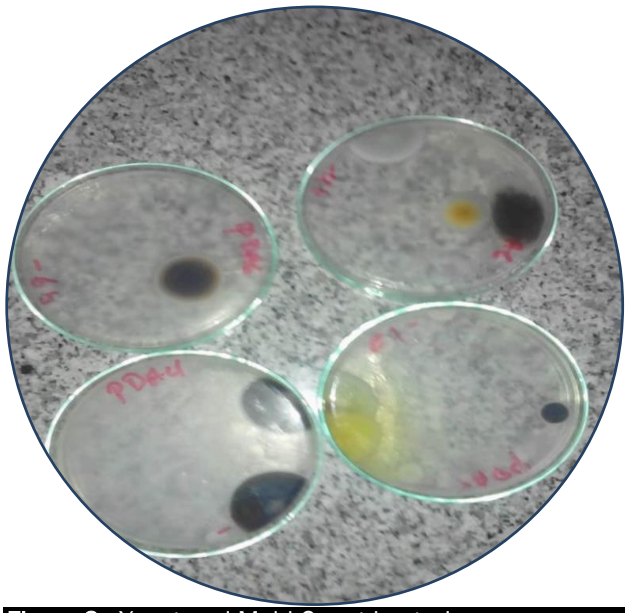

Figure 3 - Yeast and Mold Count bacteria.

DISCUSSION

The increase of standard plate count, coliform count and yeast and mold count thorough the market milk chain of raw camel milk could be associated with contaminated transporting and storage containers, water and the dust soil. Transferring of milk from container to the next during bulking towards the market makes milk sweep over wide container surfaces, thus collecting the microorganisms from container surfaces (Mwangi et al., 2016). Yeasts and Moulds are considered to be spoilage organisms. Some yeasts and moulds are a public health concern due to their production of mycotoxins, which are not destroyed during food processing or cooking (Adugna et al., 2013). Except for the tetra table acidity of milk samples obtained from milk collection centers, the majority of the physical quality of milk obtained from milk producers' samples was within the standards.

As the milk was carried from the homes to the terminal market of milk collection sites, this rise in overall bacterial counts may have happened throughout the chain. These depended on a number of factors, including the milk itself, infection of the camels' udders, the milking staff, other factors like transportation, and the type and hygienic state of the containers. This shows that raw milk supplied by retailers often had poor sanitary quality, which might most likely be attributed to inadequate milk handling during transit and storage. High total bacterial counts in raw milk primarily are a reflection of the unsanitary conditions the milk was handled in, including storage temperature, time since milking, and the health of the milking animals (Knight et al., 2016). Lack of knowledge about clean milk production, the lack of a separate area for milking, the use of non-boiled water for cleaning milking equipment and storage containers, the use of plastic containers (since plastic containers scratch easily and provide hiding places for bacteria during cleaning), and milk residues on equipment surfaces all have the potential to significantly contribute to the contamination of milk in this study. In general, greater bacterial counts could be a sign of udder disease, dirty milk handling, or unsuitable storage conditions. High levels of coliforms in milk are an indicator of poor hygiene standards utilized in milk production and show that the milk has been contaminated with fecal debris. This could be linked to inadequate udder preparation before to milking, poor milk handling techniques, and the use of subpar, unboiled water to clean milking implements (Martin et al., 2016).

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CONCLUSIONS

In the present study, there was a significant variation between the physical quality of the milk samples collected from milk producers and milk collection centers. The majority of the Physical quality of milk obtained from the milk producers' samples was within the standards except, for the tetra table acidity of the milk samples obtained from the milk collection centers. The microbial qualities of the milk obtained in the current study were poor, as judged by the high values of standard plate count; coliform count, and yeast and mold count which were significantly higher than the international standards safe for human consumption. These microbial loads may be due to poor hygienic standards during milking, and milk handling.

DECLARATIONS

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Conflict of interest
The authors declare that they have no competing interests.

Author collaboration
All authors were contributed equally.

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UNDERSTANDING LIPID QUALITY VARIABILITY IN ASIA PACIFIC THROUGH COMPREHENSIVE LIPID EVALUATION TESTS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The objective of this study was to investigate the variability in the oxidative quality and nutritional values of different lipid samples collected across Asia-Pacific region. The oxidative quality was evaluated through the peroxide value (PV) and malondialdehyde (MDA) content, while the free fatty acid (FFA) content and degree of fatty acid saturation (U/S ratio) were two essential parameters used to understand the nutritional values or metabolizable energy (ME) values of lipid samples. A total of 1221 lipid samples were collected and analyzed over a period of 10 years. The study showed high variability in oxidative quality between the lipid samples. Due to higher unsaturated fatty acid composition, the oxidative quality for most of the fish oil and soybean oil was at a less favorable range compared to rice bran oil, crude palm oil, and refined palm oil. The standard deviation of free fatty acids (FFA) content of soybean oil and refined palm oil was smaller compared to tallow, rice bran oil, crude palm oil, and fish oil. Fish oil and soybean oil had a higher standard deviation in U/S ratio. Variations in the FFA content and U/S ratio contributed to fluctuation in ME values.

Keywords: Fatty acid composition, Hydrolysis, Lipid quality, Metabolizable energy, Oxidation.

INTRODUCTION

Lipids are organic compounds comprising triglycerides which are soluble in organic solvents such as chloroform and hexane (Brian et al., 2015). The term "lipid" is often used interchangeably with fat and oil. Fat is solid at room temperature and of animal origin while oil is liquid at room temperature and of vegetable origin (Brian et al., 2015).

Lipids are expensive commodities and yet they are highly variable in composition and quality which contribute to variability in their energy density and energy content of the animal diets. It is important to quantify lipid quality variability for precise diet formulation (Wealleans et al., 2021). Inclusion of animal and vegetable-derived lipids in animal diets increases energy density because the energy in lipids is at least twice as high as other food nutrients such as carbohydrates and proteins (Ravindran et al., 2016). Apart from energy enhancement, the addition of lipids can also i) increase the diet palatability; ii) reduce the dust during feed milling; iii) improve the absorption of fat-soluble vitamins; iv) supply metabolically essential fatty acids such as linoleic acids, and v) allow for better absorption of nutrients from the diet by reducing the rate of feed passage through the gastrointestinal tract (Baião and Lara, 2005; Ravindran et al., 2016). Despite the numerous advantages of lipid supplementation, it is important to recognize that lipids are extremely susceptible to oxidative and hydrolytic rancidification. This, combined with improper storage conditions and fatty acid composition, results in quality variation (Robards et al., 1988).

Oxidation occurs in the presence of initiators such as oxygen, light, heat, and metal ions, which can cause lipids to be oxidized to hydroperoxides and carbonyls through a cascade of chemical reactions. The chemical composition of the lipids (i.e., degree of unsaturation in the fatty acid moiety of lipids) also plays a part in their susceptibility to the oxidation process (Robards et al., 1988). The extent of oxidative rancidity is commonly evaluated using the peroxide value (PV) and malondialdehyde (MDA) content to determine the degree of primary and secondary oxidation respectively (Cong et al., 2020). Lipid oxidation gives rise to unique, unpalatable odors and flavors (Robards et al., 1988). Oxidation also impacts energy digestibility and the stability of other nutrients in animal diets such as vitamins and carotenoids (Bonnie and Choo, 1999; Choe and Min, 2009; Shurson et al., 2015). This adversely affects the animal's feed intake and growth performance leading to poor return of investment (ROI) (Shurson et al., 2015; Esmail, 2018). Therefore, antioxidants are often added to fats and oils in feed to delay the onset of oxidation.

The presence of heat, moisture, and endogenous lipase lead to hydrolysis of lipids to free fatty acids (FFA) and glycerol. FFA content is often evaluated to determine the extent of hydrolytic rancidity (Robards et al., 1988). Apart from being a hydrolytic quality indicator, FFA content is also closely related to the nutritional values (i.e., metabolizable energy (ME) values) of the lipids (Wiseman and Salvador, 1991). Higher dietary FFA content was linked to a lower fat absorption rate in chickens (Rodriguez-Sanchez et al., 2019). According to Attech and Leeson (1985), lower fat absorption was likely

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due to the formation of insoluble soaps from the reaction between the carboxylic functional group of FFA and divalent minerals such as calcium and magnesium in the intestinal tract, which led to a lower fat retention rate in their intestine and with resultant lower ME values. Besides the negative contribution of high FFA content to the ME values (Wiseman and Blanch, 1994), degree of fatty acid saturation (U/S ratio) is also strongly related to fat digestion, i.e., unsaturated fatty acids have higher digestibility than saturated fatty acids (Thng et al., 2020). Wiseman et al. (1998) expressed the relationship of FFA content and U/S ratio to ME values in a mathematical equation ("Wiseman Equation") which can be used to predict the ME values of lipids for broiler chickens of different ages.

Lipid samples were collected across the Asia-Pacific region between year 2010 and 2020, from different suppliers, to evaluate their oxidative quality and ME values. The oxidative quality of the lipids was determined using PV and MDA analyses while FFA content and U/S ratio of the lipids were analyzed to predict their ME values using the Wiseman Equation (Wiseman et al., 1998). The analyses showed that both oxidative and nutritional values of various lipid types were inconsistent, and variabilities were observed between the lipid types. This study highlights the importance of assessing lipid quality to help ensure animal performance and ROI in view of the variability of lipid quality in feed.

MATERIALS AND METHODS

Sample collection and preparation

A total of 1221 lipid samples of animal and vegetable origins (Figures 1 and 2) were collected from different suppliers across the Asia-Pacific region (Figures 3 and 4) from year 2010 to 2020. No written record with respect to the handling, storage condition, or treatment on the lipid samples were available prior to the sample receival. All lipid samples were stored in plastic containers and kept in the chiller at 2 to 6 °C upon receipt. Prior to analysis, the samples were either thawed at room temperature or melted in the oven at 60 °C. Prolonged heating of samples in the oven was avoided to minimize the impact on lipid quality. All lipid samples were analyzed within one week from the receiving date.

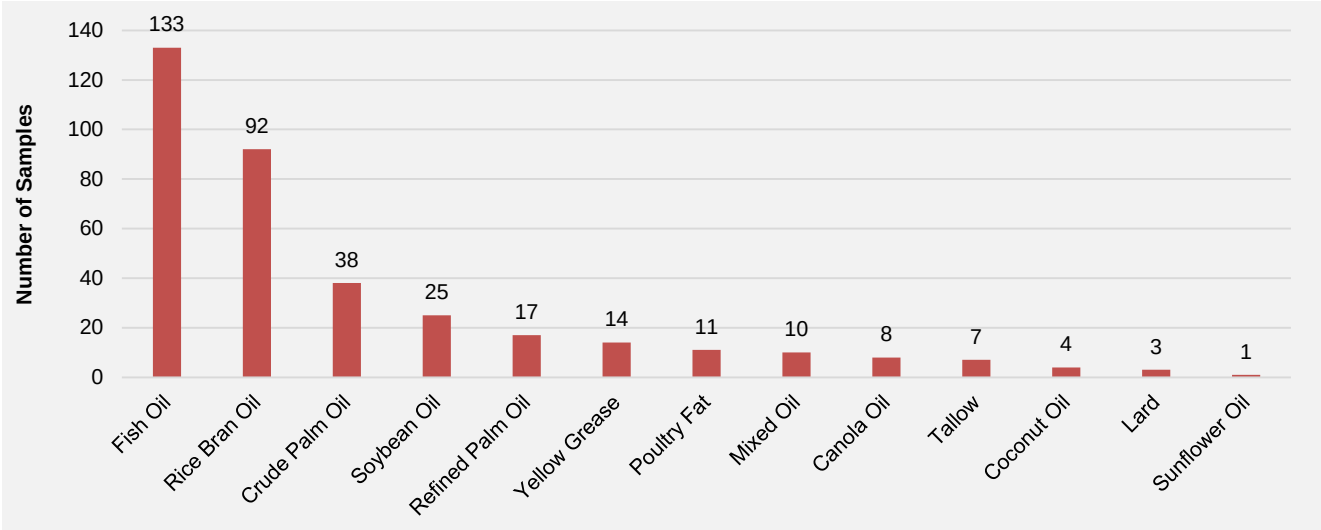

Figure 1 - Collection of different lipid types for oxidative quality study.

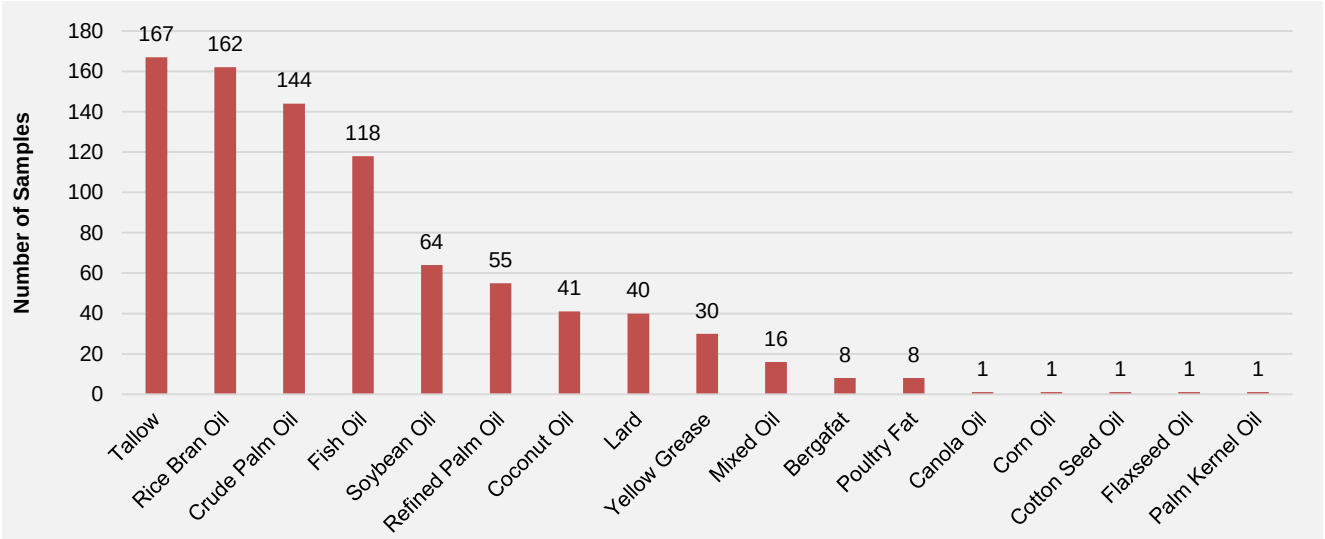

Figure 2 - Collection of different lipid types for FFA content, U/S ratio, and ME values study.

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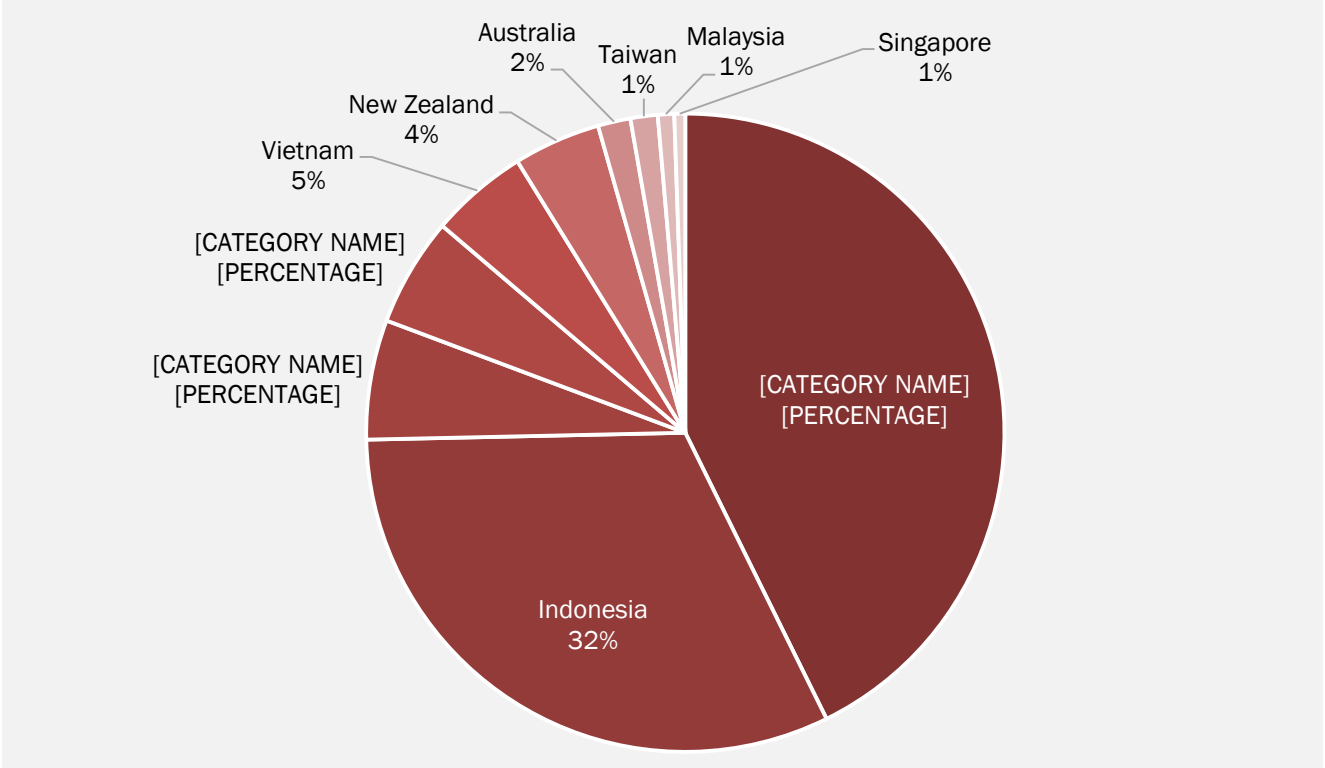

Figure 3 - Origin of lipid samples collected for oxidative quality study.

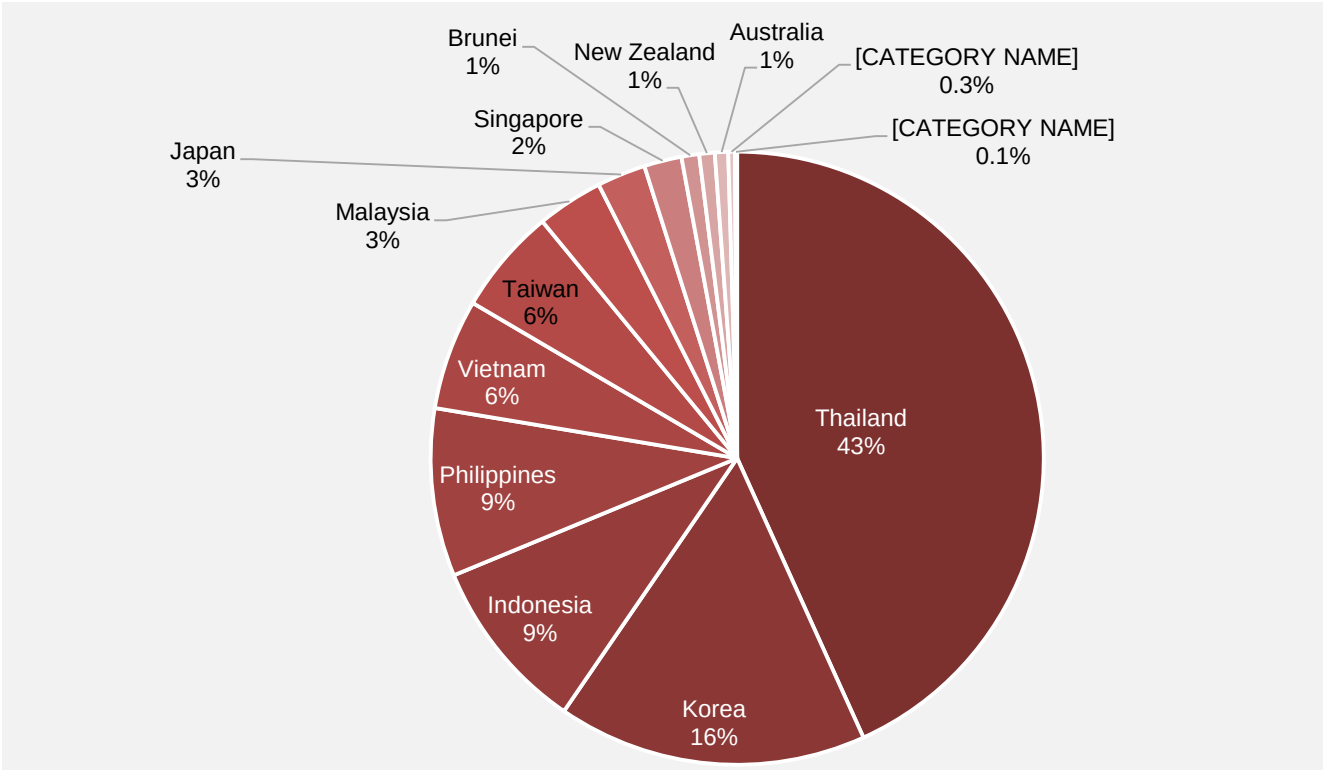

Figure 4 - Origin of lipid samples collected for FFA content, U/S ratio, and ME values study.

Instrumentation

A DL 50 GRAPHIX auto-titrator (Mettler-Toledo, United States) with DM140-SC and DG113-SG glass electrodes (Mettler-Toledo, United States) were used to determine peroxide value and free fatty acid content respectively. A 1100 Series high pressure liquid chromatography system with diode array detector (Agilent Technologies, United States) with ZORBAX StableBond C18 column (5 µm, 250 × 4.6 mm) (Agilent Technologies, United States) was used to determine the malondialdehyde content. A 7890B gas chromatography system with flame ionization detector (Agilent Technologies, United States) with Supelco SPTM-2560 column (100 m × 0.25 mm × 0.20 µm) (Sigma-Aldrich, United States) was used for chromatographic separation of the fatty acid methyl esters.

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Peroxide value (PV) determination

The PV of lipid samples was determined using an in-house method, modified from the American Oil Chemists' Society (AOCS) Official Method Cd 8-53 (AOCS, 2017). Thirty mL of acetic acid-chloroform mixture (Avantor, United States; Tedia, United States) was added to one gram of lipid sample in a titration cup and 0.5 mL of saturated potassium iodide solution (Merck, Germany) was subsequently added. The mixture was swirled for one minute and the cup was stored in the dark for another one minute. Thirty mL of distilled water was then added. The cup was attached to the auto-titrator, and the mixture was stirred at 50 % speed for five minutes before titrating it against standardized 0.01 N sodium thiosulphate solution (titrant) (Fisher Scientific, United States) to its potentiometric endpoint. The titrant was standardized against potassium dichromate (Merck, Germany) in potassium iodide solution acidified with 1 N hydrochloric acid (Merck, Germany) prior to analysis. The result was calculated based on the volume consumption of titrant and its concentration.

Malondialdehyde (MDA) content determination

The MDA content of lipid samples was determined using an in-house method, modified from the work published by Mendes et al. (2009). Dilution of 1,1,3,3-Tetraethoxypropane (TEP) (Sigma-Aldrich, United States) with 5 % trichloroacetic acid (TCA) solution (Sigma-Aldrich, United States) was used to prepare a series of standard solutions (0.2, 0.4, 0.8, 1.0, 2.0, and 5.0 µg/g). One hundred mL of 5 % TCA solution was added to 10 gram of lipid sample in a 150 mL glass beaker. The mixture was stirred for 15 minutes, and the aqueous layer of the mixture was filtered through a 0.45 µm PES syringe filter (Agilent Technologies, United States) to obtain a clear solution. Five mL of sample supernatant and standard solution were transferred to 15 mL centrifuge tubes filled with five mL of 0.2 % thiobarbituric acid (TBA) (Sigma-Aldrich, United States). The mixture was vortex agitated and the tubes were placed in a water bath with boiling water for 30 minutes. The tubes were then cooled, and the content was vortex agitated again before injection into high pressure liquid chromatography (HPLC) for analysis. The MDA-TBA adduct was separated isocratically with a HPLC mobile phase pumped at 0.7 mL/min and consisting of 0.03 M potassium dihydrogen phosphate solution (Merck, Germany), adjusted to pH 7 using 1 N sodium hydroxide solution (Merck, Germany) and methanol (Fisher Scientific, United States) in the ratio 55:45 (v/v), column thermostat at 30 °C, and detection wavelength of 532 nm. The total analysis run time was 10 minutes with post run of five minutes. The result was calculated based on the established calibration curve and 0.2 µg of TEP corresponded to 0.0065 µg of MDA.

Free fatty acid (FFA) content determination

The FFA content of lipid samples was determined using an in-house method, modified from the Association of Official Analytical Chemists (AOAC) Official Method 940.28 (AOAC, 2012). Fifty mL of 95 % ethanol (Aik Moh Paints and Chemicals, Singapore) was added to one gram of lipid sample in a titration cup. The cup was attached to the auto-titrator, and the mixture was stirred at 50 % speed for one minute before titration against 0.1 N sodium hydroxide solution (titrant) (Merck, United States) to its potentiometric endpoint. The result was calculated based on the volume consumption of titrant and its concentration. The FFA content was expressed either as % oleic acid, % palmitic acid, or % lauric acid depending on the lipid type.

Fatty acid profile analysis

The fatty acid composition of lipid samples was determined using an in-house method, modified from the Association of Official Analytical Chemists (AOAC) Official Method 969.33 (AOAC, 2012). In short, the fatty acids in the lipid samples were trans esterified into fatty acid methyl esters (FAME) to ease the chromatographic separation. Four mL of 0.5 M methanolic sodium hydroxide solution (Fisher Scientific, United States; Merck, Germany) was added to 40 mg of lipid sample in a 50 mL round bottom flask and refluxed until there were no visible lipid globules. Five mL of 14 % boron trifluoride in methanol (Sigma-Aldrich, United States) was then added and refluxed for another two minutes. Subsequently, 10 mL of heptane (Sigma-Aldrich, United States) was added and refluxed for another one minute. The content was then cooled to room temperature. Next, 15 mL of saturated sodium chloride solution (Merck, Germany) was added, and the flask was swirled vigorously. The top organic layer was filtered through an 0.2 µm RC syringe filter (Sartorius, Germany) attached to a syringe filled with sodium sulphate (Merck, Germany) and injected into a gas chromatography (GC) system for analysis. FAME were analyzed with helium as the carrier gas at a flow rate of 0.85 mL/min, split ratio of 40:1, injection volume of 0.4 µL, and inlet temperature of 260 ℃. The GC oven temperature was programmed at 140 °C for five minutes and increased to 235 °C at 5 °C/min for 15 minutes, followed by 15 °C /min to 250 ℃ for five minutes. The total run time was 45 minutes. The percentage composition of each FAME in lipid samples was calculated with reference to Supelco 37 Component FAME Mix (Sigma-Aldrich, United States). The degree of fatty acid saturation (U/S ratio) of the lipid samples was then determined based on the percentage composition results.

Metabolizable energy (ME) prediction

The ME values of lipid samples for broilers of different ages were predicted using Wiseman Equation (Equation 1) with FFA content, U/S ratio, and empirical constants A, B, C, and D (Table 1).

$$ME\ (kcal/kg) = (A+B \times FFA+C \times e^{(D(U/S))})/0.004184 \quad (1)$$

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Table 1 - The values of empirical constants A – D used in Wiseman Equation for the prediction of ME values of lipid samples for broilers of different ages (Wiseman et al., 1998).

Empirical constant	Young broilers (aged < 21 Days)	Old broilers (aged > 21 Days)
A (MJ/kg)	38.112 ± 1.418	39.025 ± 0.557
B (MJ/kg)	-0.009 ± 0.002	-0.006 ± 0.001
C (MJ/kg)	-15.337 ± 2.636	-8.505 ± 0.746
D	-0.506 ± 1.186	-0.403 ± 0.088

Statistical Analysis

Descriptive statistics were calculated using Microsoft Excel 365.

RESULTS AND DISCUSSION

Oxidative Quality (PV and MDA Content)

The peroxide value (PV) and malondialdehyde (MDA) content are two key parameters that quantify primary and secondary products generated during lipid oxidation. The results for PV and MDA content and variability are shown in Table 2 for the five major lipid types, namely fish oil, rice bran oil, crude palm oil, soybean oil, and refined palm oil. The standard deviation for PV values for these lipids ranged from 1.38 meq/kg for refined palm oil to 3.82 meq/kg for crude palm oil while that of MDA content ranged from as low as 0.12 µg/g for rice bran oil to 5.87 ug/g for fish oil. These results indicated that there were different degrees of oxidation across these five lipid types, and more importantly, that oxidative variability was present between samples belonging to the same lipid type. This could be attributed to numerous factors such as the possible presence of initiators such as heat and metal ions, as well as the chemical composition of the lipids. High temperature storage conditions and the existence of metal ions in the lipids can catalyze oxidation, leading to increased PV and MDA content. Additionally, lipids rich in unsaturated fatty acids are more prone to oxidation as compared to the more saturated lipids (Robards et al., 1988).

To describe the extent of oxidative rancidification of the lipids more effectively, Verleyen (2010) published an interpretation guideline comprising of four different oxidation stages based on the PV and MDA content of the lipids (Table 3). The oxidative stages of the samples from the five major lipid types tested in the current study were partitioned according to this guideline to examine oxidative variability (Figure 5). Accordingly, less than 40 % of the fish oil samples had PV and MDA contents in the range of acceptable oxidative quality equating to more than 60 % of the fish oil samples exhibiting signs of oxidation during the time of the study. Severe lipid oxidation accounted for 8.9 % of the fish oil samples analysed, which was the highest amongst the five major lipid types analysed. This can be attributed to the high susceptibility of oxidation in fish oil due to the presence of long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). LCPUFA are highly reactive towards oxidation due to (i.) a high number of double bonds and (ii.) position of double bonds in the backbone structure of the fatty acid as bisallylic carbons in LCPUFA have lower activation energy for hydrogen donation and free radical formation which favours lipid oxidation (Albert et al., 2013).

Table 2 - Descriptive statistical analysis for the oxidative quality of five major lipid types.

Lipid type	Sample size	PV (meq/kg) [a]				MDA Content (µg/g) [a, b]			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Fish oil	133	0.28	16.64	4.56	3.07	BDL	38.8	4.06	5.87
Rice bran oil	92	0.54	14.17	4.09	2.89	BDL	0.43	0.35	0.12
Crude palm oil	38	0.50	21.97	4.39	3.82	BDL	2.79	1.38	0.94
Soybean oil	25	0.36	15.68	6.21	3.75	BDL	0.23	NA	NA
Refined palm oil	17	0.72	5.45	2.39	1.38	BDL	0.31	NA	NA

[a] Min = minimum; Max = maximum; SD = standard deviation; [b] BDL = below detection limit; NA = not applicable; The detection limit of MDA content was 0.2 ug/g; The mean and standard deviation of MDA content for soybean oil and refined palm oil were not tabulated as there was only one sample for each lipid type with MDA content found to be greater than the detection limit.

Table 3 - Guideline on interpreting the oxidative quality of different lipid types.

Oxidative quality	PV (meq/kg)	MDA Content (µg/g)	
		Fish oil	All other oils
Acceptable	<5	<2	<1
Onset of oxidation	5-10	2-5	1-2
Progressed oxidation	10-20	5-10	2-4
Severe oxidation	>20	>10	>4

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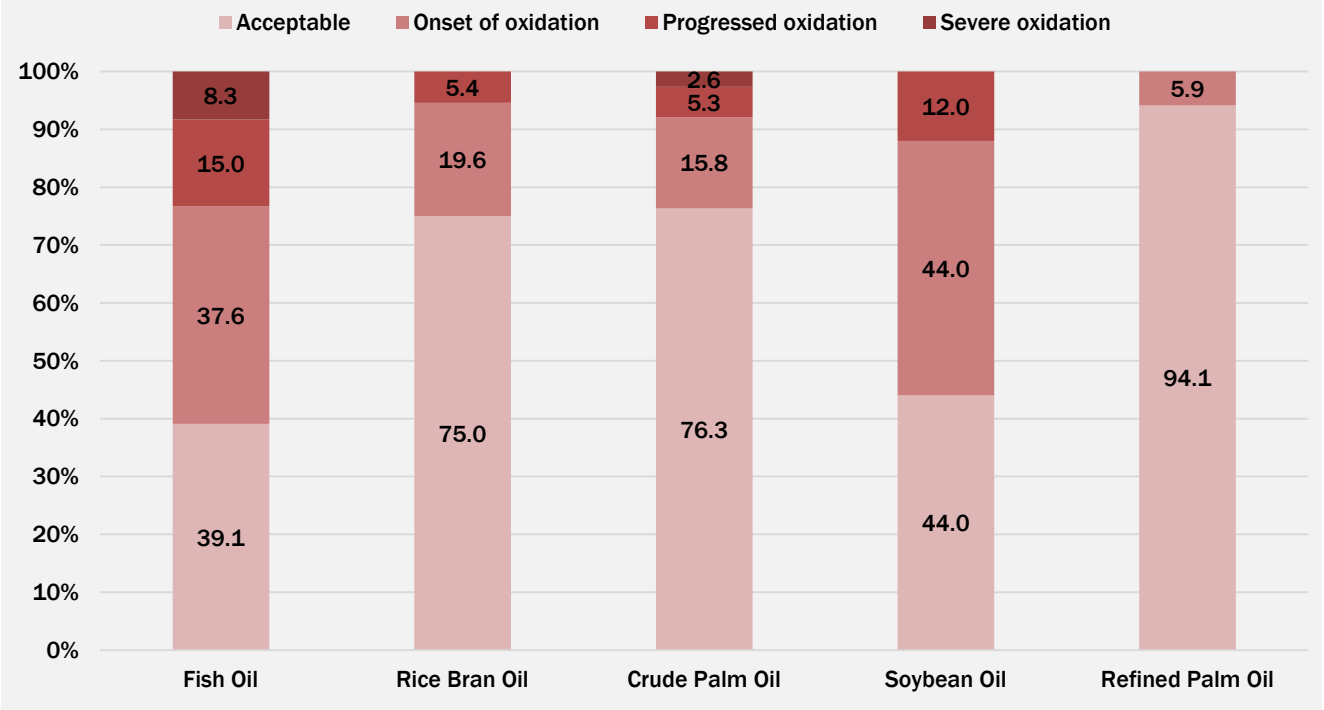

Figure 5 - Oxidative status partition percentages of 5 major lipid types.

Interestingly, the proportion of rice bran oil, crude palm oil, and refined palm oil samples with acceptable oxidative quality were higher compared to the soybean oil samples. Rice bran oil samples involved in the study were of crude oil origin, likely to contain high amounts of endogenous antioxidants such as tocopherols and oryzanols. These antioxidants help to retard oxidation rate (Yoon and Kim, 1994). The presence of natural antioxidants and the absence of highly unsaturated fatty acids in the crude palm oil and refined palm oil samples also made them comparatively stable (Berger, 2003). Severe lipid oxidative deterioration was observed in one of the crude palm oil samples, but this was likely due to the prolonged storage of the lipid under unfavorable conditions prior to receival. Due to the presence of high levels of mono- and polyunsaturated fatty acids in soybean oil (Kozłowska and Gruczyńska, 2018), it is more susceptible to oxidation as compared to rice bran oil, crude palm oil, and refined palm oil. Indeed, it was observed that 56% of the soybean oil samples were oxidized during the time of study. Apart from the partition percentages of the oxidative status of the lipids, alternative illustrations to visualize the variation in the oxidative quality of the lipids are shown in Figures 6 and 7.

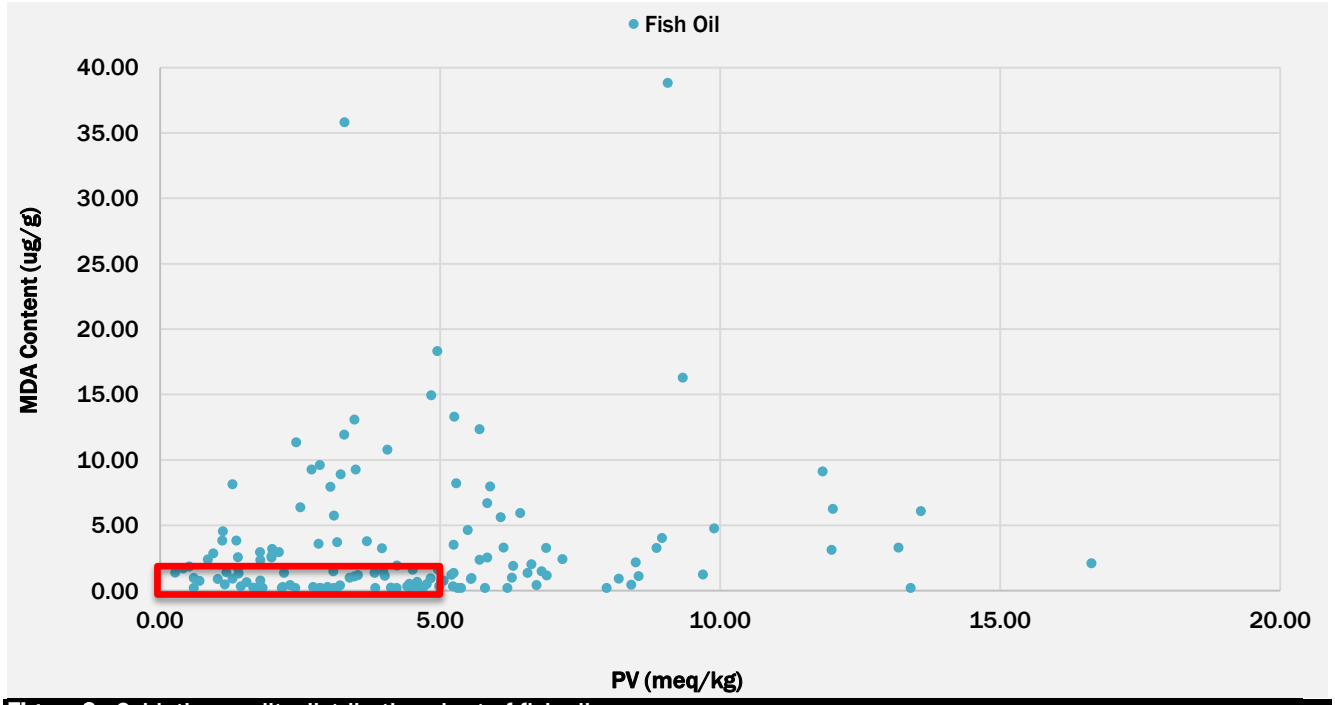

Figure 6 - Oxidative quality distribution chart of fish oil (Footnote: The marked region indicates where the oxidative quality of the fish oil samples was within the acceptable range).

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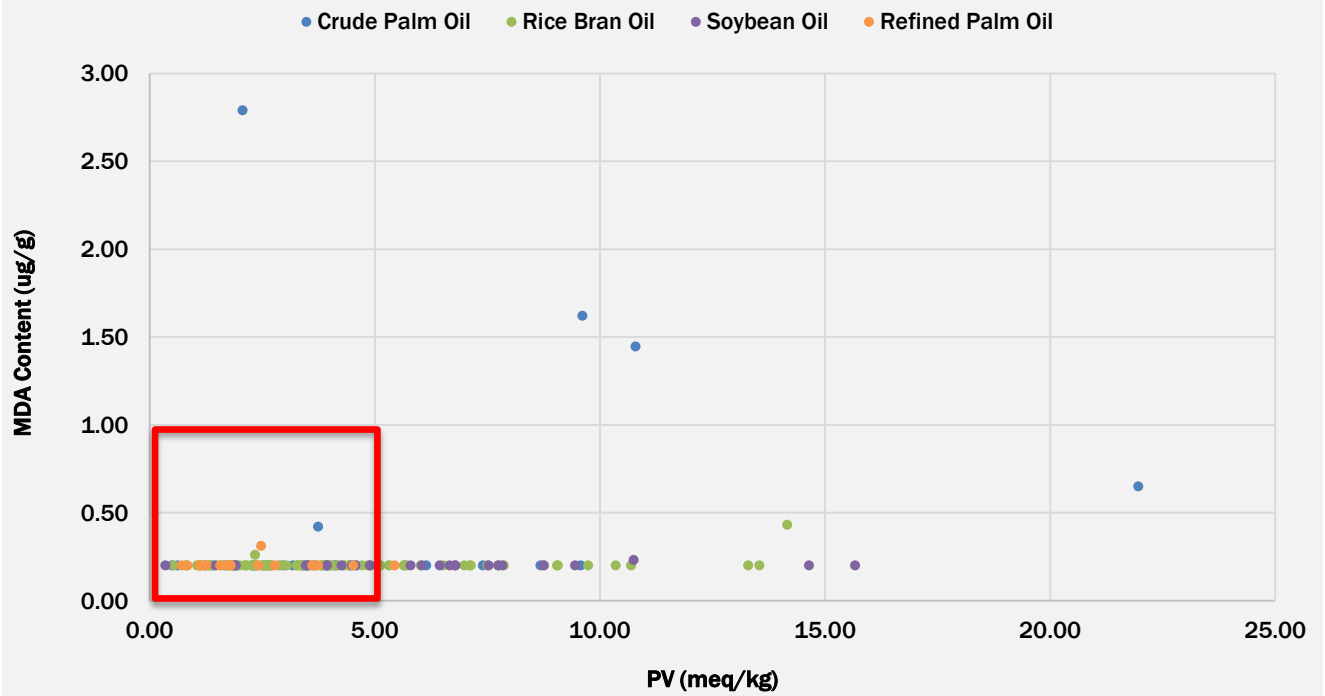

Figure 7 - Oxidative quality distribution chart of crude palm oil, rice bran oil, soybean oil, and refined palm oil samples. (Footnote: The marked region indicates where the oxidative quality of the lipid samples was within an acceptable range; The MDA content of the lipid samples which were below the detection limit were assigned with the value of 0.2 µg/g to make sure the data points were valid to be plotted.)

FFA Content and U/S Ratio

The free fatty acid (FFA) content and degree of fatty acid saturation (U/S ratio) are two variables incorporated into the Wiseman Equation to (ME) values of the lipids for broilers of different ages (Wiseman et al., 1998). The results were presented in Table 4 for the six major lipid types, namely, tallow, rice bran oil, crude palm oil, fish oil, soybean oil, and refined palm oil.

The standard deviation of FFA content for the soybean oil and refined palm oil samples were relatively small (i.e., 0.8 % oleic acid and 0.7 % palmitic acid respectively). Similarly, their average FFA content (i.e., 1.0 % oleic acid and 0.5 % palmitic acid respectively) were lower compared to the other four lipid types. The small variability and low FFA content may be attributed to the post-processing steps in the manufacturing processes used to derive these lipids. Physical and chemical refining can contribute to the removal of FFA in crude lipids, and this helps to keep the FFA content of soybean oil and refined palm oil at a more consistent level (Robert, 2021). Conversely, the FFA content of the tallow, rice bran oil, crude palm oil, and fish oil samples were more widely spread, with their average FFA contents higher than soybean oil and refined palm oil samples. For tallow and fish oil samples, higher FFA content observed may be attributed to extraction processes (i.e., rendering and wet pressing method respectively) (National Renderers Association, 2003; Bonilla-Méndez JR and Hoyos-Concha, 2018) involving high temperature conditions which subsequently increase the rate of hydrolysis in the lipids (Robards et al., 1988). The raw material for rice bran oil and crude palm oil samples (i.e., rice bran and oil palm fruit mesocarp respectively) contain high lipase activity (Cadena et al., 2012; Brunschwiler et al., 2013) which can potentially cause enzymatic degradation of the lipids, resulting in increased FFA levels (Robards et al., 1988). The extraction processes and the composition of the raw materials play a part in the variability and the level of FFA content. Figure 8 illustrates the variation in the FFA content of the 6 major lipid types in the form of box plot diagram.

From Table 4, it was observed that the fish oil and soybean oil samples had relatively high average U/S ratios (i.e., 2.21 and 4.56 respectively). This is because soybean oil is rich in mono- and polyunsaturated fatty acids (Kozłowska and Gruczyńska, 2018) and fish oil generally contains higher proportions of LCPUFA (Albert et al., 2013). The standard deviations of the U/S ratios in the fish oil and soybean oil samples were relatively large (i.e., 1.38 and 1.34 respectively) compared to other four lipid types (Table 4). This can be closely related to their highly unsaturated lipid profile which is more susceptible to oxidation. Oxidation involves the breakdown of the double bonds in the unsaturated fatty acids with oxidation being inevitable. The standard deviation of the U/S ratio in the fish oil and soybean oil samples was greater than the other lipid types. Additionally, the variation in the fatty acid composition of fish oil samples due to different fish species (such as salmon and tuna, FAO/WHO, 2017), would also contribute to the U/S ratio variability.

The average U/S ratio for tallow, crude palm oil and refined palm oil samples was close to 1.00 making these lipid types more resistant towards the oxidative rancidification process. Although rice bran oil is made up of a higher proportion of unsaturated fatty acids as shown by the relatively high average U/S ratio of 2.71 (Table 4), the inherent presence of natural antioxidants helps to retard the oxidation process and keep their U/S ratio at a more consistent level (Yoon and Kim, 1994). The standard deviation of the U/S ratio for the rice bran oil samples was only 0.50. The variation in

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measured U/S ratios may reflect the extent to which the lipid samples were exposed to the oxidation initiators. Figure 9 illustrates the variation in the U/S ratios of the six major lipid types in the form of box plot diagram.

Table 4 - Descriptive statistical analysis for the FFA content and U/S ratio of six major lipid types.

Lipid type	Sample size	FFA content (% oleic / % palmitic acid) [a, b]				U/S ratio [a]			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Tallow	167	0.3	51.8	2.6	4.9	0.34	3.22	1.29	0.40
Rice bran oil	162	3.2	68.2	12.6	7.9	1.31	4.55	2.71	0.50
Crude palm oil	144	0.1	49.6	7.2	7.8	0.81	3.62	1.14	0.41
Fish oil	118	0.4	31.1	4.6	5.8	0.56	9.52	2.21	1.38
Soybean oil	64	0.01	4.3	1.0	0.8	0.61	5.74	4.56	1.34
Refined palm oil	55	0.02	4.3	0.5	0.7	0.43	2.53	1.18	0.28

[a] Min = minimum; Max = maximum; SD = standard deviation; [b] FFA content of crude palm oil and refined palm oil was expressed in % palmitic acid while FFA content of other lipid types was expressed in % oleic acid. The results expressed in % oleic acid can be converted into % palmitic acid by dividing the former results by 282 g/mol and followed by multiplying with 256 g/mol.

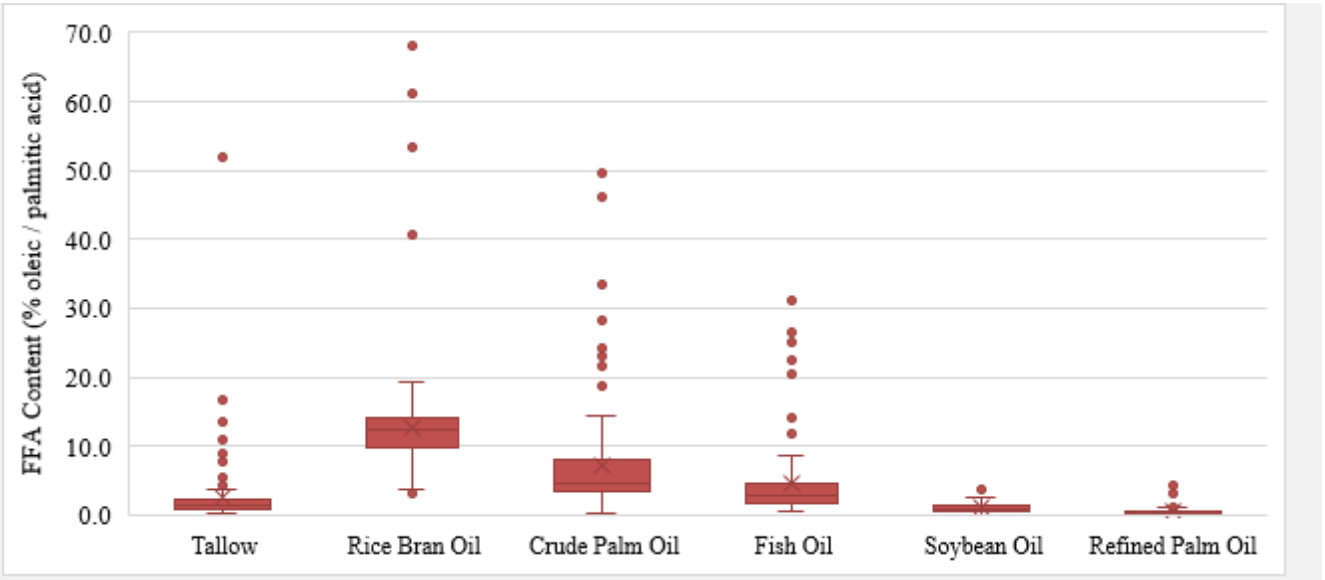

Figure 8 - FFA content variability of 6 major lipid types.

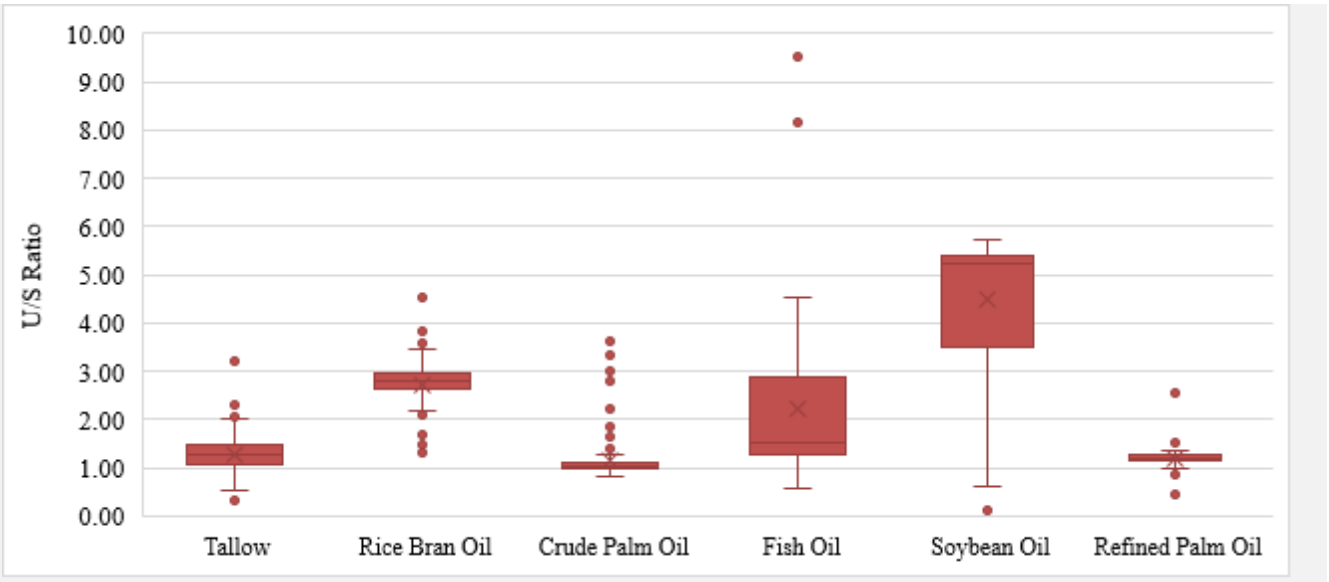

Figure 9 - U/S ratio variability of 6 major lipid types.

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Nutritional values (ME values)

Different amplitudes of fluctuation in the FFA content and U/S ratio gave rise to diverse variability in the predicted ME values between and within the lipid types (Figures 10 and 11). The standard deviation of predicted ME values for the fish oil and soybean oil samples (i.e., 639 and 517 kcal/kg respectively for young broilers; 363 and 290 kcal/kg respectively for old broilers) was relatively high compared to other lipid types (Table 5). This was due to the relatively high variation in the U/S ratio for these lipid types (Table 4). In comparison to the U/S ratio, the effect of FFA variation of predicted ME values was less notable as FFA was only linearly correlated to the predicted ME values, as demonstrated in the Wiseman Equation. This explains the observation of having a higher predicted ME value variability for the soybean oil samples even though the standard deviation of their FFA content was small (i.e., 0.8% oleic acid, Table 4). As already referred to, the presence of natural antioxidants in rice bran oil facilitated the retardation of oxidative rancidification and kept the U/S ratio at a more consistent level (Table 4). Hence the standard deviation of predicted ME values for young and old broilers for the rice bran oil samples (i.e., 264 and 152 kcal/kg respectively) was relatively small (Table 5).

The average predicted ME values for the rice bran oil, fish oil, and soybean oil samples (i.e., 7637, 7370, and 8356 kcal/kg respectively for young broilers; 8195, 8079, and 8658 kcal/kg respectively for old broilers) were higher than other lipid types (Table 5) as they contained higher mean U/S ratios than the other lipid types with unsaturated fatty acids being more digestible than saturated fatty acids (Tancharoenrat et al., 2014). The average predicted ME values of the lipids for old broilers (>21 days old) were higher than young broilers (<21 days old, Table 5). This could be attributed to the fact that young broilers produce less bile acids and pancreatic lipase than the older birds. This in turns reduces the chicks' ability to digest and absorb dietary lipids (Arshad et al., 2020). It is also noteworthy that the variability of the predicted ME values for young broilers was consistently more pronounced than for the older broilers.

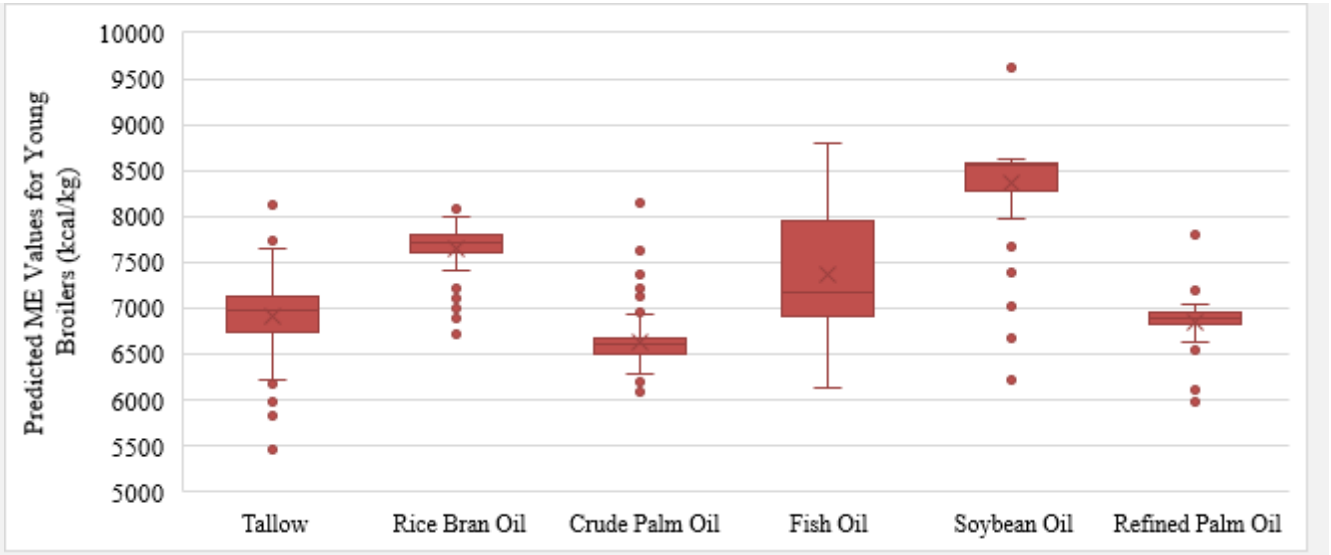

Figure 10 - Variation in the predicted ME values of six major lipid types for young broilers (aged <21 Days).

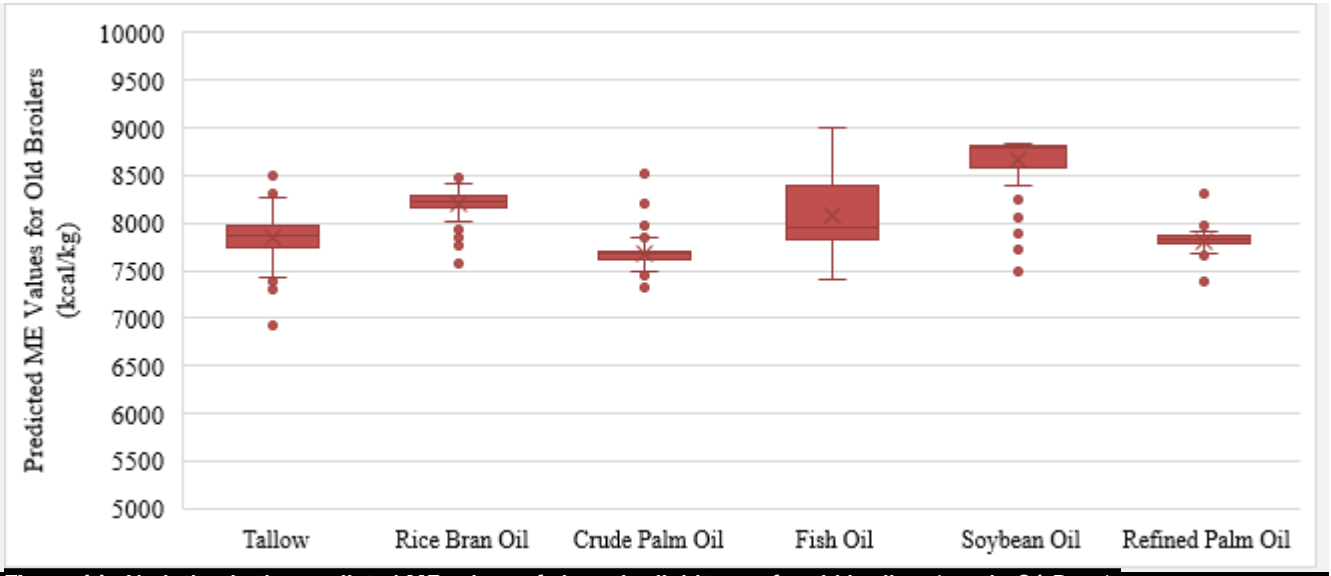

Figure 11 - Variation in the predicted ME values of six major lipid types for old broilers (aged >21 Days).

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CONCLUSION

The data from this study showed that the variability of oxidative and nutritional values was observed between the different lipid types as well between the same lipid type. The subsequent impact of these variabilities on the predicted broiler ME values, as calculated using the Wiseman Equation, were presented. To minimize the unfavorable consequences of lipid quality variability on the animal performance and return of investment, laboratory analysts and nutritionists are thus recommended to conduct thorough laboratory analyses regularly to examine the oxidative and nutritional values of lipids. Based on the lipid evaluation information, nutritionists will be able to make informed formulation decisions and apply antioxidants into the lipids when necessary to help protect the quality of their feed formulation.

DECLARATIONS

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Authors' contribution

J.X. TING proposed the design of study, prepared the manuscript, and performed the laboratory analysis. A. THNG, H.R. TAY, G.H. SOO, and H.C. ONG assisted with the laboratory analyses. All the authors read and approved the final manuscript.

Conflict of interests

The authors declared that there is no conflict of interest.

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EFFECTS OF DIETARY SUPPLEMENTATION OF TURMERIC AND BLACK CUMIN POWDER ON PERFORMANCE AND BLOOD PARAMETERS OF NATIVE CHICKENS

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ABSTRACT: This study was conducted to determine the effect of supplementation of a mixture of turmeric and black cumin powder on the production performance, blood parameters, and quality of native chicken eggs. Ninety-six female native chickens aged 17 weeks were randomly allocated in a completely randomized design into four treatments. The four treatments were differentiated based on the level of supplementation of a mixture of turmeric (TP) and black cumin powder (BCP), namely T0: Control (without a mixture of TP and BCP); T1: (2.5 g TP + 2.5 g BCP)/kg ration; T2: (5 g TP + 5 g BCP)/kg ration and T3: (7.5 g TP + 7.5 g BCP)/kg ration. Each treatment was repeated 3 times, using 8 native chickens. The variables measured were blood parameters including Hemoglobin (Hb), hematocrit, total red blood cells (RBC), white blood cells (WBC), blood cholesterol and triglycerides; and also, the performance variables of feed consumption, egg production (HDA), body weight at 34 weeks, egg weight, egg mass, feed conversion, yolk weight, shell weight, shell thickness, yolk color index, cholesterol, LDL and HDL of yolks. The Data were collected for 16 weeks and analyzed by variance analysis. The results showed that supplementation with a mixture of TP and BCP increased feed consumption; HDA; body weight; egg mass; shell weight, thickness, and yolk color ($P<0.05$). Egg weight and yolk weight were not significantly different. TP + BCP supplementation significantly reduced feed conversion, cholesterol level, HDL, and LDL. Increased TBCP supplementation increased Hb, hematocrit, red blood cells and white blood cells and significantly decreased cholesterol, lipoproteins, and triglycerides in the blood ($P<0.05$). It was concluded that TBCP mixture supplementation could improve production performance, some of blood parameters, and quality of native chicken eggs.

Keywords: Black cumin, Native chicken, Performance, Turmeric, Yolk

INTRODUCTION

The native chicken provides a significant contribution to the economy of the Indonesian people, not only as a source of meat but also of eggs. The population of native chickens in Indonesia in 2020 has reached 305,4 million heads and was able to produce 270.2 thousand tons of meat and 358,9 thousand tons of eggs (DJPKH, 2021). The pattern of farming of native chickens in Indonesia, some are developed intensively by being confined and using rational feed.

The utilization of herbal ingredients to improve poultry productivity has grown rapidly since the restrictions on the use of antibiotics in feed (Diaz-Sanchez et al., 2015). The utilization of herbal products as natural antibiotics (phytobiotics) to improve livestock performance was considered safer because it has low toxicity, is free of residues, is inexpensive, and can improve livestock performance (Diaz-Sanchez et al., 2015; Alagawany et al., 2021). The utilization of herbal ingredients in livestock has a positive effect because they contain antioxidants, can increase antibody titers, improve growth and feed conversion, suppress the growth of harmful bacteria, reduce triglycerides and cholesterol (Jouybari et al., 2009; Houshmand et al., 2012).

Black cumin (*Nigella sativa L.*) was a perennial herb from the Ranunculaceae family, which commonly found in Central Asia and Europe. Black cumin was widely used for the treatment of several diseases, has anti-cancer effects and lowers blood sugar levels (Seidavi et al., 2020). Black cumin has antibacterial and antioxidant features and provides an immune effect (Raheem et al., 2021; Zaky et al., 2021). Black cumin contains thymoquinone which known as a powerful antioxidant that contributed in disease prevention and anti-cancer (Manjunath et al., 2020). There were 18 compounds that can be identified from black cumin consisting of 99.14% of the total essential oil, namely aldehydes (23%), gamma terpine (14.5%), acetic acid (10.9%), and 1,3,8-p-menthatriene (7.9%) (Jalilzadeh-Amin et al., 2011). Other compounds found in black cumin were sabinene, carvon, carveol, flavonoids (Qinna et al., 2012), coumarins and cumin aldehyde which has antifungal activity (Yalçin et al., 2007), antisposmodic (Jalilzadeh-Amin et al., 2011), pain relief, anti-inflammatory, and anticoagulant (Mandegary et al., 2012). The usage of black cumin seeds at a dose of 1 g/kg in broiler rations could improve body weight and feed conversion (Erener et al., 2010). In laying hens rations, the usage of black cumin could protect the magnum and uterine cells (Dhama et al., 2015). At a level of 3%, it could improve the production of laying hens and lower cholesterol levels (Aydin et al., 2008).

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Turmeric plant (*Curcuma longa*) was a medicinal plant from the ginger family and grows widely in South and Southeast Asia (Sanghvi et al., 2020). The turmeric plant produced a yellow pigment called curcumin and was very potential as a natural antioxidant (Osawa et al., 1995; Rajput et al., 2013). In addition to containing curcumin, turmeric also contains demethoxycurcumin, methoxycurcumin, and taetrahydrocurcuminoid compounds (Kiuch et al., 1993). Curcumin could be used as a therapeutic and treatment including as an antioxidant, anti-inflammatory, inhibiting lipid peroxidase, antimicrobial, anti-viral, anti-tumor and nematocidal (Kiuch et al., 1993; Osawa et al., 1995; Sharma et al., 2005). In Indonesia, turmeric was used as a kitchen spice, traditional medicine in the form of "*Jamu*" and natural food coloring, improving taste, and food preservatives. Turmeric could be supplemented as feed additives to improve poultry performance. Turmeric powder supplementation at the level of 4% could improve the production and quality of duck eggs (Ismoyowati et al., 2022). Turmeric could stimulated digestive enzymes and pancreatic lipase so that it increased the digestibility and absorption of nutrients (Ammon et al., 1993; Platel and Srinivasan, 2000). A study by Rajput et al. (2013) showed that curcumin supplementation at a dose of 0.2 g/kg in broiler chicken rations could increase the length and weight of the duodenum, jejunum, and broiler ceca, which led to improved nutrient digestibility. Turmeric powder supplementation would improve egg production, egg weight, feed conversion (Gumus et al., 2018). Curcumin has the activity of inhibiting the absorption of dietary cholesterol in the gastrointestinal tract (Arafa, 2005). Curcumin supplementation will also lower blood cholesterol and triglyceride levels in quail eggs (Saraswati and Tana, 2016). Turmeric powder contains phytoestrogens of 7.97% and was estrogenic so that it stimulated the development of ovarian follicles (Saraswati et al., 2014; Azouz, 2020). The usage of mixed herbs would provide a complementary effect, so it was better than single administration. Turmeric and fenugreek powder together could improve egg production and quality, compared to single administration (Azouz, 2020). Supplementation of a mixture of turmeric and fenugreek could improve egg production and quality of chicken eggs because it increases availability (Azouz, 2020). The mixture of cinnamon and thyme in the quail ration improves egg mass and eggshell thickness (Vali and Mottaghi, 2016). A study by Suwarta and Suryani (2019) concluded that by supplementing a mixture of turmeric and cinnamon in quail rations at the level of 40g/kg could improve egg production, feed conversion, increase egg weight and increase color index. On this basis, current study was performed to examine the supplementation of a mixture of turmeric (TP) and black cumin powder (BCP) in the ration on blood composition, production performance and egg quality of native chickens.

MATERIALS AND METHODS

Ethical approval

The study was carried out without killing livestock and was carried out under the supervision of the livestock production laboratory at Universitas Mercu Buana, Yogyakarta. This research was conducted under the supervision of Teaching Farm Laboratory No. 15/KEP/TF/III/2022.

Research design

The research was conducted at the Livestock Production Laboratory, Mercu Buana University, Yogyakarta, Indonesia. The main materials of the study were turmeric powder (*Curcuma longa*), black cumin powder (*Nigella sativa*), research rations, and 8 female native chickens aged 17 weeks with a body weight of 1436±22.2 g. Turmeric powder and black cumin powder are made from turmeric rhizome and black cumin seeds purchased from a local market in the city of Yogyakarta. Turmeric (TP) is made from turmeric rhizome, sliced crosswise with a thickness of 3 mm then dried in the oven at 60 degrees Celsius for 30-36 hours, until the water content reaches 14 percent, then ground and sieved through a 25-mesh sieve. Black cumin seed powder (*Nigella sativa*) (BCP) is made from local cumin seeds which are dried at a temperature of 60-70^{o} Celsius, then ground and sieved with a size of 25-mesh. Native chickens were adapted using treatment rations for 1 week at 17 weeks of age. The treatment started at 18 weeks of age and ended at 34 weeks of age. Treatment ration formulated isoprotein and isoenergy. The composition and nutrient content of the treatment rations are presented in Table 1. Drinking water and rations were given *ad libitum*. Native chickens were kept in cages, measuring length, width, height (160×80×45 cm), and given light for 15 hours using LED lamp with an intensity of 120 lux.

The study was designed in a completely randomized design, with mixed TP and BCP supplementation treatment variations. Ninety-six native chickens were allocated to twelve research cages, each with 8 chickens. Every three research cages were used for one treatment, so there were 4 treatments. Each treatment was differentiated based on the supplementation of a mixture of turmeric and cumin powder, namely T0 (control), T1 (0.5 g TP + 0.5 g BCP)/kg ration, T2 (5 g TP + 5 g BCP) /kg ration, T3 (7.5 g TP and 7.5 g BCP)/kg ration. Performance data retrieval was carried out for 16 weeks from the age of 18 to 34 weeks. The variables of performance observed included production variables, namely feed consumption, egg production (HDA), egg weight, egg mass, feed conversion measured from each replication every week. Egg quality variables include yolk weight, shell weight, shell thickness, egg color index, cholesterol, and lipoprotein. Egg quality was observed at weeks 24, 28, and 34, where in each replication 3 eggs were taken randomly. Egg quality variables here included yolk color and egg yolk color fan (Roches, Switzerland). Egg cholesterol levels were measured from egg production at the end of the 34th week, where for each replication 3 eggs were taken using the Liebermann–Burchard method. At week 34, the final body weight of the chickens was also measured. Blood parameters were observed when the chickens were 34 weeks old, by taking 2 ml from the wing vein. The blood parameters observed included the number of red blood cells and white blood cells (hemocytometer method), hematocrit value (microhematocrit method),

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Hb (cyanmethemoglobin method), and blood cholesterol (CHOD-PAP method).

Data analysis

The data collected were analyzed using analysis of variance, followed by Duncan's Multiple range Test using SPSS version 22, with a significance level of <0.05.

Table 1 - Composition and nutrient content of treatment rations

Feed ingredients (kg) \ Treatment (%)	T0	T1	T2	T3
Corn	46.00	46.00	46.00	46.00
Rice bran	22.00	22.00	22.00	22.00
Soybean meal	23.00	23.00	23.00	23.00
Fish meal	6.00	6.00	6.00	6.00
Bone meal	2.00	2.00	2.00	2.00
$CaCO_3$	1.00	1.00	1.00	1.00
Total	100	100	100	100
Turmeric + black cumin powder (TBCP)	0	2.5g +2.5g/kg ration	5g+5g/kg ration	7.5g+7.5g/kg ration
Crude protein (%)	17.2	17.2	17.2	17.2
Metabolized energy (kcal/kg)	2720	2720	2720	2720
Crude fiber (%)	4.98	4.98	4.98	4.98
Ether extract (%)	3.98	3.98	3.98	3.98
Ca (%)	2.72	2.72	2.72	2.72
P (%)	0.86	0.86	0.86	0.86
Methionine (%)	0.56	0.56	0.56	0.56
Lysine (%)	1.16	1.16	1.16	1.16

RESULTS

Table 2 below summarizes the effect of various levels of TPBC supplementation on feed consumption, HDA, body weight, egg mass, egg weight, yolk weight, color index, shell thickness, and also cholesterol and lipoprotein levels. The rations supplemented with TBCP at various levels of 5 g/kg (T1), 10 g/kg (T2), and 15 g/kg (T3) resulted in feed consumption, egg production, body weight at 34 weeks, egg mass, shell weight significantly higher (P<0.05) than control (T0). The highest egg production, egg mass, weight and shell thickness were obtained in treatment T3 (15 g/kg). The highest egg production was obtained at T3 (62.90%) and the lowest was at control (57.53%). TBCP supplementation significantly reduced the conversion of feed, cholesterol, and lipoproteins. Feed conversion between treatments T1, T2, and T3 was not significantly different and significantly lower than control (T0). The lowest feed conversion was obtained at T3 (3.59) and the highest at T0 (3.88). TBCP supplementation at various levels (T0, T1, T2, T3) resulted in egg and yolk weights that were not significantly different (P<0.05). Cholesterol and lipoproteins level in the yolk between treatments T1, T2, and T3 were not significantly different but significantly decreased compared to control (T0). The lowest yolk cholesterol was obtained at T3 (316.5 mg/100g) and the highest was in the control (337.7 mg/100g).

The results in Table 3 show that TBCP supplementation increased the value of hematocrit, Hb, red blood cell (RBC) and white blood cell (WBC) values, and significantly decreased blood cholesterol, LDL, HDL, and triglyceride levels (P<0.05). The highest hematocrit, Hb, RBC and WBC were obtained at T3, where respectively: hematocrit (28.23%), Hb (7.8 mg/dl), RBC (1.48×106/µl) and WBC (30.83 x103/µl). T3 produces the lowest blood plasma cholesterol (121.53 mg/dl).

Table 2 - Performance of native chickens at various levels of supplementation with a mixture of turmeric and black cumin powder

Performance \ Treatments	T0	T1	T2	T3
Feed consumption (g/head/day)	94.93±1.03^{a}	96.53±0.31^{b}	97.13±0.70^{b}	97.60±0.46^{b}
HDA (%)	57.53±1.03^{a}	59.60±1.06^{b}	62.23±0.38^{c}	62.90±0.46c
Body weight age 34 weeks (g/head)	1546±22.74^{a}	1563±25.79^{ab}	1601±8.50^{b}	1598±36.39^{b}
Egg weight (g/egg) (ns)	42.87±0.31	43.50±0.36	42.70±0.66	43.44±0.38
Egg mass (kg)	2.74±0.07^{a}	2.90±0.06^{b}	2.96±0.04^{bc}	3.06±0.01^{c}
Feed conversion	3.88±0.13^{a}	3.73±0.07^{b}	3.65±0.04^{b}	3.59±0.02^{b}
Yolk weight (g/egg) (ns)	13.13±0.31	13.15±0.16	13.15±0.21	12.98±0.07
Egg shell weight (g/egg)	4.47±0.10^{a}	4.58±0.11^{ab}	4.61±0.10^{b}	4.44±0.04^{c}
Shell thickness (mm)	0.264±0.004^{a}	0.268±0.006^{ac}	0.270±0.002^{bc}	0.280±0.004^{c}
Yolk color	3.67±0.12a	3.73±0.58^{ab}	3.83±0.58^{ab}	3.93±0.15^{b}
Cholesterol (mg/100 g)	337.7±10.12^{a}	320.3±5.22^{b}	317.9±0.95^{b}	316.5±1.75^{b}
HDL (mg/100 g)	46.7±0.75^{a}	44.9±0.70^{b}	44.8±0.37^{b}	44.0±0.72^{b}
LDL (mg/100 g)	61.36±1.75^{a}	58.05±0.76^{b}	57.94±0.28^{b}	57.31±0.35^{b}

$^{a, b, c}$ in the same line shows significantly different results (P<0,05)

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Table 2 - Chicken blood parameters at various levels of supplementation with a mixture of turmeric and black cumin powder

Blood parameters \ Treatments	T0	T1	T2	T3
Red blood cells (106/µl)	1.32±0.45[a]	1.39±0.15[b]	1.45±0.21[c]	1.48±0.025[c]
White blood cells (x103/µl)	23.93±0.64[a]	26.33±0.50[b]	28.53±0.70[c]	30.83±0.40[d]
Hb (mg/dl)	7.1±0.1[a]	7.4±0.3[b]	7.6±0.1[a]	7.8±0.2[a]
Hematocrit (%)	25.27±0.42[a]	26.20±0.26[b]	27.2±0.11[b]	28.23±0.15[b]
Blood cholesterol (mg/dl)	140.06±1.66[a]	136.13±0.31[b]	127.66±1.07[c]	121.53±2.59[d]
Blood LDL (mg/dl)	56.03±0.38[a]	53.23±0.93[b]	46.83±0.59[c]	42.87±0.31[d]
Blood HDL (mg/dl)	111.56±0.80[a]	98.20±1.63[b]	76.77±0.90[c]	58.03±1.00[d]
Blood triglycerides (mg/dl)	867.0±5.7[a]	801.1±9.2[b]	712.6±23.0[c]	589.7±3.0[d]

[a, b, c] in the same line shows significantly different results (P<0,05)

DISCUSSION

Increased supplementation of turmeric black cumin powder (TBCP) in the ration, significantly increased feed consumption (P<0.05). It is believed that this is associated to several the active compounds found in TP and BCP. Turmeric curcumin has activity to stimulate digestive enzymes, including amylase, trypsin, chymotrypsin, and lipase. Likewise, BC also contains several compounds that could stimulated the secretion of digestive enzymes thereby improving nutrient absorption (Akylidiz and Denli, 2016). Turmeric contains several active compounds curcumin, turmeric also contains demethoxycurcumin, methoxycurcumin and tetrahydrocurcuminoid compounds (Kiuch et al., 1993). In BC, there were 18 compounds that can be identified from black cumin oil consisting of 99.14% essential oils, namely aldehydes (23%), gamma terpinene (14.5%) acetic acid (10.9%) and 1,3,8-p-menthatriene (7.9%) (Jalilzadeh-Amin et al., 2011; Hashemi and Davoodi, 2011). Supplementation of curcumin at a dose of 0.2 g/kg would improve feed consumption and increase the length and weight of the duodenum, jejunum and ceca of broilers, which led to improved digestive process and increase nutrient utilization (Rajput et al., 2013). Black cumin, contains several compounds including essential fatty acids, and could affect the size of the villi and the composition of microbes in the gastrointestinal tract, and increase the secretion of digestive enzymes (Kumar et al., 2017; Kumar and Patra, 2017). Single use of BC would generally result in feed consumption not so significant (Aydin, 2006).

Increased supplementation of the TBCP mixture increased egg production, body weight at 34 weeks, and egg mass. This happens because nutrients are more available either due to increased feed consumption or increased nutrient digestibility and absorption. Turmeric contains several active compounds, especially curcumin, demethoxycurcumin and bisdemethoxycurcumin. BC contains the essential fatty acids linoleic, linolenic and arachidonic which are required for egg synthesis (Qader et al., 2020). Phytoestrogens in herbs affect poultry reproduction, through the mechanism of vitellogenin synthesis in hepatocytes so that it would increase the number and weight of yolks (Levi et al., 2009). In addition, these two herbs also have activity as anti-microbial, anthelmintic and antifungal (Azeem et al., 2014). According to Malekizadeh et al. (2012), supplementation of turmeric powder at the level of 10 and 30 g/kg ration did not affect the egg weight of SCWL chickens. The use of turmeric powder at the level of 4%, increased the egg production of laying hens significantly (Rahardja et al., 2016). A study by Park et al. (2012) showed that the usage of turmeric powder on Lohmann brown laying hens singly at the level of 0.01; 0.025 and 0.5% would increase egg production, weight and egg mass. Akhtar et al. (2003) also revealed that BC supplementation at the level of 1.5% could increase egg production from 59 to 77 percent. Aydin et al. (2006) stated that supplementation of BC level 2 or 3% could improve egg production and feed conversion. Hence, it can be said that mixing herbal ingredients gives a better effect. A mixture of phytobiotics is not only used to improve absorption, it can also be used to increase immunity so that it can improve growth (Hashemi and Davoodi, 2011).

Besides being used to improve egg production and egg mass, increasing TBCP supplementation will also improve feed conversion. This was due to improvements in digestibility as well as increased nutrient utilization. The same condition was also shown in a research done by Suwarta and Suryani (2019) that TCP supplementation would increase egg production and feed conversion in quail. The usage of black cumin seed powder in broiler chickens at levels of 2 g/kg and 4 g/kg can improve feed efficiency (Toghyani et al., 2010). Aydin et al. (2006) stated that the utilization of BC in laying hens could increase egg production. Seidavi et al. (2020) highlight that supplementation of cumin oil in quail rations by 2 and 5 percent could improve growth and egg production.

Increased TBCP supplementation did not affect egg weight and yolk weight, but improved color index, shell weight and thickness index. This is different from BC which can increase egg weight, but the yolk weight is not significantly different (Tahan and Bayram, 2011). BC supplementation at the level of 1% and 2% in the ration of laying hens produced egg weights that were not significantly different (Aydin et al., 2006). Likewise, 4% turmeric supplementation did not affect egg weight (Rahardja et al., 2016). Supplementation of a mixture of turmeric and sumac in each ration at the level of 0.25% resulted in an egg weight that was not significantly different (Gumus et al., 2018).

The increase in yolk color index was due to the presence of a yellow pigment from curcumin which was deposited in the yolk. The use of BC alone could not improve the color of the yolk, but when combined with parsley (*Petroselinum crispum*), it increased the color index of the yolk (Tahan and Bayram, 2011). The use of 1% turmeric flour will increase the

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color of the yolk from 18.87 to 15.62% (Radwan Nadia et al., 2008). Hassan (2016) stated that the utilization of turmeric powder could be used to improve the color of laying hen's yolks if used at 4% in the ration of laying hens. Suwarta and Suryani (2019) concluded that supplementation of a mixture of turmeric and cinnamon at a level of 10 g/kg to 40 g/kg could improve yolk color.

TBCP supplementation would increase the weight and thickness of the shell. The increase was in accordance with some previous studies. Supplementation of turmeric powder at the level of 150 mg, would increase the strength and thickness of Hy-line brown chicken shells (Liu et al., 2020). BC supplementation in laying hens rations at levels 2 and 3%, increased shell thickness and weight (Aydin et al., 2008). Cumin seed supplementation of 1.5% also increased the thickness of the shell from 0.32 to 0.34 mm in chicken eggs (Akhtar et al., 2003). Cumin seed supplementation of 1.5% also increased the thickness of the shell from 0.32 to 0.34 mm in chicken eggs (Akhtar et al., 2003). The utilization of 1% turmeric powder would numerically increase the weight of the shell (Radwan Nadia et al., 2008). Suwarta and Suryani (2019) highlight that supplementation of a mixture of turmeric and cinnamon each at 40 g/kg sausage could improve quail eggshell weight. Turmeric improves the condition of the uterus, in terms of calcium deposition so that it will affect the thickness and weight of the eggshell. Turmeric supplementation in duck rations can improve the internal condition of the oviduct, especially in eggshell calcification, so that the eggshell of the ducks increased from 0.43 mm to 0.51 mm (Radwan Nadia et al., 2008; Ismoyowati et al., 2022). The increase in eggshell thickness was due to the increase in Ca intake due to increased feed consumption, and also curcumin can reduce the heat stress (Liu, 2020). Aydin et al. (2008) showed that BC supplementation at levels of 2 or 3% could improve eggshell thickness and strength.

Cholesterol and lipoprotein levels decreased with increasing of TBCP supplementation. This was in accordance with previous research that the utilization of herbs could lower cholesterol levels of yolk. Park et al. (2012) stated that turmeric supplementation in laying hens at the levels of 0.10, 0.20, and 0.25% reduced cholesterol, triglyceride, and lipoprotein levels in the blood plasma of laying hens. Supplementation of BC powder in laying hens at a level of 1-2% could improve health in broilers and lower cholesterol in yolks (Al-Sanabani and Al-Hothaify, 2022). According to Aydin (2008), BC supplementation at levels 2 and 3% lowers cholesterol. BC oil supplementation at the level of 3 ml/kg ration would reduce triglycerides and cholesterol (Bölükbaşi et al., 2009). Likewise, BC supplementation at the level of 3% significantly reduced yolk cholesterol levels in laying hens (Aydin et al., 2008). Supplementation of a mixture of turmeric and cinnamon powder at a level of 15 g/kg could reduce quail egg cholesterol from 1303 to 1134.8 g/100 g (Suwarta and Suryani, 2019).

This was because curcumin could increase cholesterol-7 alpha-hydroxylase activity in the liver and increase cholesterol catabolism (Riasi, 2012). Alkaloids in curcumin and black cumin inhibit cholesterol absorption from the gastrointestinal tract and inhibit cholesterol synthesis in the liver (Ismoyowati et al., 2022). Curcumin inhibits cholesterol absorption from the feed (Arafa, 2005).

TBCP supplementation increased the value of hematocrit, hemoglobin, and also the number of red blood cells (RBC) and white blood cells (WBC). The increase is related to the presence of the active components of black cumin and turmeric. Black cumin contains thymoquinone and thymohydroquinone which have strong anti-oxidant activity (Arslan et al., 2017), as well as turmeric also contains the active compound curcuminoid which has activity as an antioxidant and as an immune modulator (Dalal et al., 2018). Antioxidants produce low levels of peroxides in the membrane, thereby reducing the degree of susceptibility to hemolysis in red blood cells (Arslan et al., 2017) . The value of PVC, hemoglobin, RBC and WBC in rats increased when supplemented with BC (Ekanem and Yusuf, 2008). Supplementation of herbal ingredients can increase the production of antibodies and immunoglobulins (Dalal et al., 2018), so that the white blood cell content increases. Increased TP and BCP supplementation reduced cholesterol, lipoprotein, and triglyceride levels in blood plasma. Antioxidants will affect catabolism and fat accumulation (Fallah and Mirzaei, 2016). The use of phytobiotics can reduce blood plasma cholesterol levels (Jouybari et al., 2009). The use of 1% turmeric will lower total cholesterol. Turmeric will inhibit the activity of HMG Co-A, stimulate the conversion of cholesterol into bile acids and increase cholesterol excretion (Dalal et al., 2018). Black cumin contains thymoquinone and is an active compound that plays a role in lowering cholesterol (Al-Sanabani and Al-Hothaify, 2022).

CONCLUSION

From this study it can be concluded that supplementation with a mixture of turmeric and black cumin powder (TBCP) at the level of 7.5 g/kg in native chicken rations will improve feed consumption, egg production, egg mass, feed conversion and body weight. TBCP supplementation did not affect egg weight and yolk weight but increased yolk color, shell weight and thickness, and reduced cholesterol and yolk lipoprotein levels. Blood parameters, namely RBC, WBC, Hematocrit and Hb will increase, while Cholesterol, Lipoprotein and triglycerides will decrease. In general, TBCP supplementation will improve the performance, egg quality and blood parameters of native chickens.

DECLARATIONS

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Authors' contribution

FX Suwarta contributes on research and the write up of the manuscript; Chatarina Lilis Suryani, Niken Astuti, and Lukman Amin contribute on ration formulation and analysis, statistical analysis, and logistics.

Conflict of interests

The authors have not declared any conflict of interests.

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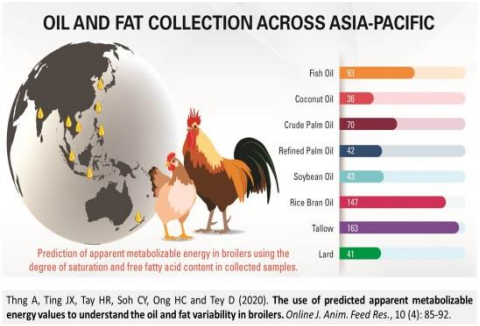

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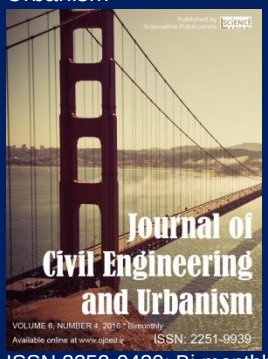

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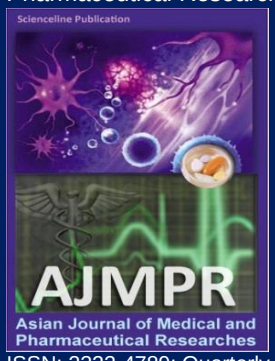

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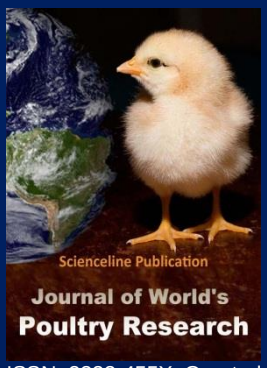

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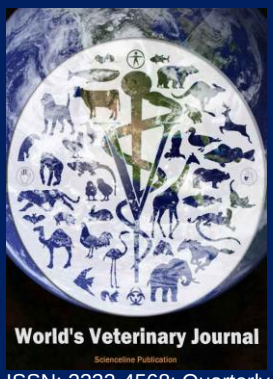

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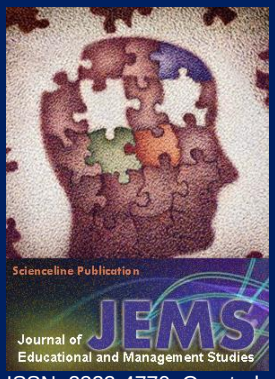

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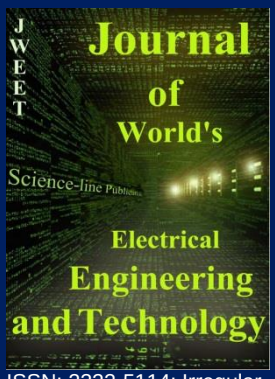

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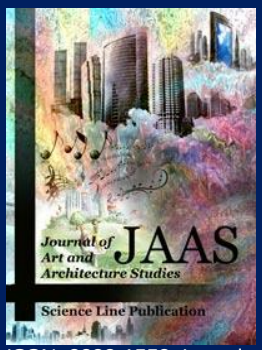

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