ONLINE JOURNAL OF ANIMAL AND FEED RESEARCH

An International Peer-Reviewed Journal which Publishes in Electronic Format

Volume 10, Issue 4, July 2020

ISSN 2228-7701

Online Journal of Animal and Feed Research

An international peer-reviewed journal which publishes in electronic format (online)

Online J. Anim. Feed Res., 10 (4): July 27, 2020

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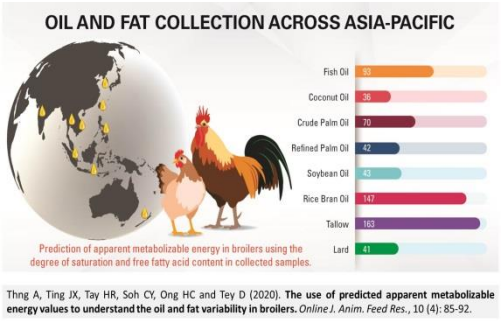
Volume 10 (4); July 27, 2020

Research Paper

The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers.



Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). **The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers.** *Online J. Anim. Feed Res.*, 10 (4): 85-92.

Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D.

Online J. Anim. Feed Res., 10(4): 150-157, 2020; pii: S222877012000021-10

Abstract: The objective of this study was to analyze the predicted apparent metabolizable energy (AME) of different oil samples across Asia Pacific region and investigate the AME values in broilers of different ages (< 21 or > 21 days old). A total of 635 oil and fat samples consisting of 93 fish oils, 36 coconut oils, 70 crude palm oils, 42 refined palm oils, 43 soybean oils, 147 rice bran oils, 163 tallows and 41 lards were collected and analyzed over a span of eight years (2011 to 2018). The free fatty acid (FFA) content of oil and fat samples were analyzed through acid-base titration and the degree of saturation (ratio of unsaturation to saturated fatty acids; U:S) were determined with Gas Chromatography with Flame Ionization Detector (GC-FID). The FFA and U:S of the samples were then incorporated into the Wiseman equation to correlate the oil and fat qualities with the AME. Our survey revealed AME variations were prevalent in most of the oil types studied, with fish oils and tallows showing the largest energy gap within oil samples. The results showed that the predicted AME values for oil and fat samples differ across countries, even within batches from the same supplier. Taken together, our investigation suggests that there is a considerable variation in the AME values of oils and fats, which may affect the feed formulation precision.
Keywords: Dietary energy, Fatty acid composition, Lipids, Oil quality, Poultry

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Review

Treatment trials of epizootic lymphangitis with local medicinal plants: a review.

Asfaw M and Fentahun T (2020). **Treatment trials of epizootic lymphangitis with local medicinal plants: a review.** *Online J. Anim. Feed Res.*, 10(4): 93-101.

Asfaw M and Fentahun T

Online J. Anim. Feed Res., 10(4): 158-166, 2020; pii: S222877012000022-10

Abstract: The aim of this paper was to review the use of local herbal medicines to treat Epizootic lymphangitis (EZL) and challenges related with safety, efficacy and quality control of herbal medicines. EZL has deleterious effect on both welfare and health of the horses and mules. In addition it has a serious negative impact on mainly the livelihoods of cart-horse owners/drivers. Basically, antifungal drugs for the treatment of EZL are costly and mostly unavailable in such areas especially in developing countries like Ethiopia. Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice, and current and future studies on medical activities can be effective in treating this disease. Furthermore, there is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional pharmaceuticals. These days, there are several trials on local plants like *Xanthium stramorium (X. stramorium)*, *Combretum molle (C. molle)* seed and *Phytolacca dodecandra (P. dodecandra)* extracts inhibited the growth of *Histoplasma capsulatum var farciminosum (H. capsulatum var farciminosum).* Among these, the aqueous and n-butanol extracts of *P. dodecandra* with minimum inhibitory concentration (MIC) of (0.078%-0.156%) and (0.039%–0.078%) respectively have been inhibiting the growth of *H. capsulatum var. farciminosum*. In vivo, over 58.3% horses with the disease responded to treatment then the other two plant extracts. In conclusion, *P. dodecandra* extracts showed a significant effect to inhibit the growth of *H. capsulatum var farciminosum* in vitro and EZL *in vivo*.
Keywords: *Combretum molle*, Epizootic lymphangitis, *Xanthium stramorium*, *Phytolacca dodecandra*, Medicinal herbs.

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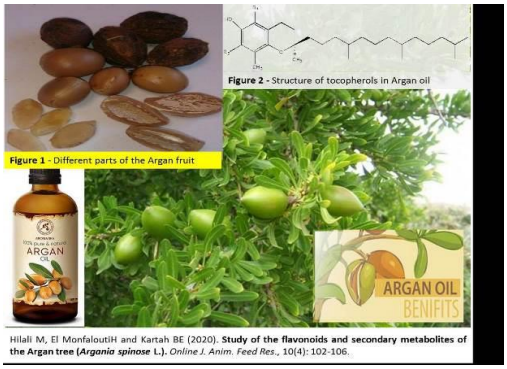
Research Paper

Study of the flavonoids and secondary metabolites of the Argan tree (*Argania spinose* L.).



Hilali M, El MonfaloutiH and Kartah BE.

Online J. Anim. Feed Res., 10(4): 167-171, 2020; pii: S222877012000023-10

Abstract: The separation and identification of the main phenolic compounds present in the co-product of the Argan tree (*Argania spinosa*) were carried out using high performance liquid chromatography techniques coupled with mass spectrometry (LC-ESI-MS/MS).The study was based on the retention times of the peaks of the phenolic compounds in samples and was compared to those of the controls (reference compounds) and supplemented by an analysis of the fragmentations of the molecules by mass spectrometry. Phenolic compounds in the pulp of the Argan namely such as catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenin (0.07%) were found. Phenolic acid is consisted of gallic acid (5%) and protocatechuic acid (21.1%). These compounds are more dominant than flavonoids. The flavonoids-O-rhamnoglucosides the most dominant compounds is isorhoifoline (7.2%) and hesperidin (4.5%) against rutin (0.1%) and rhamnetin-O-rutinoside (0.5%) are less dominant. The main compounds are the hyperoside (13.4%) and isoquercetin (10%). On the other hand, naringenin-7-O-glucoside constituted the most minority compound of this type of flavonoid in the pulp of the fruit of the Argan tree (the percentage of naringenin-7-O-glucoside and quercetin-3-O-arabinose is 15.3%). There are other phenolic compounds in the pulp of the Argan namely such as catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenin (0.07%). The main flavonoids found in the leaves of the Argan tree are Quercetin (21.73%), Myricetin (54.34%), Hyperoside (8.69%), and also Myricetin-3-Ogalactoside (9.78%). Argan cake is rich in flavonoids. Among these, epicatechin (110 mg/kg), catechin (11 mg/kg), protocatechic acid (15.2 mg/kg), vanillic acid (16.3 mg/kg) and 4-hydroxybenzyl alcohol (8.6 mg/kg) are higher in Argan cake. Argan oil is richer in tocopherol (597 to 775 mg/kg), Argan oil is rich in gamma tocopherol (631 mg/kg), and that make Argan valuable nutraceutical. The study of the secondary metabolites and especially the flavonoids of the Argan tree was undertaken with the aim of identifying new metabolites making it possible to increase the industrial than commercial value of the Argan tree.
Keywords: Argan tree, Biological activity, Flavonoids, Metabolism, Phenolic acids.

[Full text-PDF] [HTML]

Research Paper

Postmortem study on indigestible foreign bodies in rumen and reticulum of cattle (case: Haramaya and Awaday municipal abattoirs, Eastern Ethiopia).

Amin I and Fentahun T.

Online J. Anim. Feed Res., 10(4): 172-179, 2020; pii: S222877012000024-10

Abstract: A cross-sectional study was conducted from November, 2017 to March, 2018 at Haramaya and Awaday Municipal Abattoirs of Oromia Regional State, Eastern Ethiopia, with the objectives of assessing the prevalence of rumen and reticulum foreign bodies, identifying types of foreign bodies and associated risk factors for the occurrences of foreign bodies. Following appropriate ante-mortum examinations, postmortem examinations were employed for the recovery of foreign body from rumen and reticulum. The study animals were selected by using systematic random sampling using regular interval to study animal from the total slaughtered animals. From a total of 384 (207 female and 177 male) cattle examined, 41.7% (n=160) were found to contain foreign bodies at slaughter. When the prevalence was compared between genders, breed, among different age groups, and different body condition score, higher prevalence of foreign bodies 50.7%, 75.0%, 80.0%, 83.3%, were observed in female, cross breed, age older than 10 years, and animal having poor body condition score respectively. These aforementioned factors are considered as potential risk factors were highly significantly associated with the occurrence of foreign bodies. Rumen harbored mostly plastic materials while reticulum was the major site for the retention of metallic objects. The non-penetrating foreign bodies have higher prevalence than penetrating foreign bodies. The commonly recovered non-penetrating foreign bodies were plastics (46.9%), cloth (30.0%), rope (21.3%) and leather (18.8%). The penetrating foreign bodies were metals (5%). Plastics were recovered as the most common foreign bodies and followed by cloths, Ropes, and leathers. It is concluded that the detection of this level of prevalence of foreign bodies in cattle causes high mortality and morbidity, reduced production and productivity. Therefore, appropriate solid waste disposal system need to implement in the study area to prevent health risk of ruminants and also to protect the environment.
Keywords: Body Condition Score, Cattle, Foreign body, Reticulum, Rumen.

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Mini Review

Influence of dietary manipulations and milking frequency on production of dairy cows.

Khaskheli AA.

Online J. Anim. Feed Res., 10(4): 180-184, 2020; pii: S222877012000025-10

Abstract: Dairy cow responses to various types of diets differently and dairy farmers can use knowledge of its behavior to improve the cow well-being and yield. This review was carried out in order to better understanding the influence of dietary manipulation and milking frequency on the dairy cows' production. The results obtained from review of already conducted studies revealed that the dairy cow is significantly affected by composition, quality, amount and regimes of the diet. Maximum daily milk production, milk protein, milk lactose, milk fat, total solids are recorded in dairy cows when ad-libitum feed and water is provided. Further, sufficient water intake is necessary for maintaining body fluids and proper ion balance, digestion, absorption, metabolization of nutrients, elimination and body cooling. Feeding and water frequency stimulate the mammary functions and milk synthesis, which is actually a non-invasive method. Reducing feeding frequency from 2x daily to 1x daily decreases milk yield from 7 to 38% in dairy cows, however changing feeding frequency from 2x to 3x daily results about 18% increase in milk production that can be economically acceptable. On the other hand, increasing milking frequency from 2x to 3x daily increase milk production up to 30%. Therefore, in addition of dietary manipulation and milking frequency, high quality feed and ad-libitum water plays always a key role for improving the performance and production of dairy cows.
Keywords: Ad-libitum, Diet, Performance, Production.

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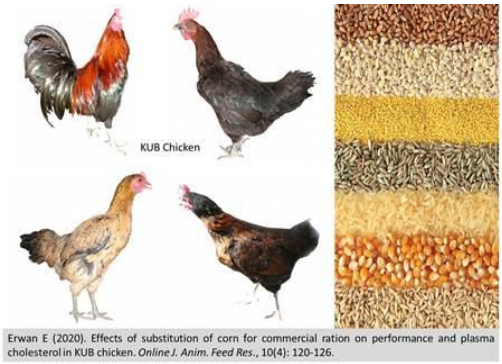
Research Paper

Effects of substitution of corn for commercial ration on performance and plasma cholesterol in KUB chicken.



Erwan E (2020). Effects of substitution of corn for commercial ration on performance and plasma cholesterol in KUB chicken. *Online J. Anim. Feed Res.*, 10(4): 120-126.

Erwan E.

Online J. Anim. Feed Res., 10(4): 185-190, 2020; pii: S222877012000026-10

Abstract: The aim of the present study was to evaluate growth performance and plasma total cholesterol (TCHO) concentration of KUB chickens fed by substitution of commercial feed with corn in 1 of day-old chick of KUB were raised for 10 weeks in two dietary groups including only commercial feed (group A) and a commercial feed substituted by 30% corn (group B). Data were analysed by T-test. The results showed that there was no significant effect of the treatments on feed intake, body weight (BWG) and feed conversion ratio (FCR) in KUB chickens. Similarly, plasma TCHO concentration did not show any difference between two experimental rations. However, total income of commercial feed substituted with 30% corn was higher than commercial feed. It was concluded that corn could be used at 30% to substituted commercial feed without significantly affecting the KUB chicken performance and TCHO. Present research considered usefulness of corn as a potential alternative of commercial feeds in KUB chickens in Indonesia.
Keywords: KUB chickens, Feed Intake, Body Weight Gain, Feed Conversion Ratio, Commercial Feed, Corn

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Online Journal of Animal and Feed Research

ISSN: 2228-7701

Frequency: Bimonthly

Current Issue: 2020, Vol: 10, Issue: 4 (July 27)

Publisher: SCIENCELINE

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2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 150-157; July 27, 2020 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.21

THE USE OF PREDICTED APPARENT METABOLIZABLE ENERGY VALUES TO UNDERSTAND THE OIL AND FAT VARIABILITY IN BROILERS

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ORIGINAL ARTICLE

PII: S222877012000021-10

Received: May 26, 2020

Revised: July 03, 2020

ABSTRACT: The objective of this study was to analyze the predicted apparent metabolizable energy (AME) of different oil samples across Asia Pacific region and investigate the AME values in broilers of different ages (< 21 or > 21 days old). A total of 635 oil and fat samples consisting of 93 fish oils, 36 coconut oils, 70 crude palm oils, 42 refined palm oils, 43 soybean oils, 147 rice bran oils, 163 tallows and 41 lards were collected and analyzed over a span of eight years (2011 to 2018). The free fatty acid (FFA) content of oil and fat samples were analyzed through acid-base titration and the degree of saturation (ratio of unsaturation to saturated fatty acids; U:S) were determined with Gas Chromatography with Flame Ionization Detector (GC-FID). The FFA and U:S of the samples were then incorporated into the Wiseman equation to correlate the oil and fat qualities with the AME. Our survey revealed AME variations were prevalent in most of the oil types studied, with fish oils and tallows showing the largest energy gap within oil samples. The results showed that the predicted AME values for oil and fat samples differ across countries, even within batches from the same supplier. Taken together, our investigation suggests that there is a considerable variation in the AME values of oils and fats, which may affect the feed formulation precision.

Keywords: Dietary energy, Fatty acid composition, Lipids, Oil quality, Poultry

INTRODUCTION

Vegetable oils and animal fats are usually added to animal diets to increase dietary energy concentration (Ravindran et al., 2016). Since oils and fats confer at least twice as much energy as other food nutrients such as carbohydrates and proteins (Ahiwe et al., 2018; Blair, 2018), there is a greater demand in optimizing the use of these products to meet the energy requirements of poultries (Ravindran et al., 2016). Furthermore, high fat feeding in poultry has been proven to improve the digestibility and absorption of non-lipid constituents (Blair, 2018). However, the quality of oils and fats are highly variable, and their digestibility are dependent on their chemical structures (Codony et al., 2017). Poor processing and storage conditions can also cause structural changes in oils and fats, leading to high fluctuations in the nutritional values (FAO/WHO, 2001; Gibson and Newsham, 2018).

Fat digestion consists of the emulsification of dietary fat with bile salt, followed by the enzymatic hydrolysis of triglycerides. The 2-monoglycerides, formed from partial hydrolysis of triglycerides, improve the solubility and absorption of free fatty acids through the formation of micelles (Pond et al., 2004; Scanes et al., 2019). As such, low levels of 2-monoglycerides will result in incomplete micellar solubilization of free fatty acids. It was previously reported that the total micellar fatty acids were lowest in the duodenum of free fatty acid (FFA) – fed chicks where monoglycerides were present at trace level (Hofmann and Borgstrom, 1962; Sklan, 1979). In addition, fat digestion is also highly dependent on the degree of fatty acid saturation where Tancharoenrat et al. (2014) reported a higher digestibility with unsaturated fatty acids such as oleic acid and linoleic acid in comparison to saturated fatty acids such as palmitic and stearic acids. Additionally, the natural emulsifying properties of unsaturated fatty acids could also aid in mixed micelle formation and absorption, resulting in better utilization of saturated fatty acids (Rodriguez-Sanchez et al., 2019). Given the importance of FFA and the degree of saturation of oil (ratio of unsaturated to saturated fatty acids; U:S) in oil digestion and absorption, the Wiseman equation incorporates both of these parameters into one general equation to predict the energy values of different sources of oils and fats (Wiseman and Blanch, 1994).

As these macromolecules are important energy sources for animals, it is imperative for us to understand the variation of oil quality based on apparent metabolizable energy (AME) across countries and oil types, and its impacts on broilers. Previous reports showed that the fat utilization in broilers was age dependent where fat utilization improved with age (Rodriguez-Sanchez et al., 2019). Animal nutritionists often struggle to formulate feed with adequate energy intake due to the variation of the nutritional values in oil and fat samples that can lead to reduction in the performances of the

To cite this paper: Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers. *Online J. Anim. Feed Res.*, 10 (4): 150-157.

animals and substantial economic losses (Niu et al., 2009; Ahiwe et al., 2018). A better understanding of the AME of different oil and fat samples can be gained by incorporating the FFA and U:S data into the Wiseman equation to generate information on the quality of the oil and fat samples; this will allow nutritionists to make informed decisions on their use for feed formulation to achieve consistent animal performance. In this study, the AME for oil and fat samples were determined based on the Wiseman equation to highlight the importance of accurate information on dietary energy value of feed.

MATERIALS AND METHODS

Instrumentation

DL 50 GRAPHIX auto-titrator (Mettler-Toledo, Ohio, United States) and DG113-SC glass electrode (Mettler-Toledo) were used to determine free fatty acid content. 7890B GC-FID (Agilent Technologies, California, United States) with Supelco SPTM-2560 (L × I.D. 100 m × 0.25 mm, df 0.20 µm thickness) (Sigma-Aldrich, Missouri, United States) was used for chromatographic separation of fatty acid methyl esters (FAME).

Sample collection and preparation

A total of 635 oil and fat samples with plant and animal origins were collected across the Asia Pacific region and analyzed over a span of eight years from year 2011 to 2018. These samples included tallow, rice bran oil, fish oil, palm oil (crude and refined palm oil), soybean oil, lard and coconut oil. All samples were stored in plastic containers upon receipt and kept in the chiller at 2 °C to 6 °C. Before analysis, the samples were either thawed at room temperature or melted in the oven at 60 °C. All samples were analyzed within one week from the collection date.

Free Fatty Acid (FFA) content

The FFA content of oil and fat samples were determined with an in-house method, modified from the Association of Official Analytical Chemists (AOAC) method (AOAC, 2012). Fifty (50) mL of 95% ethanol (Aik Moh Paints and Chemical Pte Ltd, Singapore) was added to 1.0 g of oil or fat sample in a titration cup. The sample was stirred for 60 s under stirring speed of 50% with an auto-titrator. After stirring, titration was done with 0.1 N sodium hydroxide (Merck KGaA, Darmstadt, Germany) as the titrant using a pH sensor with measurement mode set as equilibrium controlled. The result was calculated from the volume consumption of the sodium hydroxide titrant and its concentration. Based on the oil type, the FFA content is expressed either as % oleic acid, % palmitic acid or % lauric acid.

Fatty Acid Methyl Esters (FAME) composition analyses

FAME composition of oil and fat samples were determined using an in-house method, with modification from Association of Official Analytical Chemists (AOAC) method (AOAC, 2012). Four mL of 2% (w/v) methanolic sodium hydroxide (Merck) was added to 40 mg of fat or oil sample and refluxed until there were no visible fat globules. 5 mL of 14% boron trifluoride in methanol (Sigma-Aldrich) was added and refluxed for another 2 mins. Finally, 10 mL of heptane (Sigma-Aldrich) was added and refluxed for another 1 min. Subsequently, the content was cooled to room temperature. Next, 15 mL of 26% (w/v) sodium chloride (Merck) was added and swirled vigorously. The top organic layer (heptane) was filtered through sodium sulphate (Merck) and injected into the GC-FID for chromatographic separation. Extracted samples were analyzed with helium at a flow rate of 0.85 mL/min as carrier gas and a split ratio of 40:1. Injection volume was set at 0.4 µL with injection port temperature set at 260 °C. The GC oven temperature was programmed at 140 °C for the first 5 mins and raised to 235 °C at 5 °C/min for 15 mins, followed by 15 °C/min to 250 °C for 5 mins. The total run time was 45 mins. Percentage composition of each FAMEs in oil and fat samples were calculated with Supelco 37 component Fatty Acid Methyl Esters (FAME) Mix certified reference material (CRM) (Sigma-Aldrich) as reference standard.

Data analysis

Prediction of Apparent Metabolizable Energy (AME) using Wiseman equation

AME of samples were predicted using a general equation (Equation 1) with A, B, C and D based on the values shown in Table 1 (Wiseman and Blanch, 1994; Wiseman et al., 1998).

$$AME\ (MJ/kg\ fat) = A + B*FFA + C*e^{D(U/S)} \quad (1)$$

Apparent Metabolizable Energy (AME) variation

AME range was calculated as the difference between the highest and lowest predicted AME values whereas relative variations were calculated as the ratio of calculated range against lowest predicted AME or literature AME.

Statistical analyses

Single measurement data were calculated for the AME of each oil type. Descriptive statistics were calculated using Microsoft Excel 365 and presented in Table 3.

To cite this paper: Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers. *Online J. Anim. Feed Res.*, 10 (4): 150-157.

Table 1 – Empirical values of constants A – D used in Wiseman equation to predict the apparent metabolizable energy (AME) values of poultry at different ages

Constant (unit)	Young broilers (< 21 days)[a]	Old broilers (> 21 days)[a]
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

[a] Empirical values of constants A – D were categorized into two groups, young broilers (aged < 21 days) and old broilers (aged > 21 days). All young broilers (aged < 21 days) followed the same empirical values for constants A – D, likewise for old broilers (aged > 21 days).

RESULTS

Predicted Apparent Metabolizable Energy (AME) values for all samples

Using GC-FID and acid-base titration, all samples were analyzed for their lipid composition and FFA content (Table 2). Descriptive analysis of eight different oil types were presented in Table 3. AME of young broilers (aged < 21 days) and old broilers (aged > 21 days) were studied in this paper. Based on the GC-FID analyses, it was determined that the U:S for crude palm oil was lowest amongst all samples analyzed while the U:S for soybean oil was the highest, with relatively low FFA content of 1.01% oleic acid recorded (Table 3). When the data was further extrapolated using Equation 1, it was found that the highest predicted mean AME values were from soybean oil, at 8362 kcal/kg (young broilers) and 8672 kcal/kg (old broilers). On the other hand, the lowest predicted mean AME values were from crude palm oil with the predicted AME values at 6617 kcal/kg (young broilers) and 7669 kcal/kg (old broilers) (Table 3).

It was apparent that the predicted AME values were inconsistent across all oil samples. In particular, a large spread of AME for fish oil samples for different age groups of broilers was observed. The energy gaps for young (< 21 days old) and old broilers (> 21 days old) were 2295 kcal/kg and 1417 kcal/kg, with a relative variation of 36% and 19% respectively (Table 3). The AME gap for crude palm oil for young (< 21 days old) and old broilers (> 21 days old) were found to be 1057 kcal/kg and 540 kcal/kg with relative variations of 17% and 7% (Table 3). Comparatively, refined palm oil also showed a smaller AME spread relative to crude palm oil, with 506 kcal/kg for young broilers (8% variation) and 250 kcal/kg for old broilers (3% variation) (Table 3). As the three major oil groups (e.g. tallow, rice bran oil, and fish oil) accounted for 63% of the total oil and fat samples collected and represented the majority of the oil and fat products (Table 2), the data for these groups were further analyzed (Table 4).

Tallow

Large AME discrepancy of 2670 kcal/kg for young broilers with relative variation of 49% and 1565 kcal/kg for old broilers with a relative variation of 23% were observed (Table 3). Out of 163 samples, 85% of the samples were received from five different sources originating from South Korea (Table 4). Majority of the samples were from the same source, supplier 1, where it accounted for approximately 78% of the tallow samples received from South Korea. Large spread of AME was observed for supplier 1, at 1248 kcal/kg with a relative variation of 20% for young broilers and 626 kcal/kg with a relative variation of 8% for old broilers (Figure 1). As such, supplier 1 from South Korea was singled out with samples collected in eight batches over a span of five years, from year 2012 to 2016. The AME values observed were inconsistent even within batches where the energy spread was in the range of 230 kcal/kg to 1063 kcal/kg with relative variation of 3% to 17% (Table 5). Likewise, AME values for tallow samples from supplier 3 were inconsistent as well, with energy spread at 1362 kcal/kg for young broilers (aged < 21 days) and 740 kcal/kg for old broilers (aged > 21 days) (Figure 1). This translated to relative variations of 20% for young broilers (aged < 21 days) and 10% for old broilers (aged > 21 days).

Rice bran oil

All rice bran oil samples received were from Thailand since 2012. From 2012 to 2014, the predicted ME values were highly variable as shown in Figure 2. However, from 2015 onwards, the predicted AME values were calculated to be more consistent where the energy values ranged from 7500 kcal/kg to 8000 kcal/kg (relative variation of 7%) with only seven outlier samples. High FFA content of 12.50% oleic acid was observed (Table 3).

Fish oil

Majority of the fish-based oil samples were from Indonesia and Thailand (63% of fish oil samples). Figure 3 showed that fish oils from Thailand consisted of large energy gaps of 2295 kcal/kg (young broilers) and 1417 kcal/kg (old broilers). Similarly, when the AME for different batches of fish oils from the same supplier (supplier A) in Thailand were determined, it was found that the AME ranged from 6442 kcal/kg to 8738 kcal/kg with relative variation of 36% in young broilers (Table 4). Likewise, a difference of 1926 kcal/kg in terms of AME variation (30%) was observed between fish oil samples from Indonesia (Table 4).

To cite this paper: Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers. *Online J. Anim. Feed Res.*, 10 (4): 150-157.

DISCUSSION

The quality and efficiency of feed formulations are highly dependent on two main factors, the extent and accuracy of animal nutritionists' knowledge on raw materials' qualities and compositions, as well as the nutrient requirements of targeted species (Lall and Dumas, 2015). Animal nutritionists struggle to formulate feed with adequate energy when lipid energy values stated in traditional feed tables often deviate from the actual energy value due to various reasons such as poor storage and processing conditions. It is also likely that these values did not account for the species and age dependent metabolism. While Baião et al. (2005) reported that the AME for tallow was in the range of 7000 kcal/kg, Figures 1(A) and 1(B) indicated that regardless of animal age groups, inconsistency in AME values of tallow were apparent where energy variations occurred even within the same supplier with relative variation as high as 17% for the same batch of tallow samples. Without proper lipid quality evaluations, this will eventually lead to poorer animals' performances and economic losses.

The predicted mean AME of soybean oil is the highest as compared to other oil types. One of the main contributing factors is the presence of high unsaturated fatty acids where the recorded U:S ratio was 4.71 (Table 3). Consistent to a previous study conducted by Rodriguez-Sanchez et al. (2019) where it was reported that hydrolysis in unsaturated diets were relatively more efficient than saturated diets which results in higher digestibility and absorption. The AME of soybean oil ranged from 6665 kcal/kg to 8796 kcal/kg for young broilers (aged < 21 days) and 7716 kcal/kg to 8997 kcal/kg for old broilers (aged > 21 days). In comparison, a study showed that the AME of soybean oil is at 8790 kcal/kg (Baião et al., 2005), demonstrating 12% relative variation from the literature value. Low fatty acid content (1.01% oleic acid) was also observed for soybean oil. The presence of high FFA decreases bile secretion which in turns reduces micellar formation, leading to poor absorption of digested materials (Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019). A study conducted by Wiseman and Salvador (1991) showed that AME is inversely proportional to the FFA content, with the effect being more pronounced in younger broilers. In agreement with the study conducted by Wiseman and Salvador, our survey also showed that the AME values of young broilers were more divergent as compared to older broilers, demonstrating their sensitivity to oil quality variations possibly due to less developed physiological capacity in fat utilization (Rodriguez-Sanchez et al., 2019).

Our results showed that from year 2015 onwards, the AME values for rice bran oil samples collected from Thailand were more consistent (Figure 2). One plausible reason could be due to technological improvements made to the manufacturing or transporting processes in Thailand. While more consistent AME values were observed over the years, the FFA content of rice bran oil remains the highest among the eight oil types analyzed (Table 3). As rice bran contains endogenous lipase capable of digesting and hydrolyzing the triglycerides present to form FFA (Goffman and Bergman, 2003, Vallabha et al., 2015), it is possible that the samples collected were likely to be extracted from poor quality rice bran where the triglycerides had been hydrolyzed (Rajan and Krishna, 2009). Interestingly, while high FFA content was observed in rice bran oil (Table 3), its AME remains one of the highest among the other oil types. One of the reasons could be due to the relatively higher U:S where the presence of unsaturated fats aid in the solubilization and absorption of FFA (Hofmann and Borgstrom, 1962).

Large energy gaps were observed in fish oil with 36% variation in young broilers (< 21 days) and 19% variation in old broilers (> 21 days). This is likely due to the presence of different fish oils with different oil quality grades such as salmon fish oil and crude tuna fish oil. There are different standards for different fatty acid compositions of different fish origins. For instance, while the standard for C22:6 (n-3) docosahexaenoic acid of tuna oil ranges from 21.0 – 42.5% of total fatty acids, similar standard for wild salmon oil ranges from 6.0 – 14.0% (FAO/WHO, 2017). Fish oils are also susceptible to lipid oxidation due to the high degree of unsaturation (European Food Safety Authority (EFSA, 2010) where unsaturated fatty acids are prone to oxidation (Dominguez et al., 2019). Comparatively, refined palm oil has lower AME spread as compared to crude palm oil. This is likely due to the refining processes that may have possibly removed the impurities, and therefore confers a more consistent oil quality in refined palm oil.

Given these analyses, it is evident that having a proper lipid analysis in place is fundamental for accurate estimation of dietary energy in feed formulations.

Table 2 – Number of oils and fats collected across Asia Pacific region, per oil type

Oils and fats	Count
Tallow	163
Rice bran oil	147
Fish oil	93
Crude palm oil	70
Soybean oil	43
Refined palm oil	42
Lard	41
Coconut oil	36

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Table 3 – Descriptive analysis data of the eight different oil types for broilers

Item	Apparent Metabolizable Energy (AME) of poultry (broiler)															
	Tallow		Rice bran oil		Fish oil		Crude palm oil		Soybean oil		Refined palm oil		Lard		Coconut oil	
	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21
Statistics (kcal/kg)																
Minimum	5448	6930	6709	7566	6442	7530	6359	7524	6665	7716	6533	7652	6002	7399	6685	7705
Maximum	8118	8495	8138	8503	8738	8947	7416	8064	8796	8997	7038	7902	7825	8320	7914	8375
Range	2670	1565	1429	937	2295	1417	1057	540	2131	1282	506	250	1822	922	1229	670
1st quartile	6742	7744	7608	8332	6912	7826	6509	7616	8321	8624	6858	7810	6996	7878	7159	7926
Median	6949	7853	7710	8488	7183	7965	6601	7666	8579	8807	6908	7835	7115	7934	7493	8121
3rd quartile	7087	7913	7787	8567	7916	8373	6674	7704	8589	8814	6964	7862	7347	8060	7664	8229
Mean	6886	7820	7635	8194	7370	8076	6617	7669	8362	8672	6886	7824	7084	7925	7411	8075
SE of mean	29	15	38	12	60	34	21	11	49	43	18	9	62	31	54	32
Standard deviation, σ	374	191	265	147	581	329	172	88	463	279	114	56	398	200	323	192
Nutritional parameters																
FFA (%)[a]	2.66		12.50		4.62		7.07		1.01		0.34		1.35		8.45	
U:S ratio	1.28		2.70		2.14		1.10		4.71		1.20		1.48		2.10	

< 21 = Young broilers of age less than 21 days; > 21 = Old broilers of age more than 21 days; SE = Standard error; FFA = Free fatty acid content; U:S = unsaturated: saturated fatty acid. [a] Free fatty acid content is expressed as % palmitic acid for crude and refined palm oil, % lauric acid for coconut oil and % oleic acid for the rest of the oil types.

Table 4 – Details of samples collected from the different countries for the three major oil types (Tallow, rice bran oil and fish oil), with minimum, maximum, range and mean apparent metabolizable energy (AME) of young broilers (< 21 days)

Oil types / Country	Tallow						Rice bran oil						Fish oil					
	AME (< 21 days) (kcal /kg)						AME (< 21 days) (kcal/kg)						AME (< 21 days) (kcal/kg)					
	Min	Max	R	Mean	n	n_s	Min	Max	R	Mean	n	n_s	Min	Max	R	Mean	n	n_s
Thailand	-	-	-	-	0	-	6709	8138	1429	7635	147	7	6442	8738	2295	7367	25	5
Indonesia	-	-	-	-	0	-	-	-	-	-	0	-	6499	8425	1926	7416	34	4
Vietnam	6299	6333	35	6316	2	-	-	-	-	-	0	-	6575	8321	1746	7103	24	9
Philippines	5448	6561	1112	5989	6	1	-	-	-	-	0	-	7919	8233	313	8028	3	1
Singapore	5826	6372	546	6138	3	1	-	-	-	-	0	-	7199	7199	0	7199	1	1
Taiwan	6002	6640	638	6345	10	2	-	-	-	-	0	-	7440	7826	386	7633	2	1
South Korea	6240	8118	1878	7003	138	5	-	-	-	-	0	-	7948	7948	0	7948	1	1
New Zealand	6073	6520	448	6370	3	2	-	-	-	-	0	-	7861	8309	448	8031	3	1
India	6503	6503	0	6503	1	1	-	-	-	-	0	-	-	-	-	-	0	-
Total	5448	8118	2670	6886	163	12	6709	8138	1429	7635	147	7	6442	8738	2295	7370	93	23

AME (< 21 days) = Apparent metabolizable energy for young broilers of age less than 21 days; Min = Minimum; Max = Maximum; R = Range; n = number of observations; n_s = number of suppliers.

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Table 5 – Details of tallow samples collected from South Korea, Supplier 1, with minimum, maximum, range calculated for apparent metabolizable energy (AME) of young broilers (< 21 days)

Batch \ AME (< 21 days) (kcal/kg)	Count	Min	Max	R	Percentage variation (%)
Batch 1	26	6240	7303	1063	17
Batch 2	7	6546	7124	578	9
Batch 3	24	6612	7428	815	12
Batch 4	22	6710	7488	778	12
Batch 5	18	6630	7413	783	12
Batch 6	1	7258	7258	0	NA
Batch 7	1	7173	7173	0	NA
Batch 8	9	6908	7137	230	3

AME (< 21 days) = Apparent metabolizable energy for young broilers of age less than 21 days; Min = Minimum; Max = Maximum; R = Range; NA = Not Applicable

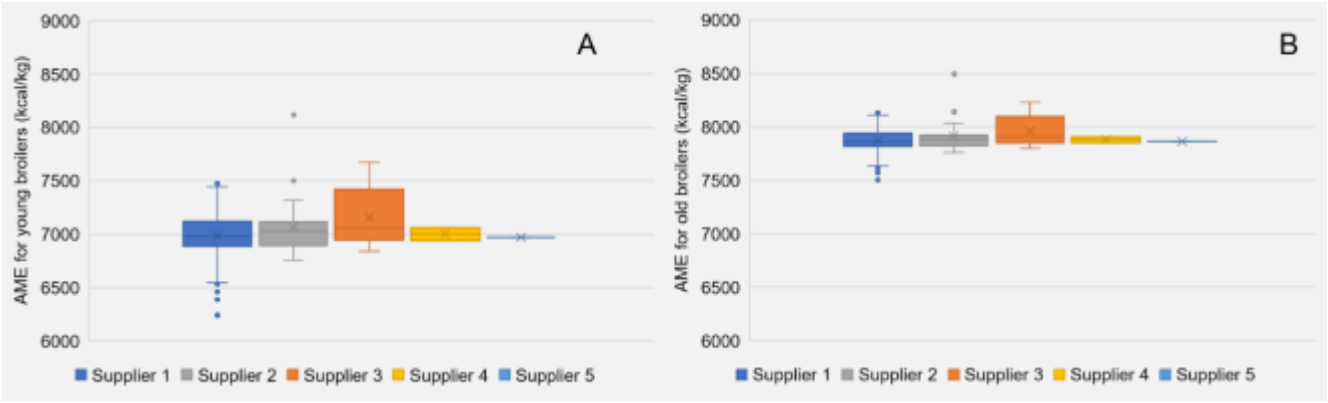

Figure 1 - Variations in minimum, first quartile, median, third quartile and maximum in predicted apparent metabolizable energy (AME) for both (A) young broilers (aged < 21 days) and (B) old broilers (aged > 21 days) differentiated by the different tallow suppliers in South Korea.

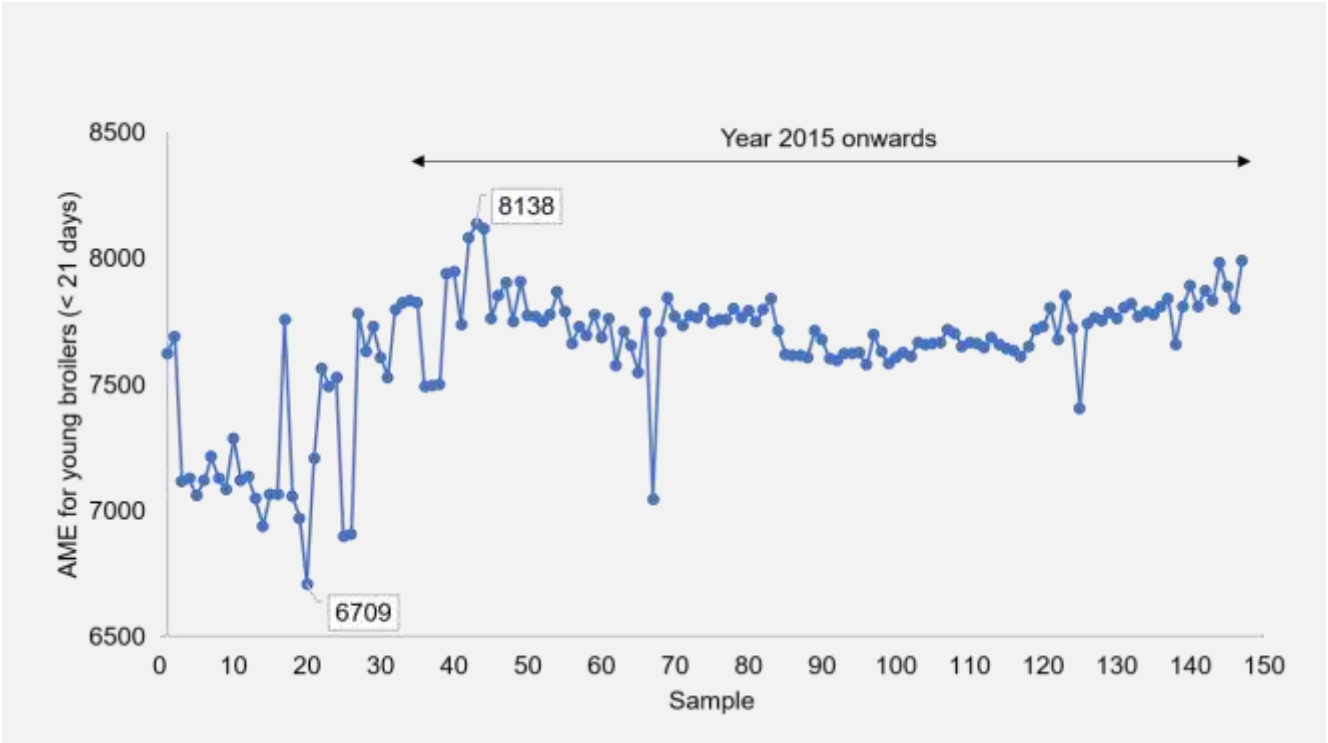

Figure 2 - Predicted apparent metabolizable energy (AME) trend graph for young broilers (aged < 21 days) with emphasis on year 2015 onwards, for rice bran oils.

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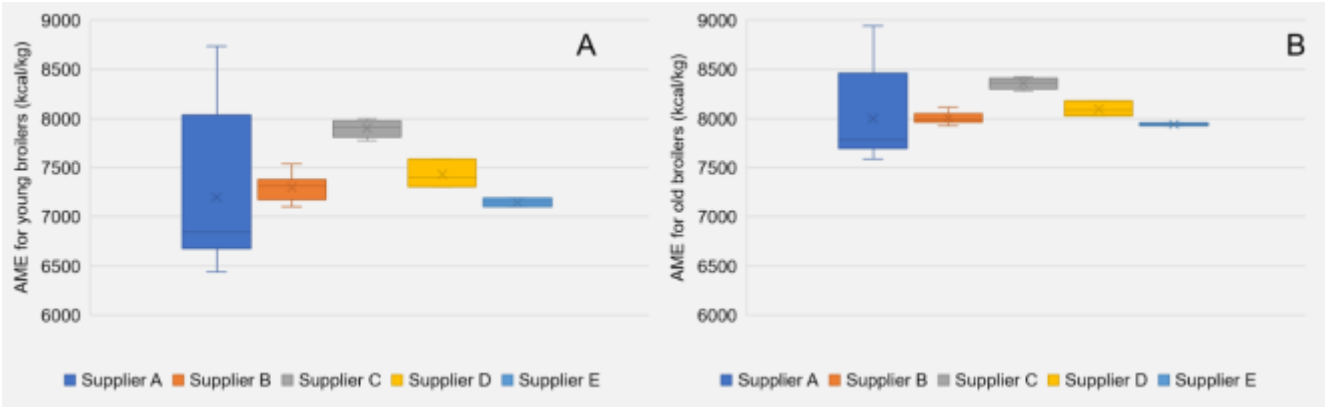

Figure 3 - Variations in minimum, first quartile, median, third quartile and maximum in predicted apparent metabolizable energy (AME) for both (A) young broilers (aged < 21 days) and (B) old broilers (aged > 21 days) differentiated by the different fish oil suppliers in Thailand.

CONCLUSION

In conclusion, our data suggested that there is a considerable variation of the AME values in oils and fats. The AME variation that existed across oil samples from different regions and even within batches from similar suppliers may affect the feed formulation precision if the variation remains unaccounted for. Generic lipid energy values extracted from the traditional feed table were typically inaccurate as the animal species and age dependent metabolism were likely not considered in these tables. Furthermore, poor storage and processing conditions may deteriorate the oil quality as well. Inevitably, inconsistent AME values will not only contribute to huge economic losses but may also impact the animal performances adversely due to inaccurate feed formulations that fail to meet the caloric requirements of the animals. In view of these concerns, it is important to have a proper lipid evaluation tests in place for a more accurate lipid profile (e.g. AME value) estimation. Additionally, oil quality parameters such as peroxide and *p*-anisidine values should also be considered for oxidative stability evaluation as oil and fat quality may deteriorate over time. To improve oil and fat qualities, bio-emulsifiers and antioxidants can be used concurrently to improve oil and fat qualities in the context of oxidative stability and feed fat variability control.

DECLARATIONS

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Authors' Contribution

A. Thng proposed the design of study, prepared the manuscript and performed the laboratory analysis. J.X. Ting, H.R. Tay, C.Y. Soh and H.C. Ong assisted with the laboratory analyses. D. Tey reviewed and edited the manuscript.

Conflict of Interests

The authors declared that there is no conflict in this study.

Acknowledgements

The authors thank the suppliers from the Asia Pacific region for the collection of oil and fat samples. We also thank Dr C. Sugumar for his scientific contributions in this study, and Ms R.Lye, Drs J. Tan, K.P. Chan and H. Chirakkal for their constructive feedbacks on the manuscript draft. This study was supported by Kemin Industries (Asia) Pte Limited, Animal Health and Nutrition.

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To cite this paper: Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers. *Online J. Anim. Feed Res.*, 10 (4): 150-157.

2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 158-166; July 27, 2020 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.22

TREATMENT TRIALS OF EPIZOOTIC LYMPHANGITIS WITH LOCAL MEDICINAL PLANTS: A REVIEW

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Supporting Information

REVIEW

PII: S222877012000022-10

Received: May 26, 2020

Revised: July 10, 2020

ABSTRACT: The aim of this paper was to review the use of local herbal medicines to treat Epizootic lymphangitis (EZL) and challenges related with safety, efficacy and quality control of herbal medicines. EZL has deleterious effect on both welfare and health of the horses and mules. In addition it has a serious negative impact on mainly the livelihoods of cart-horse owners/drivers. Basically, antifungal drugs for the treatment of EZL are costly and mostly unavailable in such areas especially in developing countries like Ethiopia. Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice, and current and future studies on medical activities can be effective in treating this disease. Furthermore, there is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional pharmaceuticals. These days, there are several trials on local plants like *Xanthium strumarium (X. strumarium)*, *Combretum molle (C. molle)* seed and *Phytolacca dodecandra (P. dodecandra)* extracts inhibited the growth of *Histoplasma capsulatum var farciminosum (H. capsulatum var farciminosum).* Among these, the aqueous and n-butanol extracts of *P. dodecandra* with minimum inhibitory concentration (MIC) of (0.078%-0.156%) and (0.039%–0.078%) respectively have been inhibiting the growth of *H. capsulatum var. farciminosum*. In vivo, over 58.3% horses with the disease responded to treatment then the other two plant extracts. In conclusion, *P. dodecandra* extracts showed a significant effect to inhibit the growth of *H. capsulatum var farciminosum in vitro* and EZL *in vivo*.

Keywords: *Combretum molle*, Epizootic lymphangitis, *Xanthium stramorium*, *Phytolacca dodecandra*, Medicinal herbs.

INTRODUCTION

Epizootic lymphangitis (EZL) is a contagious, chronic disease which mainly affects horses, mules, and camels (Biyashev et al., 2019; Adedokun et al., 2020). It is caused by *Histoplasma capsulatum var. farciminosum* (*H. capsulatum var. farciminosum*). The disease is characterized clinically by a suppurative, ulcerating, and spreading pyogranulomatous, multifocal dermatitis and lymphangitis. It is seen most commonly in the extremities, chest wall and the neck, but it can also be manifested as an ulcerating conjunctivitis of the palpebral conjunctiva, or rarely as a multifocal pneumonia. The organism may also invade open lesions including ruptured strangles abscesses and castration wounds (OIE, 2009).

The source of *the H.capsulatum var. farciminosum* can be the skin lesions, nasal and ocular exudates of infected animals or the soil. This organism can also spread on fomites (common utensil) such as grooming or harnessing equipment. Biting flies in the genera Musca and Stomoxys are thought to spread the conjunctival form. The pulmonary form probably develops when the animal inhales the organism (Public Health Agency of Canada, 2001).

Epizootic lymphangitis is more common in tropical and subtropical regions than in temperate zones (Alsaad et al., 2016). *H.capsulatum var. farciminosum*is endemic in some countries in the Mediterranean region, and in parts of Africa and Asia including India, Pakistan and Japan (OIE, 2009). Many treatment types have been tried, largely without success. Parenteral iodides and amphotericin B have been reported as effective. However, although the disease is highly prevalentand economically important in Ethiopia (Ameni, 2006), the treatment options mentioned have not been employed because of the cost of the drugs and their absence in Ethiopia. This warrants for the need for other approaches including the use of traditional remedies.

Traditional medicine is used throughout the world as it is heavily dependent on locally available plant species and plant-based products and capitalizes on traditional wisdom-repository of knowledge (Awas and Demissew, 2009). The wide spread use of traditional medicine could be attributed to cultural acceptability, economic affordability and efficacy against certain type of diseases as compared to modern medicines. Knowledge of medicinal plants of Ethiopia and of their uses provides vital contribution to human and livestock health care needs throughout the country (Belayneh et al.,

Citation: Asfaw M and Fentahun T (2020). Treatment trials of epizootic lymphangitis with local medicinal plants: a review. *Online J. Anim. Feed Res.*, 10(4): 158-161.

2012). The plant- based human and livestock health care persists and remains as the main alternative treatment for different ailments in Ethiopia, largely due to shortage of pharmaceutical products, prohibitive distance of the health service stations, unaffordable prices by small holder farmers and pastoralists for conventional drugs, emergence and re-emergence of certain diseases and appearance of drug resistant microbes and/or helminthes (Bekele et al., 2012).

Whole plant of *Xanthium strumanrium* (*X. strumanrium*) as well as all parts separately is used in medicine (Bhogaonkar and Ahmad, 2012; Fan et al., 2019). The genus xanthium also possess antibacterial, antiviral, antimalarial, fungicidal, insecticidal and cytotoxic activities against cancer cell lines (Sravani et al., 2010; Passos et al., 2019). *Phytolacca dodecandra* (*P. dodecandra*) is one of the many plants claimed to have antifungal secondary metabolites. The antifungal effect of the crude aqueous extract of *P. dodecandra* was demonstrated *in- vitro* against different genera of dermatophytes of human pathogen and four clinical isolates of *Candida albicans* (Woldeamanuel et al., 2005; Tura et al., 2017).

Combretum molle was used as a medicinal plant since ancient times (Grønhaug et al., 2008). The test of *C. molle* seed extract as antifungal property has been demonstrated in various studies (Masoko et al., 2007; Anato and Ketema, 2018). Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice, and current and future studies on medical activities can be effective in treating diseases (Singh, 2015).

In terms of population exposure alone, it is essential to identify the risks associated with the use of herbal medicines, and in this regard, the safety of these products has become an issue of great public health importance (WHO, 2004; WHO, 2005). There is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional or orthodox pharmaceuticals (WHO,2005; Zhou et al., 2013). Thus, the general requirements and methods for quality control of finished herbal products remain far more complex than for other pharmaceuticals (WHO, 2003; WHO, 2004; WHO, 2005). Therefore the main objectives of this paper is to review the commonly used local herbal medicine to treat EZL and challenges related with safety, efficacy and quality control of local herbal medicines.

TREATMENT TRIALS FOR EZL USING HERBAL MEDICINE

The Xanthium strumarium leaf extract

The *X. strumarium,* a rough cocklebur is broad leaved, tap rooted herbaceous annual plant. This is in a family of asteraceae, sub family asteroideae, tribe heliantheae, and genus Xanthium and species *X. strumarium*. It grows as weed throughout on waste lands. Cockleburs are short day plants and they can also flower in the tropics where the day length is constant. The herb is reputed as medicine in Europe, China, Indo-china, Malaysia and America also (Bhogaonkar and Ahmad, 2012). Stem is erect, ridged, rough and hairy and frequently branched which results somewhat bushy plants from 30-120 cm tall. It has small greed unisexual flower occurring in separate cluster at the end of the brunches and main stems. The fruit is brown, hard, woody, bur from 0.4-0.8 inch long and coved with stout, hooked bristle. Its seed are produced in hard, spiny, globes or oval double chambered single seeded bur (Agharkar, 1991).

Beside its medicinal values if a small quantity of parts of the mature plants is consumed, the seeds and seedlings will cause intoxication because extremely toxic chemical carboxyatratyloside is contained in them (Madalln and Sing, 2001). Whole plant of *X. strumanrium* as well as all parts separately is used in medicine (Bhogaonkar and Ahmad, 2012). The genus *xanthium* also possess antibacterial, antiviral, antimalarial, fungicidal, insecticidal and cytotoxic activities against cancer cell lines (Sravani et al., 2010).

Antifungal activity can be determined by the agar diffusion method. Test samples are diluted in Sabouraud dextrose agar followed by solidification in slanting positions. Test fungal cultures are inoculated on the slant and are incubated at 29°C for 3-7 days (Paxton, 1991; Nisaret al., 2010). The principal compounds isolated from *X. strumanrium*leaves are found to contain, isoxanthanol, hydroquinone, caffeyolquinic acids, xanthanol, anthraquinone, cardenolide, leucoanthocyanin, simple phenolic striterpenoids and thiazinedione (Bhogaonkar and Ahmad, 2012). *X. strumanrium* produces secondary metabolites such as alkaloids, tannins, terpenoids, flavonoids, chloroform and n-hexane fractions whose activity has been demonstrated to be antifungal (Gujar and Talwankar, 2012). Antifungal activity of these molecules from *X. strumarium* exhibited 60% and 50% inhibition activity against the major dermatophyte fungi, *Microsporum canis* (Bharathi et al., 2010).

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Figure 1 - Lesions of Epizootic lymphangitis (Wondmnew and Teshome, 2016).

The Phytolacca dodecandra extracts

Antifungal effect is one of the effects of secondary metabolites produced by plants. It is one of the many plants claimed to have antifungal secondary metabolites. Many studies indicated that, saponins are responsible for its antifungal effect. The antifungal effect of the crude aqueous extract of *P. dodecandra*is demonstrated *in vitro* against different genera of dermatophytes of human pathogen and four clinical isolates of *Candida albicans* (Woldeamanuel et al., 2005). The crude aqueous extract is also found to have effect against *H. capsulatum var farciminosum* both *in- vitro* and *in- vivo* (Ameni and Tilahun, 2003; Hadush et al., 2008). The n-butanol and aqueous extracts of *P. dodecandra* are evaluated for their effects on the isolates of *H. capsulatum var. farciminosum* and for the treatment of cases of epizootic lymphangitis. The phytochemical analysis of *P. dodecandra* shows the presence of saponins, alkaloids, and phenolic compounds in the berries of *P. dodecandra*. Thus, the secondary metabolites identified in the berries are all active antifungal compounds (Arif et al., 2009), which could imply that these secondary metabolites could be responsible for the antifungal activity of the berries observe in the n-butanol extract of the berries.

The antifungal effect of n-butanol extract is observed to be much greater than that of the aqueous extract. The minimum inhibitory concentration (MIC) of n-butanol extracts range from (0.039%–0.078%); whereas that of the aqueous extract is in the range of (0.625%–1.250%). Similar finding for the aqueous extract is reported in which the MIC of *P. dodecandra* against the yeast forms of different *Candida* species is higher than 0.5% (Woldeamanuel et al., 2005). Another study shows that the MIC of the aqueous extract of *P.dodecandra* is 1% (Ameni and Tilahun, 2003). The MIC for novel pharmacological compounds should be <0.1% (Kuete, 2010).

The minimum fungicidal concentration (MFCs) of aqueous and n-butanol extracts of *P. dodecandra* ranges from (1.250%–2.500%) and (0.078%–0.156%), respectively. The prepare ointment is topically applied and the result shows that, 58.3% are completely healed, while 41.7% did not cure. Comparable results are reported with the aqueous extracts (Ameni and Tilahun, 2003; Hadush et al., 2008). The n-butanol extract of *P. dodecandra*is effective against *H. capsulatum*

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var. farciminosum. Previous toxicity studies on *P. dodecandra* indicated that human and guinea pigs can tolerate skin irritation of *P. dodecandra*. Moreover, oral LD50 are found to be 2.6 and 2.2 g/kg in mice and rats, respectively (Ameni and Tilahun, 2003; Hadush et al., 2008).

Combretum molle extracts

Combretum molle (*C. molle*) is used as a medicinal plant since ancient times (Grønhaug et al., 2008). The test of *C.molle* seed extract as antifungal property has been demonstrated in various studies (Masoko *et al*., 2007). Phytochemical studies carried out in the genus *Combretum* have shown the occurrence of many classes of constituents, including triterpenes, flvonlds, lignans, non-protein amino acid and tannins from different parts of the plant (Pietrovsk *et al*., 2006). *C. molle* has been widely used as a medicinal plant to treat various diseases such as parasitic, protozoan, and fungal infectious diseases in East and West Africa (Grønhaug et al., 2008). Antifungal activity is reported in numerous fungal models that used *Candida albicans*, *Candida neoformans, Epidermophyton flccosum, Microsporum gypseum, Trichophyton mentagrophytes, Aspergillus fumigatus, Sporothrix schenckii and Microsporum canis* (Masoko et al., 2007).

The minimum inhibitory concentration (MIC) of *C.molle* seed extracts obtain in the study is 0.0156 %. However, the difference between the MIC (0.0156%) and the maximum none inhibitory concentration (0.0078%) suggests that the MIC could be lower. As Kuete (2010) stated, the MIC for novel pharmacological compounds should be <0.1%. So, the MIC (0.0156 %) is below 0.1% and hence this extract can be considered active. The positive control Ketaconazole is found to be more potent than *C.molle* seed extracts in inhibiting the growth of the mycelia form of *H. capsulatum var. farciminosum* (Asres et al., 2006). The *C.molle* seed extracts has inhibitory effect on *H. capsulatum var. farciminosum*. The Minimum inhibitory effect of *C.molle* seed extracts obtained from the study by Wondmnew and Teshome (2016) is also in harmony with previous studies made on fresh garlic extract (0.5 mg/ml, 0.05%) by Mesfi (2012), *P. dodecandra* (0.03%) by Mekonnen et al. (2012). According to a study by Mekonnen et al. (2012), the MICs of n-butanol and aqueous extracts of *P. dodecandra* are (0.039%-0.078%) and (0.625%-1.250%), respectively. The difference in MIC of the two extracts of *P. dodecandra*can be ascribing by the difference in the polarity of the solvent used in the extraction process. This is supported by Masoko et al. (2007); Eloff et al. (2005); and Cowan, (1999), as the polarity of the solvent has great effect on the quantity and types of bio-molecules extract.

The main antifungal molecule in *C.molle* seed extract is tannin (Mishra et al., 2009). The presence of phenolic hydroxyl groups on the surface of tannin molecules participate strongly in the biological activities of tannins. It combines with protein and other polymers to form stable complexes through nonspecific forces such as hydrogen bonding, hydrophobic effects and covalent binding (Stern et al., 1996). This is done by hydrolysis of ester linkage between gallic acid which eventually affects the biosynthesis of cell wall and cell membrane. Impairment of biosynthesis of cell wall and cell membrane cause to increase the permeability of cell membrane and alterations of cell wall .This leads to decrease cell volume and disjunction of cell membrane from the cell wall (Suraya and Darah, 2002). Moreover, this leads to leakage of internal contents and no more exchange of molecule between cell wall and cell membrane.

As Haslam (1996) tannins have two forms, and these are hydrolysable and condensed tannins which affect fungal growth. In the same study make by Ndip et al. (2007) both hydrolysable and condensed tannins have been found to possess antifungal effect. However, the hydrolysable tannins are found to be more effective against fungi. This is because hydrolysable tannins (gallic acid and ellagic acid) are linked to esters of core molecules which will be hydrolyzed easily while condensed tannins are not susceptible to hydrolysis (Haslam, 1996). In other research done by indicated that the fungicidal effect of the extract is due to the presence of high amount of hydrolysable tannins. In addition to its fungicidal effect, when *C.molle* seed extract is used topically, it will promote tissue healing, stop bleeding, stop further infection and heal the wound internally. As mentioned by Stephane et al. (2004), the ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more.

Challenges associated with monitoring safety of local Herbal Medicine for EZL

In terms of equine exposure alone, it is essential to identify the risks associated with the use of local herbal medicines, and in this regard, the safety of these products has become an issue of great animal health importance (WHO, 2004; WHO, 2005). There is no doubt that the increasing cases of poisoning associated with the use of local herbal medicines in many parts of the world in recent times, is necessitating the need to ensure thorough toxicity assessment alongside active pharmacovigilance on these products in order to promote their safe use and protect animal health (Zhou et al., 2013).

Challenges related to the assessment of safety and efficacy of local Herbal Medicine

There is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of local herbal medicines are much more complex than those required for conventional or orthodox pharmaceuticals (WHO, 2005; Zhou et al., 2013). A single local herbal medicine or medicinal plant may contain hundreds of natural constituents, and a mixed local herbal medicinal product may contain several times that number. Suppose every active ingredient is to be isolated from individual herb from which the local herbal medicine is formulated or produced, the time and resources required would be tremendous. Such an analysis may practically be impossible especially where local herbal product is a mixture of two or more herbs (WHO, 2005).

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Medicinal herbs in the future perspectives

Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice, and current and future studies on medical activities can be effective in treating such diseases (Singh, 2015). The use of medicinal plants has a long history; however, the use of the whole plant or raw materials for treatment or experimentation has many drawbacks, including changes in the plant's compounds in different climates, simultaneous development of synergistic compounds that lead to adverse effects of antagonists, or other unexpected changes in bioactivity, and changes or loss of bioactivity due to the variability and accumulation, storage and preparation of raw materials; therefore, advancing towards the isolation of compounds and the use of pure substances with bioactivity, instead of the plant benefits, has certain benefits including convenient examination of therapeutic effects and determination of toxic doses to control the quality of the therapeutic formulation (Zhang, 2011). The beginning of the development of herbal medicines is concurrent with the development of chemistry and isolation, purification, and determination of plant compounds (Shakya et al., 2012).

In the past, the drug discovery of the biological compounds from plant materials and the process of identifying the structures of active compounds from the extracts are problematic depending on the complexity of the compounds and might take weeks, months or even years. Nowadays, the rate of bioassay-guided fractionation has been significantly enhanced by the development of precision instruments such as high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LCMS), magnetic field and nuclear magnetic resonance (NMR) is a recent major breakthrough for the categorization (NMR) is a recent major breakthrough for the categorization of compounds that are extremely limited in quantity in their organisms of origin (Schroeder and Gronquist, 2006). Despite the success of research to produce medicinal plants over the past few decades, future efforts face many challenges. The quality of the herbal product has been studied. Standardization of raw materials is an important issue for the plant industry (Yadav et al., 2014).

Herbaceous plants can be easily infected during growth, processing and collection. Contamination and pollution with heavy metals are two main problems with herbal drugs. It is therefore necessary to improve the quality and quantity of bioactive compounds for the production of herbal drugs while making effort to discover more new herbal drugs (Clark, 1996). Due to expanding the use of natural substances around the world, the quality and safety ofplant-derived medicines should be comprehensively and accurately studied issues and the traditional and the millennial beliefs about these issues cannot be surely trusted; therefore, scientific and enlightening studies are essential to obtain reliable information for the use of medicinal plants in health care (Firenzuoli and Gori, 2007).

Table 1 - Results of the *in vitro* evaluation of methanol extracts of *P. Dodecandra*, *C. longa*, and *D. stramonium* on *H. capsulatum var. farciminosum*

C. longa		*P. dodecandra*		*D. stramonium*		Ketoconazole	
Conc.	Growth	Conc.	Growth	Conc.	Growth	Conc.	Growth
5mg/mL	X	5mg/mL	X	5mg/mL	+	0.8µg/mL	X
2.5mg/mL	X	2.5mg/mL	X	2.5mg/mL	+	0.4µg/mL	X
1.25mg/mL	X	1.25mg/mL	X	1.25mg/mL	+	0.2µg/mL	X
0.625mg/mL	X	0.625mg/mL	X	0.625mg/m	+	0.1µg/mL	X
0.312mg/mL	X	0.312mg/mL	X	0.312mg/m	+	0.05µg/mL	X
0.156mg/mL	X	0.156mg/mL**	X	0.156mg/m	+	0.025µg/mL**	X
0.07mg/mL**	X	0.07mg/mL	+	0.07mg/mL	+	0.0125µg/mL	+
0.03mg/mL	+	-	-	-	-	-	-

Key: x= No growth observed
+= Growth observed

Source: Hawi (2019)

Table 2 - Growth of HCF in different concentrations of *C.molle* seed extract and ketoconazole

Concentration %	Growth	Concentration %	Growth
2%	-	2 × 10⁻¹%	-
1%	-	1 × 10⁻¹%	-
0. 5%	-	5 × 10⁻²%	-
0.25%	-	2.5 × 10⁻²%	-
0.125%	-	1.25 × 10⁻²%	+
0.0625%	-	6.25 × 10⁻³%	+
0.0312%	-	3.12 × 10⁻³%	+
0.0156%	-	1.56 × 10⁻³%	+
0.0078%	+	7.8 × 10⁻⁴%	+
0.0039%	+	3.9 × 10⁻⁴%	+
0.0019%	+	1.95 × 10⁻⁴%	+
0.0009%	+	9.75 × 10⁻⁵%	+
0.0004%	+	4.88 × 10⁻⁵%	+
0.0002%	+	2.44 × 10⁻⁵%	+
0%	+	0%	+

*MIC=no visible growth **visible growth

Source: Wondmnew and Teshome (2016)

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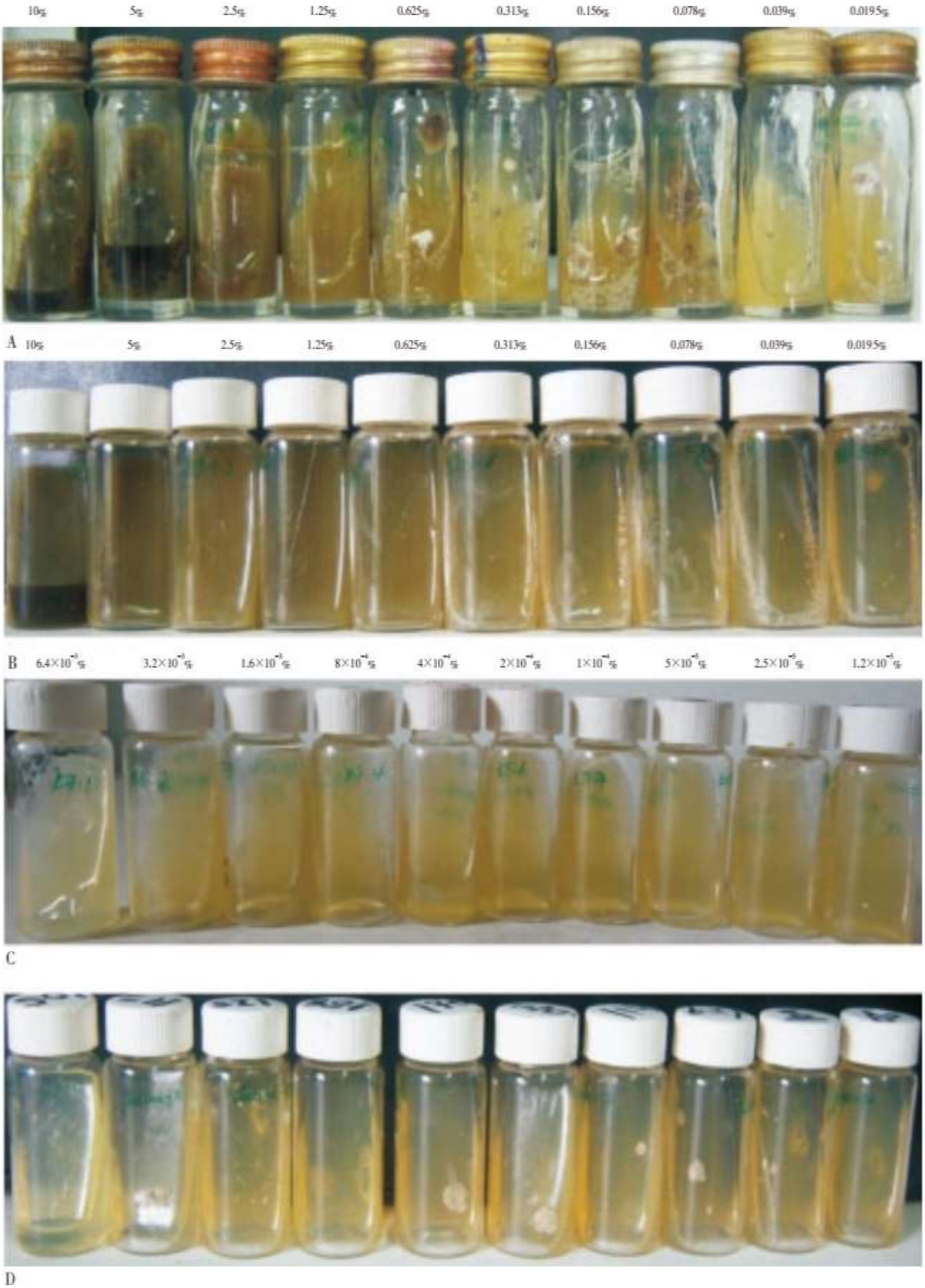

Figure 2 - The MICs of aqueous and n-butanol extracts of *P. dodecandra* against *H. capsulatum var. farciminosum*. A: MIC of aqueous extract: growth was observed starting at 0.625%; B: MIC of n-butanol extract: growth was observed starting from 0.039%; C: MIC of ketoconazole (standard): growth was observed at a concentration of 1.2×10^{-5}%; D: Saline diluted Sabourauds dextrose agar (negative control): growth was observed in all agar plates (Mekonen et al., 2012).

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CONCLUSION and RECOMMENDATION

The *X. strumarium* leaf extract has strong inhibitory effect on the growth of the mycelial form of *H. capsulatum var. farciminosum*. The *X. strumarium* leaf extract can be included in the treatment of epizootic lymphangitis provided that convenient methods of preparation, dose and route of administration should be established through rigorous *in -vitro* and *in -vivo* trials. The n-butanol extract of *P. dodecandra* demonstrated minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values that are considered to have antifungal properties. Therefore, since antifungals are not available for veterinary use in Ethiopia and also as they are expensive; searching for available and affordable antifungals such as n-butanol extract of *P. dodecandra* is recommended for the treatment of epizootic lymphangitis. The investigation of chemical compounds from natural products is fundamentally important for the development of new drugs. Ethanol macerated *C. molle* seed extract have a promising anti-fungal effect on mycelial form of *H. capsulatum var. farciminosum*. The main anti-fungal molecule in *C. molle* seed extract is hydrolysable tannins. The main action of tannin on *H. capsulatum var. farciminosum* is inhibition of cell wall and cell membrane biosynthesis. In topical application tannin has haemostatic and wound closure effect. The *C. molle* seed can be used for the treatment of epizootic lymphangitis if convenient methods of preparation, dose, and route of administration are established through meticulous *in vitro* and *in-vivo* trials. Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice, and current and future studies on medical activities can be effective in treating diseases. Those all parts of the plant have the chemicals upon extraction, the seed of the plants primarily and leaves secondly are preferable than the other parts of the particular plant because the seeds have higher concentration of the ingredients or chemicals required to use. The combination of the results got from both in vivo and in vitro trials are mandatory to witness the effectiveness of the particular plant's medicinal value.

Based on the above conclusions the following recommendations are forwarded: The appropriate treatment decision should be achieved to avoid suffering of equine from EZL. Challenges related with safety, efficacy and quality control of local herbal medicines for EL should be avoided. Any other local herbal medicines should be tried on EZL like ''embuay and ambacho''. *In-vivo* studies must be conducted so that the safety margin, toxicity and cure rates will be known in order to use them commercially. Study on the mechanism action of the local herbal medicine extracts and their toxic effect on lab animals should be reported .Antifungal drug should be produced by using herbal medicines that serve to cure the disease and shall be scale up later at industry level.

DECLARATIONS

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Authors' contribution
All the three authors reviewed the paper and contributed in developing the content.

Conflict of Interest
The authors declare they have no competing of interests.

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Citation: Asfaw M and Fentahun T (2020). Treatment trials of epizootic lymphangitis with local medicinal plants: a review. *Online J. Anim. Feed Res.*, 10(4): 158-161.

2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 167-171; July 27, 2020 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.23

STUDY OF THE FLAVONOIDS AND SECONDARY METABOLITES OF THE ARGAN TREE (*Argania spinosa* L.)

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Supporting Information

RESEARCH ARTICLE

PII: S222877012000023-10

Received: June 05, 2020

Revised: July 16, 2020

ABSTRACT: The separation and identification of the main phenolic compounds present in the co-product of the Argan tree (*Argania spinosa*) were carried out using high performance liquid chromatography techniques coupled with mass spectrometry (LC-ESI-MS/MS).The study was based on the retention times of the peaks of the phenolic compounds in samples and was compared to those of the controls (reference compounds) and supplemented by an analysis of the fragmentations of the molecules by mass spectrometry. Phenolic compounds in the pulp of the Argan namely such as catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenin (0.07%) were found. Phenolic acid is consisted of gallic acid (5%) and protocatechuic acid (21.1%). These compounds are more dominant than flavonoids. The flavonoids-O-rhamnoglucosides the most dominant compounds is isorhoifoline (7.2%) and hesperidin (4.5%) against rutin (0.1%) and rhamnetin-O-rutinoside (0.5%) are less dominant. The main compounds are the hyperoside (13.4%) and isoquercetin (10%). On the other hand, naringenin-7-O-glucoside constituted the most minority compound of this type of flavonoid in the pulp of the fruit of the Argan tree (the percentage of naringenin-7-O-glucoside and quercetin-3-O-arabinose is 15.3%). There are other phenolic compounds in the pulp of the Argan namely such as catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenin (0.07%). The main flavonoids found in the leaves of the Argan tree are Quercetin (21.73%), Myricetin (54.34%), Hyperoside (8.69%), and also Myricetin-3-Ogalactoside (9.78%). Argan cake is rich in flavonoids. Among these, epicatechin (110 mg/kg), catechin (11 mg/kg), protocatechic acid (15.2 mg/kg), vanillic acid (16.3 mg/kg) and 4-hydroxybenzyl alcohol (8.6 mg/kg) are higher in Argan cake. Argan oil is richer in tocopherol (597 to 775 mg/kg), Argan oil is rich in gamma tocopherol (631 mg/kg), and that make Argan valuable nutraceutical. The study of the secondary metabolites and especially the flavonoids of the Argan tree was undertaken with the aim of identifying new metabolites making it possible to increase the industrial than commercial value of the Argan tree.

Keywords: Argan tree, Biological activity, Flavonoids, Metabolism, Phenolic acids.

INTRODUCTION

The Argan tree (*Argania spinose* L. Skeels) is used by the local populations : the wood and the woody shell of the fruit for heating. The almond of the fruit is used in the production of Argan oil. The foliage and the pulp of the fruit and also the oil cake (residue from the production of Argan oil) are designed for animal nutrition (Pumareda et al., 2006). The study of the chemical composition of Argan derivatives was undertaken with the aim of identifying new metabolites allowing increasing the industrial than commercial value of the Argan tree. Secondary metabolites are compounds naturally biosynthesized by plants but which do not directly participate in plant metabolism. Many secondary metabolites have therapeutic properties and are (or have been) used in human medicine (Gitton-Ripoll, 2009; Khallouki et al., 2017). A systematic study of the secondary metabolites of the Argan tree has been developed since the 1990s in order to see to what extent it is possible to increase the economic value of the Argan grove and hence promote its extension in the long term. The results of this study have revealed a wide variety of secondary metabolites within the different parts of the Argan tree. Besides molecules frequently encountered in higher plants (triterpenes, sterols, flavonoids, etc.), molecules of original structure and belonging to the group of flavonoids have been isolated. Multiple flavonoids, also extracted from plant species other than Argan (El Kabouss et al., 2001; Zhar et al., 2016; Falode et al., 2019), have therapeutic properties which are sufficiently encouraging to warrant further investigation (Guillaume et al., 2005). Some other flavonoids seem to be involved in phytoprotective phenomena (Jiang et al., 2020). Applications in the food or cosmetology fields are also being studied. All this clearly indicates that this chemical family of secondary metabolites has interesting potential in many fields. As a result, the analysis of several parts of the Argan tree (wood, oil cake, shell and fruit pulp) was carried out and many flavonoids of different and often original structures could be isolated and then identified, and the properties of certain d 'have been evaluated (Safer, 2018; Hilali et al., 2020). This study briefly describes the flavonoids of the Argan tree.

Citation: Hilali M, El MonfaloutiH and Kartah BE (2020). Study of the flavonoids and secondary metabolites of the Argan tree (*Argania spinose* L.). *Online J. Anim. Feed Res.*, 10(4): 167-171.

MATERIALS AND METHODS

Plant material

The fruits of the Argan tree and their leaves are harvested in the village of Tidzi province of Essaouira, The collected samples are dried in the open air then pulped, crushed and finally reduced, separately, in fine powder (Bellefontaine et al., 2011).

Figure 1 - Different parts of the Argan fruit

Identification of phenolic compounds

The separation and characterization of the main phenolic compounds present in the Argan fruit pulp were carried out using high performance liquid chromatography techniques coupled with mass spectrometry (LC-ESI-MS/MS). This method already applied on other plants (cocoa, lepechinia graveolens) (Sánchez-Rabaneda et al., 2003) is important for the study of polyphenols. It makes it possible to determine the molecular weight and to give certain structural information of the molecules (Adlouni, 2010). In our study, we based ourselves on the retention time of the peaks of the phenolic compounds in our sample and compared to those of the control peaks of the reference compounds.

RESULTS AND DISCUSSION

Phenolic compounds of the pulp of the Argan fruit

The study of the phenolic composition of the pulp of Argan fruit has identified 16 phenolic compounds (Table 1). This study was approached as a biochemical approach to establish a polyphenolic identity card and allows highlighting a fairly significant structural diversity encompassing four main groups of phenolic compounds (El Kabouss et al., 2001; Charrouf et al., 2007). Phenolic acids consisting of gallic acid (5%) and protocatechuic acid (21.1%). These compounds are more dominant than flavonoids. In our study we did not find p-hydroxybenzoic acid among the phenolic acids in Argan pulp. The flavonoids-O-rhamnoglucosides the most dominant compounds is isorhoifoline (7.2%) and hesperidin (4.5%) against rutin (0.1%) and rhamnetin-O-rutinoside (0.5%) are less dominant. Flavonoids-O-glycosides: The main compounds are the hyperoside (13.4%) and isoquercetin (10%). On the other hand, naringenin-7-O-glucoside constituted the most minority compound of this type of flavonoid in the pulp of the fruit of the Argan tree (the percentage of naringenin-7-O-glucoside and quercetin-3-O-arabinose is 15.3%), (compounds 11 and 12). There are other phenolic compounds in the pulp of the Argan namely such as catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenin (0.07%). It is noted that epicatechin was the most affordable compound in the pulp of the Argan fruit after Protocatechuic acid (21.1%). The pulp of Argan fruit has been found to be rich in epicatechin and other catechic derivatives whose natural antioxidant power is important according to numerous studies (Ba et al., 2010). However, such combinations of natural phenolic compounds could be used for better preservation of Argan oil.

Phenolic compounds of Argan leaves

The study of the extracts of the leaves of the Argan tree by chromatography shows a higher content of flavonoids in the leaves of the Argan tree (Table 2) which are flavonic molecules including (Myricetin 3-O-galactoside, the hyperoside, quercitrine and myricitrine). The main flavonoids found in the leaves of the Argan are Quercetin, Myricetin and their glycosides (Quercetin, Myricetrine, Hyperoside, and Myricetin 3-O-galactoside) (Tahrouch et al., 1998). The derivatives of these aglycones represent 16.5% of the total flavonoids. The flavonoid extract of the leaves has a very interesting anti-radical and antioxidant activity. It is currently marketed as a protective cosmetic active ingredient for extracellular macromolecules in the skin such as collagen, glycoproteins, etc. (Charrouf et al., 2009).

Phenolic compounds in Argan oil

The proportion of phenols in Argan oil is low but their impact on its biological properties is very important. These are caffeic acids, 4-hydroxybenzoic, vanillic, syringic, ferrulic 4-o-glycosylated, oleuropein, 3-hydroxypyridine (3-Pyridinol), 6-

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methyl-3-hydroxypyridine and catechol, resorcinol, vanillyl alcohol, tyrosol, 4 hydroxy-3-methoxyphenethyl, epicatechin and catechin (Guillaume et al., 2005).

Table 1 - Phenolic compounds of the pulp of the Argan fruit

N°	Compounds (%)	Retention time (min)	[M-H]	Fragments	MS/MS (Experimental method) Neutral loss scan	MS/MS (Experimental method) Precursor scan	MS/MS (Experimental method) Production
1	Gallic acid (5.0)	0.82	169	125	-	-	-
2	Protocatechuic acid (21.8)	1.44	153	109	-	-	-
3	Catechine (2.8)	4.06	289	-	-	289	245
4	Isorhoifoline (7.2)	7.13	577	-	-	-	-
5	Epicatechin (14.7)	7.65	289	-	-	289	245
6	Procyanidin (2.7)	7.67	579	-	-	579	289,245
7	Rutin (0.1)	10.87	609	-	308	609	301
8	Hesperidin (4.5)	11.19	609	463.301	308	609	301
9	Hyperosid (13.4)	11.46	463	-	162	463	301
10	Isoquercitrine (10)	11.70	463	-	162	463	301
11	Quercetine-o-pentose	12.33	433	-	132	433	301
12	Naringenine-7-o-glucoside	12.69	433	-	162	433	271
13	Rhamnetine-o-rutinoside (0.5)	13.37	623	-	308	623	315
14	Quercetin (1.6)	17.83	301	-	-	301	151, 121, 107
15	Luteolin (0.2)	17.94	285	-	-	-	-
16	Naringenin (0.07)	18.51	271	-	-	271	119,109

Table 2 - Phenolic compounds of the Argan leaves

N°	Compounds (%)	Retention time (min)
1	Myricetine 3-Ogalactoside (9,78%)	3.4
2	Myricitrine (54,34%)	3.9
3	Hyperoside (8,69%)	5
4	Quercitrine (21,73%)	6

Compounds of tocopherols from Argan oil

The tocopherols were analyzed by HPLC on a column in the normal phase, directly from vegetable oil without saponification. They were identified by comparison of their chromatogram with controls injected under the same conditions. Their dosage was possible by the use of α-tocopherol. The results obtained are grouped in Table 3. Tocopherols are natural antioxidants, they are molecules with carbon chains linked to a quinone function (Bouhadjra, 2011). In vegetable oils there are four groups of tocopherols (a, b, g and d). Tocopherols have both a vitamin power (vitamin E, in particular a tocopherol) and antioxidant properties (Landrier, 2011). In addition, these compounds can constitute an analytical criterion for controlling the purity of oil. Argan oil is richer in tocopherol (597 to 775 mg/kg) than olive oil (50 to 150 mg/kg) and also than hazelnut oil (300 to 550 mg/kg) (Hilali et al., 2005, 2020). Tocopherols are natural antioxidants, gamma tocopherol has the highest antioxidant power (Bourre and Clement, 1996). Rich in gamma tocopherol, Argan oil is a valuable nutraceutical. Tocopherols (vitamin E) and polyphenols are natural antioxidants. These play an essential role in the prevention of several diseases, because they are anti-free radicals (Carrouf, 2002).

Table 3 - Composition of tocopherols in Argan oil (mg / kg).

Tocopherols	γ-tocopherol	δ-tocopherol	α-tocopherol	β-tocopherol	Total
Argan oil	631,3 mg/kg	59,5mg/kg	26,6 mg/kg	-	717.4 mg/ kg

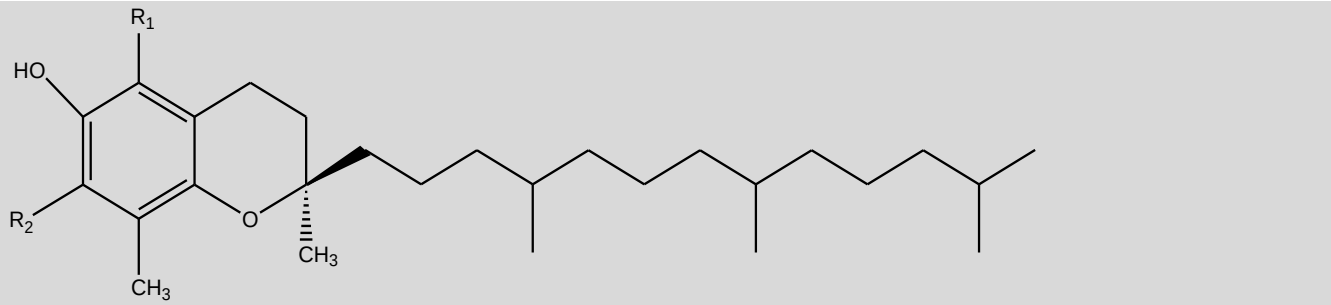

Figure 2 - Structure of tocopherols in Argan oil (R1 = R2 = CH3: α- tocopherol; R1 = CH3, R2 = H: β- tocopherol; R1 = H; R2 = CH3: γ- tocopherol; R1 = R2 = H: δ- tocopherol)

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Phenolic compounds of Argan cake

The cake from the extraction or meal is currently used as feed for fattening cattle. It is rich in carbohydrates and proteins (46.6% to 49%) and contains an important pharmacodynamic group consisting of saponins (Guillaume et al, 2005) and also contains an important group of flavonoids (Table 4). The Argan cake is rich in flavonoids, sixteen flavonoids were found. Among these, epicatechin, catechin, protocatechic acid, vanillic acid and 4-hydroxybenzylic alcohol are higher in Argan cake. On the other hand, epicatechin is a powerful antioxidant from the flavonoid family. A recent American study shows that epicatechin has positive health effects and shows that it can significantly reduce the risks of coronary heart disease and stroke during regular consumption (Rees et al., 2018).

Table 4 - Polyphenols from Argan cake

N°	Phenolic compound	Concentration (mg / kg)
1	Catechol	1.4
2	Resorcinol	1.3
3	4-hydroxybenzyl alcohol	8.6
4	Vanillin	1.1
5	Tyrosol	6.2
6	P-hydroxybenzoic acid	14.1
7	(4-hydroxyphenylacetic) acid Alcohol	1.0
8	vanillic alcohol	3.6
9	3.4-dihydroxybenzyl alcohol	0.9
10	Methyl 3.4-dihydroxybenzoate	1.6
11	vanillic acid	16.3
12	Hydroxytyrosol	0.9
13	Protocatéchic acid	15.2
14	Syringic acid	6.6
15	Epicatechine	110.1
16	Catechin	11

CONCLUSION

Argan cake is rich in flavonoids. Among these, epicatechin (110 mg/kg), catechin (11 mg/kg), protocatechic acid (15.2 mg/kg), vanillic acid (16.3 mg/kg) and 4-hydroxybenzyl alcohol (8.6 mg/kg) are higher in Argan cake. Argan oil is richer in tocopherol (597 to 775 mg/kg), Argan oil is rich in gamma tocopherol (631 mg/kg), and this metabolite making Argan oil is a valuable nutraceutical. The flavonoid extract of the co-products of the Argan tree has a very interesting anti-radical and antioxidant activity. It is currently marketed as a cosmetic active protector of the skin's extracellular macromolecules such as collagen, glycoproteins for this reason the co-product of Argan has become very important in the cosmetic field and its most widespread marketed.

DECLARATIONS

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Authors' contribution

The author reviewed the document and contributed to the development of the content.

Availability of data

The data can be availed to the journal upon request.

A conflict of interest

The author declare that there is no conflict of interests regarding the publication of this paper

Acknowledgments

The author would like to thank my fellow plant chemistry lab colleagues for their invaluable help and especially Mr. Mohamed Greih. To all the people who contributed directly or indirectly to the realization of this work, I send them my warmest thanks. The author has stated that he is not taking any funding to do this work or to publish this article.

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Citation: Hilali M, El MonfaloutiH and Kartah BE (2020). Study of the flavonoids and secondary metabolites of the Argan tree (*Argania spinose* L.). *Online J. Anim. Feed Res.*, 10(4): 167-171.

2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 172-179; July 27, 2020 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.24

POSTMORTEM STUDY ON INDIGESTIBLE FOREIGN BODIES IN RUMEN AND RETICULUM OF CATTLE (CASE: HARAMAYA AND AWADAY MUNICIPAL ABATTOIRS, EASTERN ETHIOPIA)

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Supporting Information

RESEARCH ARTICLE

PII: S222877012000024-10

Received: May 20, 2020

Revised: July 16, 2020

ABSTRACT: A cross-sectional study was conducted from November, 2017 to March, 2018 at Haramaya and Awaday Municipal Abattoirs of Oromia Regional State, Eastern Ethiopia, with the objectives of assessing the prevalence of rumen and reticulum foreign bodies, identifying types of foreign bodies and associated risk factors for the occurrences of foreign bodies. Following appropriate ante-mortum examinations, postmortem examinations were employed for the recovery of foreign body from rumen and reticulum. The study animals were selected by using systematic random sampling using regular interval to study animal from the total slaughtered animals. From a total of 384 (207 female and 177 male) cattle examined, 41.7% (n=160) were found to contain foreign bodies at slaughter. When the prevalence was compared between genders, breed, among different age groups, and different body condition score, higher prevalence of foreign bodies 50.7%, 75.0%, 80.0%, 83.3%, were observed in female, cross breed, age older than 10 years, and animal having poor body condition score respectively. These aforementioned factors are considered as potential risk factors were highly significantly associated with the occurrence of foreign bodies. Rumen harbored mostly plastic materials while reticulum was the major site for the retention of metallic objects. The non-penetrating foreign bodies have higher prevalence than penetrating foreign bodies. The commonly recovered non-penetrating foreign bodies were plastics (46.9%), cloth (30.0%), rope (21.3%) and leather (18.8%). The penetrating foreign bodies were metals (5%). Plastics were recovered as the most common foreign bodies and followed by cloths, Ropes, and leathers. It is concluded that the detection of this level of prevalence of foreign bodies in cattle causes high mortality and morbidity, reduced production and productivity. Therefore, appropriate solid waste disposal system need to implement in the study area to prevent health risk of ruminants and also to protect the environment.

Keywords: Body Condition Score, Cattle, Foreign body, Reticulum, Rumen.

INTRODUCTION

Ingestion of foreign bodies is one of the major bottle necks to livestock development in the tropics (Gupta and Single, 2013; Fasil, 2016). In Ethiopia, ruminants are kept under an extensive type of management are likely to be exposed to the ingestion of indigestible garbage from various sources due to a wide spread environmental contamination with plastic bags, absence of policy to protect environment and frequent occurrence of drought that predispose animals to nutritional deficiency and pica (Abebe and Nuru, 2011).

Gastrointestinal foreign bodies are among the most common surgical emergency in veterinary medicine. Cattle are more susceptible to foreign body syndrome than small ruminants because cattle do not use their lips for prehension, they are more likely to ingest foreign bodies than small ruminants as they are more likely to eat chopped feed in which foreign bodies may be incorporated (Bayne and Edmondson, 2020).

According to different studies, the common non-piercing foreign bodies commonly ingested by ruminants are plastic bags, sack thread, ropes, leather, rubber, bed linen, pieces of lead pipe, straw baskets, hair and plant fibers (bezoars) (Anwar et al., 2013). While wire, needles, nails and stones are the major penetrating foreign bodies isolated from ruminants (Bwatota et al., 2018). The indiscriminate feeding habits and mineral deficiency make them susceptible to inadvertent ingestion of foreign materials (Priyanka and Dey, 2018).

Environmental pollution is one of the growing problems for grazing animals due to absence of recycling industries, cleaning of environment cultures, improper disposal of plastic bags; free grazing animals eat plastic bags especially in towns and villages (Bhaskara and Sasikala, 2012; Reuters, 2019). These plastic bags are indigestible and their accumulation in the rumen of grazing animals may lead to adverse effect on health (Ghurashi et al., 2009); plastic bags resist to biodegradation and pollute for decades and centuries and pose great risk to human health and environment (Ramaswamy and Sharama, 2011). Feed shortage usually occurs at specific time of the year in most part of Ethiopia. Moreover, most owners do not provide supplementary feed to animals. These in turn may predispose the animals to negative energy balance and force them to feed on unusual materials including plastics, clothes, ropes and even metallic substances (Bhaskara and Sasikala, 2012; Tesfaye et al., 2012). In addition, industrialization and mechanization of agriculture have further increased the incidence of foreign bodies in the animals (Semieka, 2010). The ingestion of foreign bodies causes various problems in different organ of the animal mainly in rumen and reticulum. The problem that are caused vary with the duration that the foreign body

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has been present, the location of foreign body, the degree of obstruction that is caused as well as problems associated with the material of the foreign body. Glossitis, esophagitis, ruminitis, impaction of rumen, traumatic pericarditis (TP) and traumatic reticulo peritonitis (TRP) are the possible health problems which can be caused by the ingestion of foreign bodies by the cattle (Desiye and Mersha, 2012). Traumatic reticulo-peritonitis disease in cattle is caused by the ingestion of foreign bodies in the reticulum swallowed metallic objects such as nail or pieces of wire fall directly on the reticulum or pass into the rumen and subsequently carried over the rumeno-reticular folds into the cranioventral part of the reticulum (Radostits et al., 2007; Braun et al., 2018).

The presence of foreign bodies in the rumen and reticulum hampers the absorption of volatile fatty acids and consequently reduction in the rate of animal fattening (Bassa and Tesfaye, 2017) the perforation of the wall of the reticulum can also allow leakage of ingest and bacteria which contaminates the peritoneal cavity, resulting in local or diffuse peritonitis (Anwar et al., 2013).

Animals with large amount of blunt foreign bodies show anorexia, depression, intermittent respiratory distress, recurrent rumen tympany, rumen stasis, dehydration, reduced milk yield, distended left paralumbarfossa and sometimes vomiting (Reddy and Sasikala, 2012; Abu-Seida and Al-Abbadi, 2014). Tachycardia, muffled heart sounds, distended jugular veins, pericardial sounds like splashing, rubbing or squeaking sounds, brisket and ventral edema were observed in cattle with traumatic reticulopericarditis (Ramin et al., 2011). Shrunken rumen, strangulated foreign bodies, congested ruminal mucosa and ulceration are the common necropsy findings in animals with foreign body syndrome (Abu-Seida and Al-Abbadi, 2016; Serem et al., 2019).

This disease is of high economic importance and serious due to severe reduction in milk and meat production, treatment costs, potential fatalities and fetal losses in affected animals (Sileshi et al., 2013). The condition is usually common in urban and peri- urban areas where extensive building are carried out and proper plastic material disposal is not conditioned and so thrown on roads and near the fence or anywhere and that is way our dairy cattle are dying mainly associated with foreign bodies (Ramaswamy and Sharama, 2011). Many efforts were made to study infectious disease prevalent in the country, however, solid environmental pollution (Foreign Body's) have been given lesser attention to be treated as a separate health problem. Therefore, the objectives of current study were to assess the prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Haramaya and Awaday Municipal Abattoirs. As well as, the type of rumen and reticulum foreign bodies will be identified.

MATERIALS AND METHODS

Study area

The study was conducted in Haramaya and Awaday municipal abattoirs, Eastern Hararghe zone, and Oromia region. The Awaday town is located 9021'10'' N, 42013'46'' E with average altitude of 1962 m at a distance of 510 km from capital Addis Ababa (Mekonnen and Uttama, 2014).The Haramaya town is located at 509 Km from Addis Ababa at an elevation of 1400 to 2340 meter above sea level. The town situated between a latitude and longitude of 42°01′E and 9°24′N respectively. The mean annual rainfall received range from 600 to 1260 mm with bimodal nature Minimum and maximum annual temperature range from 6^0C to 12^0C and 17^0C to 25^0C respectively. The relative humidity varies between 60% to 80%. The farming system in the area is mixed type (crop-livestock production). The total population of people in the area is estimated to be 352,031 according to (CSA, 2013). The livestock population of the study district is estimated to be 76 336 cattle, 65 083 sheep, 84 916 goats, 22 355 donkeys, 356 camels and 89 800 chickens (CSA, 2012).

Study animals

The study was conducted on 384 apparently healthy slaughtered cattle in Haramaya and Awaday Municipal Abattoirs from November, 2017 to April, 2018. It has been difficult to trace back the origin of animals, since the animals pass a chain of markets. Nevertheless, attempts made in this regard revealed that majority of them were bought from nearby markets. Animals from both local and cross breed cattle were brought to both municipal abattoirs. Even though, the study animals were kept under broad range of management and animals in most of the rural areas were kept to graze pasture on grassland and supplementary feedings of crop residue when pasture in scarce especially during long dry season.

Study design

A cross sectional study was conducted from November, 2017 to April, 2018 to assess the prevalence of the rumen and reticulum foreign bodies, to identify the types of foreign bodies, and their associated risk factors for the occurrence of the foreign bodies were sex, age, Breed, and body conditions were considered as risk factor for occurrence of foreign bodies.

Sampling method

A cattle slaughtered during each visit day were selected by systematic random sampling using regular interval to study the prevalence of foreign body and identification of types of foreign bodies in rumen and reticulum in cattle slaughtered at Haramaya and Awaday Municipal Abattoirs of Eastern Ethiopia.

Sample size determination

The study was carried out by determining the sample size according to Thrusfield (2005), for an infinite population with 95% confidence level, 5% desired absolute precision by considering expected prevalence of the rumen and reticulum foreign bodies in cattle in the area. Therefore, the sample size was as follows:

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$n = 1.96^2 \times P^{exp}(1-P^{exp}) / d^2$

Where:

n = required sample size; P^{exp} = expected prevalence; d^2 = desired absolute precision.

Based on the above formula 384 animals were needed but there was no previous study on the occurrence of rumen and reticulum foreign bodies of cattle slaughtered at haramaya and awaday Municipal Abattoirs. The sample size for this work were determined using 50% expected prevalence and 5% absolute precision at 95% confidence level using the above formula, 384 cattle are intended to be sampled.

Data collections

Ante mortem examination. Ante mortem examination on individual animals was done for assessment of age, sex, breed, body condition. Sex (female and male), age was categorized into young (<5 years), adult (5-10 years) and old (>10 years), body condition (good, medium and poor). Body condition of cattle was recorded as poor, medium and good based on the appearance of the animal and manual palpation of the spines proses and transverse processes of the lumbar vertebrae described by Nicholson and Butterworth (1986) and breeds was classified as local and cross based on the species of animal brought to abattoir. The age of the animal was also scored according to Mari (1989) based on dentition. Each animal selected for the study was further identified by providing a unique identification number that could be used for both ante-mortem and post-mortem examinations of the animal and each animals mark for the identification by writing a code on its gluteal muscle by using ink.

Postmortem Examination. In the postmortem examination rumen and reticulum were examined. Immediately after slaughtered in the evisceration stage, the stomach was carefully removed from the abdominal cavity and open and explored for the presence of any foreign material by visualization and palpation. Any foreign bodies obtained during inspection are washed by water to removing feed material and identified. When the finding is positive, the location and type of the foreign bodies was recorded otherwise recorded as negative postmortem recorded sheet.

Data management and statistical analysis

The data collected was entered and scored in Microsoft excel worksheet. Before subjected to statistical analysis, the data were thoroughly screened for errors and properly coded. For analysis SPSS Microsoft software Version 20 was used. Descriptive statistical analysis was used to summarize and present the data collected. The prevalence of rumen and reticulum foreign bodies were calculated as percentage by dividing total number of cattle positive for foreign bodies to the total number of cattle examined. Pearson chi square (χ^2) test was employed to assess the existence of association between prevalence of the foreign bodies and potential risk factors considered. For (χ^2) test, p-value < 0.05 were considered significant whereas p-value > 0.05 considered non-significant.

RESULT

A cross sectional study was conducted from November 2017 to March, 2018 at Haramaya and Awaday Municipal Abattoir. From the total of 384 cattle's examined for the presences of any foreign bodies in their rumen and reticulum, 41.7% (160/384) of them were found positive. From 160 positive cases of foreign body, 129 (80.6%) were occurred in rumen while 16 (10%) in reticulum and 14 (8.8%) in rumen and reticulum. The types of foreign bodies were detected plastics, leathers, clothes, ropes, Stone or Calcified and Metal. From this plastics 75 (46.9%), cloth 48 (30.0%), rope 34 (21.3%) and leather 30(18.8%) were more frequently encountered of the positive cases respectively.

Foreign body regarding to breed

From the total 384 animals 352 local breeds and 32 cross breeds were examined and 136(38.6%) and 24(75.0%) of foreign bodies were detected in both breeds respectively. So, the prevalence of rumen and reticulum foreign bodies was higher in cross breed cattle. The statically analysis also showed that there exist highly significant differences among different breed (p=0.000) which is P<0.05 in the occurrences of foreign bodies (Table 1).

Table 1 - Breed distribution of rumen and reticulum foreign bodies in cattle at Haramaya and Awaday Municipal Abattoirs.

Foreign body	Breed		Total
	Local	Cross	
Plastic	49 (13.9%)	7 (21.9%)	56
Rope	15(4.3%)	-	15
Cloth	22(6.2%)	1(3.1%)	23
Leather	10(2.8%)	4(12.5%)	14
Stone or Calcified	4(1.1%)	-	4
Metal	8(2.3%)	-	8
Plastic and rope	10(2.8%)	-	10
Cloth and leather	7(2.0%)	4(12.5%)	11
Plastic and cloth	5(1.4%)	-	5
Leather and plastic	-	4(12.5%)	4
Cloth and rope	5(1.4%)	4(12.5%)	9
No foreign body	217(61.6%)	8(25.0%)	225
Total	352	32	384

χ^2= 15.958; p=0.00

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Foreign bodies relation with age

Study animals were grouped in to three as young (< 5 years), adult (5-10 years) and old (> 10 years) From 160, 194 and 30 animals were examined with age in these age groups, 38(23.8%), 98(50.5%) and 24(80.0%) were found positive, respectively. Foreign bodies were more frequently encountered in old animals than other two groups. The statically analysis also showed that there exist highly significant differences among the three age groups (p=0.000) which is P<0.05 in the occurrences of foreign bodies as shows in (Table 2).

Foreign bodies in relation to sex

From 160 positive animals, 105 (50.7%) and 55 (31.1%) were detected in female and male animals, respectively. Foreign bodies were observed at the maximum of 50.7% in female cattle compared to male 31.1%. There was statistically strongly significant association (p= 0.00) in the frequency of occurrence between male and female (Table 3).

Table 2 - Age distribution of rumen and reticulum foreign bodies in cattle at Haramaya and Awaday Municipal Abattoirs.

Foreign body	Age			Total
	≤5 year	5-10 year	≥10years	
Plastic	22(13.8%)	32(16.5%)	2(6.7%)	56
Rope	6(3.8%)	9(4.6%)	-	15
Cloth	3(1.9%)	17(8.8%)	3(10.0%)	23
Leather	-	10(5.2%)	4(13.3%)	14
Stone or Calcified	-	2(1.0%)	2(6.7%)	4
Metal	4(2.5%)	4(2.1%)	-	8
Plastic and rope	3(1.9%)	4(2.1%)	3(10.0%)	10
Cloth and leather	-	11(5.7%)	-	11
Plastic and cloth	-	4(2.1%)	1(3.3%)	5
Leather and plastic	-	4(2.1%)	-	4
Cloth and rope	-	-	9(30.0%)	9
No foreign body	122(76.2%)	97(50.0%)	6(20.0%)	225
Total	160	194	30	384
χ^2= 45.518 ; p=0.00				

Table 3 - Sex distribution of rumen and reticulum foreign bodies in cattle at Haramaya and Awaday Municipal Abattoirs.

Foreign body	Sex		Total
	Female	Female	
Plastic	29(14.0%)	27(15.3%)	56
Rope	9(4.3%)	6(3.4%)	15
Cloth	22(10.6%)	1(0.6%)	23
Leather	11(5.3%)	3(1.7%)	14
Stone or Calcified	3(1.4%)	1(0.6%)	4
Metal	1(0.5%)	7(4.0%)	8
Plastic and rope	7(3.4%)	3(1.7%)	10
Cloth and leather	7(3.4%)	4(2.3%)	11
Plastic and cloth	3(1.4%)	2(1.1%)	5
leather and plastic	4(1.9%)	-	4
Cloth and rope	9(4.3%)	-	9
No foreign body	102(49.3%)	123(69.5%)	225
Total	207	177	384
χ^2 = 15.159 ; p=0.00			

Foreign body with regard to body condition score

From total of 384 cattle 24,208 and 152 animals were examined with poor, medium and good body condition, 20 (83.3%), 130 (62.5%) and 10 (6.6%) were positive for foreign body, respectively. The statically analysis also showed that there exist highly significant differences among the three body condition score groups (p=0.000) which is P<0.05 in the occurrences of foreign bodies (Table 4).

Foreign bodies with regard to Lodgment sit

From 160 positive cases of foreign body, 129(80.6%) were occurred in rumen while 16(10.0%) in reticulum and 14(8.8%) in both rumen and reticulum. Prevalence of foreign bodies to lodgment sites was highly statistically significant (p < 0.05) (Table 5).

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Table 4 - Prevalence and frequency of rumen and reticulum foreign bodies in cattle slaughtered at Haramaya and Awaday Municipal Abattoirs in association with body condition.

Foreign body	Body Condition Score			Total
	Poor	Medium	Good	
Plastic	1(4.2%)	49(23.6%)	6(3.9%)	56
Rope	-	12(5.8%)	3(2.0%)	15
Cloth	5(20.8%)	18(8.7%)	-	23
Leather	4(16.7%)	10(4.8%)	-	14
Stone or Calcified	1(4.2%)	3(1.4%)	-	4
Metal	3(12.5%)	5(2.4%)	-	8
Plastic and rope	2(8.3%)	8(3.8%)	-	10
Cloth and leather	4(16.7%)	7(3.4%)	-	11
Plastic and cloth	-	5(2.4%)	-	5
Leather and plastic	-	4(1.9%)	-	4
Cloth and rope	-	9(4.3%)	-	9
No foreign body	4(16.7%)	78(37.5%)	143(94.1%)	225
Total	**24**	**208**	**152**	**384**

χ^2= 131.22; p=0.00

Table 5 - Frequency of occurrence of rumen and reticulum foreign body in cattle slaughtered at Haramaya and Awaday Municipal Abattoirs.

Foreign body	Location site of Foreign Body				Total
	Reticulum	Rumen	Rumen and Reticulum	None	
Plastic	3(19.0%)	44(34.11%)	9(64.3%)	_	56
Rope	_	11(8.53%)	4(28.6%)	_	15
Cloth	1(6.25%)	21(16.3%)	1(7.14%)	_	23
Leather	_	14(10.9%)	_	_	14
Stone or Calcified	4(25.0%)	_	_	_	4
Metal	8(50.0%)	_	_	_	8
Plastic and rope	_	10(7.8)	_	_	10
Cloth and leather	_	11(8.53%)	_	_	11
Plastic and cloth	_	5(3.90%)	_	_	5
Leather and plastic	_	4(3.10%)	_	_	4
Cloth and rope	_	9(7.0%)	_	_	9
No foreign body	_	_	_	225	225
Total	**16**	**129**	**14**	**225**	**384**

χ^2= 379.9; p=0.00

DISCUSSION

Ingestion of indigestible foreign materials by ruminants is a common problem as reported by Ghurashi et al. (2009). The present study revealed an overall prevalence of 160 (41.7%) of rumen and reticulum foreign bodies in cattle slaughtered at Haramaya and Awaday Municipal abattoirs. This occurrence of foreign bodies is almost similar with report of Sheferaw et al. (2014) who reported 41.8% in cattle from the Amhara region of Ethiopia and slightly lower than the report from eastern Ethiopia at Haramaya University and Haramaya municipal abattoirs Negash et al. (2015) who reported 43.4% in cattle and significantly lower than the prevalence (77.41%) which was reported by Ismail et al. (2007) in adult dairy cattle having indigestible foreign bodies in their fore-stomach, as the result they are suffering from recurrent rumen tympani in Jordan. These differences could be due to the differences in availability of predisposing factors is those area, and the problem of waste management system, poor management of animals and industrialization increased the incidence of foreign bodies between countries. Moreover, the time of the study also could play a role for the differences where in recent times the rate of intensification of animal management is increasing and as a result the probability of animals to be exposed to foreign materials might be declined as the animals are staying in a limited confinement for longer time.

In present study, the higher prevalence (50.7%) of foreign bodies was detected in female cattle than male (31.1%). These results are in agreement with the findings of Vanitha et al. (2010) stating that, the foreign bodies were found more frequently in female cattle than male in their study on 30 stray cattle having clinical symptoms suggestive of ruminal impaction. Similarly, Zegeye (2011) reported that higher level of occurrence of foreign bodies in female cattle in retrospective study of clinical cases of farm animal in three years period in University of Gondar Veterinary Clinic. Roman and Hiwot (2010) have also reported that higher degree of occurrence of foreign bodies in female ruminants compared to male. This may be due to female animals are more exposed to the environmental

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pollution as they kept for production purpose for longer period of time and there might be increased appetite of female animals due to the nutritional demands during pregnancy and lactation.

The highest frequency of occurrence of rumen and reticulum foreign bodies were detected in animal's ≥10 year (80.0%) followed by 5-10 years (50.5%) and ≤5 years (23.8%) age group of animals. Highest prevalence (80.0%) of foreign bodies was detected in cattle greater than 10 year than other age group. This finding is in agreement with Desiye and Mersha (2012) who recover (81.25%) of foreign bodies in cattle greater than 10 year age. Rahel (2011) also reported (17.85%) of the animals had higher frequency of foreign bodies in rumen and reticulum in the old age. The present Prevalence is relatively high since it may be that ingestion of foreign bodies is associated with shortage of forage during the long dry season, owners were not supply supplementary feed and increased demand of feed, and pollution of grazing land with indigestible foreign bodies.

In this finding, the prevalence was higher in the cross breed cattle (75.0%) compare to local breeds (38.6%). This findings are agree with the work of Desiye and Mersha (2012) who found 70% in cross breed and 10.77% in local breed and Rahel (2011) who reported forestomach foreign bodies with the high prevalence of 58.82% in crossbreeds. Sileshi et al. (2013) reported that cross breed animals are more exposed for indigestible foreign bodies than local breeds. The higher prevalence in cross breed might be associated with their higher productivity which requires high demand of nutrition that enforces cattle to indiscriminately feeding and hence increased exposure to foreign bodies.

This study also identified the highest prevalence of rumen and reticulum foreign bodies were detected in animals with poor body condition (83.3%) followed by medium (62.5%) and good body condition (6.6%) score animals. This finding agree with the work of Desiye and Mersha (2012) who recovered foreign body at higher prevalence from the rumen and reticulum of poor body conditioned animal (72.72%) than medium (35.95%) and good (7.33%) body condition. Poor body condition by itself might be due to the contribution of the foreign body that is the animal loss weight after it has been exposed or it might be due to the interference of foreign body with the absorption of volatile fatty acid (VFA) and thus causes reduced weight gain reported by Rahel (2011).

Metallic foreign bodies were most frequently recovered from reticulum. This finding agrees with the report of (Sileshi et al., 2012) who recovered metallic foreign bodies at highest prevalence from reticulum. In addition, Desiye and Mersha (2012) reported the highest prevalence (87.5%) of metallic foreign bodies from reticulum. The reason might be due to retention of these foreign bodies by the honey comb structure of the reticular mucosa and their heavy weight give chance to be attracted to the lumen of the reticulum due to gravitational attraction force of these heavy foreign bodies to the ventral part of the fore-stomach.

The types of foreign bodies detected in this study were plastic, cloth, leather, rope, metal (wire) and stone or calcified. Berrie et al. (2015) also found the same thing. This study indicated that most foreign bodies occurred in the rumen (80.6%) than reticulum (10%). This may be due to the fact that many ingested feed goes to the rumen. The results of this study further indicate that Plastic was the most commonly encountered (45.6%) foreign material in all study animals, followed by cloth (30.0 %) and rope (21.3%). This finding is in general agreement with various reports from different areas of Ethiopia (Abebe and Nuru 2011; Roman and Hiwot 2010; Sheferaw et al., 2014; Tesfaye et al., 2012). This is due to these materials used for storing wastes, shopping bags and packing food items and disposed everywhere after using, hence they were eaten by the free grazing animal.

CONCLUSTION AND RECOMMENDATIONS

The current finding is used to assess the prevalence of rumen and reticulum foreign bodies, to identify the type of foreign bodies and to identify the magnitude and occurrence of fore stomach foreign bodies done in Haramaya and Awaday municipal abattoirs. Sex, breed, age, and body condition score were considered risk factors for the occurrence of foreign bodies. And this study revealed that rumen and reticulum foreign bodies have great economic significance associated with reduced production and productivity of animals suffering from them. The overall prevalence of foreign bodies was 160(41.7%). Similarly, Plastic was found the most common foreign bodies found preferably in Rumen (34.11%). On the other hand, Female (14.0%) local breeds (13.9%), aged 5-10 years (16.5%) with medium body condition (23.6%) were mostly affected. Degree of association was highly statistically significant for the occurrence of foreign body in cattle. Both female and cross breed cattle are the most affected groups compared to that of male and local breed cattle respectively. According to body condition score of cattle, poor body conditions were more affected groups than cattle which have medium and good body conditions. Most of the nonmetallic foreign bodies lodged in rumen while metallic foreign bodies lodged in reticulum.

Awareness for owners should be implemented to avoid the risk of foreign body ingestion such as prevent nutritional deficiencies and not allowing animals in polluted grazing land. Appropriate solid waste disposal system should be implemented. Enforcement of recycling of plastic bags and other environmental pollutants so that threat to environment and life can be reduced. The finding of this study could help environmental activists, veterinarians, and livestock owners to recognize the impact of foreign bodies on cattle's health and productivity in this area.

DECLARATION

Authors' contribution

The two authors reviewed the paper and contributed in developing the content.

Availability of data

The data can be availed to the journal upon request.

Citation: Amin I and Fentahun T (2020). Postmortem study on indigestible foreign bodies in rumen and reticulum of cattle (case: Haramaya and Awaday municipal abattoirs, Eastern Ethiopia). *Online J. Anim. Feed Res*., 10(4): 172-179.

Consent to publish
Not applicable

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

Acknowledgement
The authors would wish to acknowledge Eco Fuels Kenya LTD (EFK) for their support through the whole process of developing this publication.

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Citation: Amin I and Fentahun T (2020). Postmortem study on indigestible foreign bodies in rumen and reticulum of cattle (case: Haramaya and Awaday municipal abattoirs, Eastern Ethiopia). *Online J. Anim. Feed Res*., 10(4): 172-179.

2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 180-184; July 27, 2020

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.25

INFLUENCE OF DIETARY MANIPULATIONS AND MILKING FREQUENCY ON PRODUCTION OF DAIRY COWS

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Supporting Information

MINI REVIEW

PII: S222877012000025-10

Received: May 15, 2020

Revised: July 20, 2020

ABSTRACT: Dairy cow responses to various types of diets differently and dairy farmers can use knowledge of its behavior to improve the cow well-being and yield. This review was carried out in order to better understanding the influence of dietary manipulation and milking frequency on the dairy cows' production. The results obtained from review of already conducted studies revealed that the dairy cow is significantly affected by composition, quality, amount and regimes of the diet. Maximum daily milk production, milk protein, milk lactose, milk fat, total solids are recorded in dairy cows when *ad-libitum* feed and water is provided. Further, sufficient water intake is necessary for maintaining body fluids and proper ion balance, digestion, absorption, metabolization of nutrients, elimination and body cooling. Feeding and water frequency stimulate the mammary functions and milk synthesis, which is actually a non-invasive method. Reducing feeding frequency from 2x daily to 1x daily decreases milk yield from 7 to 38% in dairy cows, however changing feeding frequency from 2x to 3x daily results about 18% increase in milk production that can be economically acceptable. On the other hand, increasing milking frequency from 2x to 3x daily increase milk production up to 30%. Therefore, in addition of dietary manipulation and milking frequency, high quality feed and *ad-libitum* water plays always a key role for improving the performance and production of dairy cows.

Keywords: *Ad-libitum*, Diet, Performance, Production.

INTRODUCTION

Dairy cow responses to various types of diets differently and dairy farmers can use knowledge of animal behavior for improving the well-being and yield of cow. For instance, feeding and watering systems must be placed appropriately. Accessibility of feed and water may be more important than the actual amount of nutrients provided (Wilde et al., 1987; Erickson and Kalscheur, 2020). Efforts must be made to reduce the competition for feed, water, minerals, and shelter. Also, cow space, cow density, and distribution of feed and water are closely related factors. Feed intake and consequent milk yield are improved by provision of feed on cows need and want to eat (VanBaale et al., 2005). When one cow eats, another might be stimulated to do likewise, whether she is hungry or not. This behavior is an example of social facilitation when cows eat in groups; they eat more than when they are fed separately. Furthermore, cows kept in groups are likely to be less fearful, and hence, more contented, healthier, and more productive. The common practice of feeding and milking cows in groups thus has a sound psychological basis (Thokal et al., 1985).

Water is an essential component to sustain life and optimize growth, lactation, and reproduction of dairy cattle. However, unlike the careful and continuous attention paid by dairy producers and nutritionists to other nutrients in the ration, oftentimes the quality and provision of free drinking water does not receive the attention necessary to ensure optimal nutrition and cattle performance. In high producing dairy cows, the water requirement is greater compared other land based animals (Tyrrell et al., 1982). This higher need is actually related to large quantity of milk which actually contains 87% water. In addition, water is also used up in digestion, metabolism, circulation, excretion, ionic balance, fluid balance etc. (Stelwagen et al., 1994). Adult dairy cattle contain 56 and 81% water in the body depending on lactation cycle. Loss of even 20% water is considered fatal (Thokal et al., 2004). In order to produce optimum milk dairy cows must drink sufficient amount of water and the feed. The quantity of water which a dairy cow would consume, is largely depends on the environmental temperature, types of consumed feed, amount of milk production and the water temperature (Tulloh, 1966). Water need is directly related to the dry matter intake. Increased intake of dry matter results increased water consumption (Negrao et al., 2001). Keeping in view the importance of diet in life of dairy cow current study was planned, whereby the main objective was to understand the dietary influence on dairy cows worldwide.

INFLUENCE OF WATER FREQUENCY ON THE PRODUCTION OF DAIRY COWS

Water constitutes 60 to 70 percent of the body of dairy cow. Water play role in the cows body such as ionic balance, digesting, absorption, metabolization, excretion, maintaining body fluids balance, thermoregulation, transportation of nutrients. The water need of cows is fulfilled by drinking and metabolic resources. Metabolic water is produced as a result

Citation: Khaskheli AA (2020). Influence of dietary manipulations and milking frequency on production of dairy cows. *Online J. Anim. Feed Res.*, 10(4): 180-184..

of oxidation of organic compounds. Saliva, urine, feces, and milk are main routes whereby water is lost from the body of dairy cows. In addition to that some water is also lost through sweating, evaporation and the respiratory tract. The quantity of loss of water from cow's body is related to the activities performed the animal, temperature and humidity of air, respiratory rate, water and feed intake, and milk yield etc. (Pearson et al., 1979).

It has been revealed that water need of a high producing dairy cow is significantly higher compared other land mammals. It is because of fact that cow produces a large quantity of liquid milk, which actually contains 87 percent water. In order to produce such as higher amount of liquid product, dairy cows must need sufficient supply of water in the diet otherwise their production will severely be impaired (Meyer et al., 2004). Several studies have been conducted on different regimes of water supply to the dairy cows. In a study, some researcher indicated that dairy cows contain 56 and 81% water in their body. Water supply on *ad-libitum* not only supports in maintaining their total body water but also increases the milk production (Little et al., 1976). Few other reported that dairy cows in early lactating stage have higher body weight compared to later lactating stage (69% versus 62%). This change in body weight is actually related to the water level in their body. It was depicted that in early stage (1st lactation) milk production of dairy cows remain significantly lower compared to late lactation (3rd lactation). During early stage water supply on *ad-libitum* is stored in the body tissues which cause overall increase of body weight (Li et al., 2005). Few others reported that 2/3 of body water is found in the intracellular spaces, while 1/3 water is found in extracellular compartments, connective tissues, blood, digestive tract. Water present in the GIT ranges from 15 to 35% of total body weight. However water supply through drinking source play key role in maintaining these water levels in different parts of body (Linzell, 1966).

In another study, researchers observed residence time of water in the rumen of dairy cows (lactating) which was found 1 hour. It was further revealed that when water reside the rumen for long period, it helps in digestion that results higher milk production. They indicated that dairy cows produced higher milk yield on *ad-libitum* water supply compared two times/day water supply (Knight et al., 1992). Some others showed that daily water loss through milk represents 26 and 34% of total water intake (Hale et al., 2013). Water loss through feces ranges from 30 to 35% of total water intake in dairy cows. Loss in urine ranges from 15 to 22%. Cows though were provided water on *ad-libitum* coped excellently with these water losses from body. Further, fecal water loss increases with the increase of dry matter level feed and forage content of the diet. Urinary excretion of water is positively correlated to the water availability, absorption from GIT, urinary N, Na and K excretion (Jurjanz et al., 1993). Further it was found that water loss is also concerned to saliva production, sweating and respiratory evaporation. However, amount of water loss through these routes is dependent upon environmental temperature (Khan et al., 2003; Hernández-Castellano et al., 2019).

Daily water intake and need of dairy cows is influenced by several factors such as quantity of milk production, quantity of feed intake, physiological state, body size, level of activity, environmental temperature, air movement, diet composition etc. In addition to these, other main influencing factor has been reported is frequency and periodicity of watering. Water need of dairy cows is mainly met by drinking source. However, drinking of water is associated with feed supply (Khan et al., 2012). Dry matter content in feed significantly affects the total water consumption by dairy cows. When dry matter content in diet is declined from 50 to 30 percent then consumption of water by dairy cow reduces by 42 percent (Hansen et al., 2007). In study water consumption by lactating dairy cows was studied with respect to pastures. It was noticed that dairy cows consume less quantity of water when fed on green pastures. Further, diets with high level of sodium salts or protein stimulate water consumption in dairy cows (Silanikove et al., 1995).

In another research, it has been stated that higher dry forage diets increase the water requirements. It occurs due to significantly higher excretion of water in feces. They further depicted that there is direct correlation between dry matter and water intake in the dairy cows. When water consumption is below normal level, feed intake decreases. However, if water consumption is normal then cow take dry matter as per its need in order to maintain normal performance, growth, lactation and pregnancy (Carruthers et al., 2015). It has also been studied that water quality is quite important for appropriate water consumption by dairy cows. Water quality in term of odor, taste, presence of toxic elements, physical and chemical state, level of macro and micro-minerals, and microbial contamination. These all contribution factors in combination have direct influence on the acceptability of water, by dairy cows. Total dissolved solids, sulfate sulfur, nitrates, chlorides, iron, and fluoride are primary factors which influence the water quality for dairy cows (Chamberlain et al., 2011). Higher than normal level of mineral elements, microorganisms, and other toxic compounds cause deleterious effects on dairy cows (McGuffey, 2017).

INFLUENCE OF FEEDING AND MILKING FREQUENCIES ON THE PRODUCTION OF DAIRY COWS

In order to maintain regular and consistent lactation, appropriate feeding is the basic need of dairy cows. Minor changes in the feeding significantly influence the normal physiological processes as well production of dairy cow. Altering feeding frequency although appears so simple concept, but it has been reported main contributing factor for reducing the production of dairy cows (Archer, 2013). The influence of changing the frequencies of feed on milk production is variable depending the ability of individual animal to persist itself in the harsh condition. Studies have shown that reducing of feeding frequency from 2 times/ day to 1 time/ day considerably reduces the milk production (7 to 38%) in dairy cows and also increases the loss of udder tissue (AspMisra and Singh, 2012). It has also been reported that increase of milking frequency from 2 time/day to 3 times/day increases the overall milk yield (7 to 20%) (Banerjee, 2009). The exact

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mechanism behind the increase in milk production have not been elucidated yet, but some investigators reveal that the increase in milk production is actually related to increase in mammary epithelial cells, increased cellular activity, reduced apoptosis in mammary epithelial cells and frequent removal of feedback inhibitor of lactation from mammary gland. However feed supply play key role behind these mechanisms (Andrew et al., 2014).

As per previous reports of scientists increasing of feeding frequencies enhances the functioning of mammary cells though in combination supports the milk synthesis in the udder. Increase in milk production is achieved when there is little loss of body condition, while condition loss remains minimum when extra nutrients need of cow is being met by increased feed intake through increased feeding frequency (Amos et al., 2015). This argument is further supported by a study which revealed that milk production on 1 time/day feed supply remains significantly ($p<0.05$) lower compared to 2 times/day and 3 times/ day feed supply (Blake and Custodio, 2014). Some other scientists reported that varying feeding frequency from 2 times to 3 times per day in cattle results increased milk production by 18%. In another study researchers revealed that production of diary cos increases by 20% when feeding frequency is changed from 2 times/day to 3 times/day. Further it was revealed that the lactation period has significant ($p<0.001$) influence on overall milk production of dairy cows. On every next day of milking, production decreases by 2.5ml (DePeters et al., 2015). This concept was further supported by another scientist who depicted that milk yield of dairy cows declines gradually after peak period (Devendra, 2014). The decline in the milk production is mainly related to the sloughing of secretory tissues as well as decreased secretion rate/ mammary cell. In animal is non pregnant then reduction in milk production after reaching to peak period remains gradual, with average 5% reduction in every next month (Stelwagen et al., 1994). In another research it was reported that the dairy cows milked 6 times/ day consume higher quantity of dry matter compared to those who were milked 3 times/ day (Bell, 2011). In the follow up of this research, few others stated that significant increase in feed intake occurs when feeding frequency is increased that ultimately favors the rise in milk production of dairy cow (Erdman and Varner, 2015).

In another study it was noticeable that, with the progression of lactation most of the animals do not gain weight. In fact, loss of body condition occurs during the lactation period especially when animals are milked thrice a day. This indicates that dairy cows have higher dry matter need with higher milking frequencies (Franchi et al., 2019). This study was supported by another research, where researchers indicated higher intake of dry matter by cows when milked 6 times per. Increased milking times causes failure to compensate increased energy demands, therefore dairy cows continuously lose their body weight. On other hand lower body weight loses occur when cows are milked 3 times/day as that provide longer recovery period for better production (Senn et al., 1996). It was further found that dairy cows milked 3 times daily tend to be lighter in weight compared to those which are milked twice a day. They further noticed that dry matter intake decreases by 15% on 2 times/day milking compared to 3 times milking/day. Further it was reported that dairy cows milked 3 times per day have higher dry matter requirement compared to 2 times milking daily. However, the higher dry matter requirement can be fulfilled by increasing the feeding frequencies (Senn et al., 1996).

Few other researchers showed that milking 3 times daily reduces overall body weight gain in dairy cows. Dairy cows milked thrice a day possess higher tendency of losing body contrast to those who are mile twice per day. Even though, cows are provided *ad-libitum* dry matter, but the dairy cows fail to compensate due to higher energy demand related to increased milk yield. Increasing frequency of feeding, results preferential nutrients utilization for production of milk as well as higher tissue catabolism rate (Peel and Fronk, 1983). Some other researchers suggested higher milk production on higher feed intake which actually serves as long term tissues reserves for nutrients need during milk production. Lactation length also has significant ($P < 0.001$) influence on dry matter intake. Dry matter increases on every next day of lactation by 3.73g (Mengistu et al., 2012). These research outcomes were supported by few other scientists who revealed that lactation onset results dramatic increase in the nutrients requirements of dairy cows such as amino acids, glucose and fatty acids etc. The increased nutrients need is fulfilled by increased voluntary feed intake as well as by an array of metabolic strategies (Burgos et al., 2001). As per few other researchers, GIT hypertrophy, higher fatty acid metabolism in adipose tissues and higher gluconeogenesis rate, are the key changes which possess great importance for maintaining the higher milk yield. When dairy cow efficiently utilize the feed then drastic increase in production occurs. It is also noteworthy, that feed utilization by dairy cows is attributed to quality of offered feed, animal breed and overall physiological status of animal. These findings were supported by results of few other researchers who found that milk yield significantly depends on feed efficiency, environmental contributing factors and genetic capacity of the dairy cow (Royle et al., 1992).

Moreover, few others scientists noted that carbohydrate utilization efficiency can be improved by such treatment strategies which encourage the production of propionate by dairy cows instead of acetate or butyrate production. If the dairy cow is producing higher concentration of propionate, that will be useful for higher feed efficiency. For instance, the elephant grass provided to dairy cows in chopped form increases the surface area of the roughage, thus digestion is improved and as a result production is increased (Salama et al., 2003). According to some other researchers, the hormones such as growth hormone, insulin and prolactin, contribute in controlling the partitioning of energy to milk as well as body tissues. This interaction is related to genetic variations among animals (Stockdale et al., 1997). In another study it was revealed that the higher the milking frequency results higher feeding efficiency, higher feeding frequency and higher milk production. Dairy on 3 times milking utilize feed more efficiently than 2 times and 1 time milking. These results are further supported by King and Stockdale (2014) who stated that dairy cows milked 3 times per day daily have

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feeding efficiency by 14% higher compared to cows milked twice per day. Therefore, dairy cows on 3 times milking frequency have improved performance, if fed as per yield (King and Stockdale, 2014).

CONCLUSION

Present review concludes that the dietary manipulation and milking frequency always play a key role for improving the performance and production of dairy cows. In addition, a high quality feed plus *ad-libitum* water and also increased milking frequency support a good production of dairy cows.

DECLARATIONS

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Author's contribution
Both authors have contributed equivalent effort for this manuscript.

Competing interests
The author has not declared any conflict of interests.

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2020 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 10, Issue 4: 185-190; July 27, 2020 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2020.26

EFFECTS OF SUBSTITUTION OF CORN FOR COMMERCIAL RATION ON PERFORMANCE AND PLASMA CHOLESTEROL IN KUB CHICKENS

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Supporting Information

SHORT COMMUNICATION

PII: S222877012000026-10

Received: May 20, 2020

Revised: July 20, 2020

ABSTRACT: The aim of the present study was to evaluate growth performance and plasma total cholesterol (TCHO) concentration of KUB chickens fed by substitution of commercial feed with corn in 1 of day-old chick of KUB were raised for 10 weeks in two dietary groups including only commercial feed (group A) and a commercial feed substituted by 30% corn (group B). Data were analysed by T-test. The results showed that there was no significant effect of the treatments on feed intake, body weight (BWG) and feed conversion ratio (FCR) in KUB chickens. Similarly, plasma TCHO concentration did not show any difference between two experimental rations. However, total income of commercial feed substituted with 30% corn was higher than commercial feed. It was concluded that corn could be used at 30% to substituted commercial feed without significantly affecting the KUB chicken performance and TCHO. Present research considered usefulness of corn as a potential alternative of commercial feeds in KUB chickens in Indonesia.

Keywords: KUB chickens, Feed Intake, Body Weight Gain, Feed Conversion Ratio, Commercial Feed, Corn

INTRODUCTION

The use of native chicken in tropical countries varies among countries and from community to community within a region (Padhi, 2016; Yakubu et al., 2020). Recently, the poultry production in Indonesia has been given significant economic prospect especially towards provision of chicken carcasses in an effort to fulfill community demand of nutrition (Puspani et al., 2011; Harianto et al., 2019). Population of native chicken in Riau Province from year to year continues to increase along with consumer tastes towards native chicken. This fact is reflected in population growth and demand for native chickens, which has increased from year to year (Bakrie et al., 2003). According to the Central Statistics Agency the number of native chickens in 2014, 2015 and 2016 were 3,327,820, 3,746784 and 3,896,655, respectively (Central Bureau of Statistics, 2017). Indigenous chickens contributed in meat production about 292,710 tons in period 2011-2015. Recently, indigenous chicken production includes almost 16.7% of total of market share of commercial meat-type poultry in Indonesia (Tangendjaja, 1999). Considering this potential, solutions should be sought to increase population and productivity. To fulfil the demands of indigenous chicken meat in Indonesia, it has been followed by the finding of native KUB chicken as moderately improved native chicken breed. KUB chicken is a superior native chicken produced by the Indonesian Agency for Agricultural Research and Development, Indonesia (Hidayah, 2019). The KUB breed has some advantages such as high hatchability, low feed conversion ratio and high rates of egg production (160-180 eggs/year) (Sartika, 2016) as well as considering aa a meat type breed (Hidayah et al., 2019), in compared to their previous generations as well as local chickens.

One of the keys to success in maintaining KUB chicken is to meet their nutritional needs through the provision of rations that are in accordance with the standards of livestock needs. In general, farmers buy commercial rations that are marketed to have nutritional standards. The feed is the largest cost component, which is about 70% of the total production cost in poultry (Teguia and Beynen, 2005). Therefore, indirectly the ration is a determinant of the level of profits of farmers. The price of commercial rations sold in markets and poultry shops is considered very expensive by farmers. Therefore it is very important to look for ration giving strategies to reduce feed costs. One alternative that can be taken to reduce the cost of feed is to reduce the portion of the commercial ration provided by one of the raw materials that contain high calories to the performance of the chicken.

One of the main feed ingredients in poultry in preparing rations as an energy source is corn. This feed ingredient has several advantages including easy digesting, palatable and does not contain anti-nutritive substances. In addition, corn also contains xanthophyll substances which can increase the yolk on the yolk, feet and chicken carcass skin. Aside from being a feed source for carbohydrates, the ingredients of this ration are also a source of protein, namely: albumin, globulin, prolamin, glutelin, and nonprotein nitrogen. According to Scott (1982) yellow corn compound 3,370 kcal / kg of

Citation: Erwan E (2020). Effects of substitution of corn for commercial ration on performance and plasma cholesterol in KUB chicken. *Online J. Anim. Feed Res.*, 10(4): 185-190.

metabolic energy (EM), 8.6% crude protein, 3.9% fat, 2% crude fiber 0.02% calcium and 0.1% phosphorus. In addition, Suarni and Widowati (2007) stated that corn has other advantages including containing 12.19% dietary fiber which functions to reduce total cholesterol (TCHO), LDL levels and blood glucose. Another advantage of corn is that it contains vitamin A or carotenoid and vitamin E which functions as natural antioxidants that can increase the body's immunity and can inhibit degenerative cells. The content of several essential minerals, such as K, Na, P, Ca and Fe are also found in corn.

Several previous studies have shown that replacing some commercial rations with corn does not reduce chicken performance. For instance, Puspani et al. (2011) revealed that substitution commercial feed up to 20% with corn did not alter feed consumption and FCR in broiler chicken. Winarti and Wiranti (2013) fed diet substitution of broiler commercial feed with corn up to 40% did not adverse growth and FCR in native chickens. Furthermore, Munira et al. (2016) in their research results reported that there were no significant difference on feed intake, body weight gain (BWG), carcass weight and carcass percentage of super native chickens when fed fermented 10% rice bran in ration compared to control. These findings indicate that one alternative that can be done in an effort to reduce the cost of raising chickens both broilers and native chickens is to replace some commercial rations with energy source feed ingredients.

To our knowledge, there have been no reports regarding the effect of substitution of commercial feed with corn on performance and plasma TCHO in KUB chicken. Therefore, the purpose of this study was to determine the effect of substitution of commercial feed with corn on performance and plasma TCHO in KUB chickens. In addition, evaluation of corn energy source for economical broiler production also was evaluated.

MATERIALS AND METHODS

This research was carried out at the Poultry Division Field Laboratory, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia in 2018.

Ethical approval

Chickens were handled and managed accordance with the recommendations in the Guide for the Care and Use of Animal, at the Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia.

Animals and ration

This study used 50 DOC KUB chickens purchased from local breeding farms and placed in 2 enclosures (25 per plot). All birds were distributed with uniform body weight and water was provided *ad-libitum.* One day before the experiment. Placement of chickens into the cage was done randomly. Chicken was put into the cage done two weeks after the cage was cleaned and washed. Likewise, the treatment was given randomly. The size of the enclosure for each unit is 75 cm x 60 cm width and 60 cm height. Each cage was equipped with a ration container and drinking water container. This study consisted of 2 treatments, each consisting of 25 chickens. The treatment ration and water were given ad libitum. Chicken were raised for 10 weeks. Experimental rations consisted of two treatments, namely 100% of commercial feed and 70% commercial feed + 30% corn. The commercial feed was purchased from PT Charoon Pokphan Ltd, Pekanbaru (Table 1), while the composition of nutrient content of treatment is shown in Table 2. The parameters measured were performance including feed intake, (BWG) and feed conversion ratio (FCR), concentration of TCHO in blood plasma.

Table 2 - The percentage of nutrient content of Corn and Commercial ration

Nutrient	Corn*	Commercial ration
Crude Protein (%)	8.6	23.50
Crude Fiber (%)	2	1.88
Crude Fat (%)	3.9	5.87
Ca (%)	0.02	0.29
P (%)	0.1	0.15
ME (Kcal/kg)	3,370	3,050

* Scott et al. (1982). Ca: Calcium, P: Phosphor, ME: Metabolizable Energy; *Commercial feed: CP511 PT, Charoen Pokphand, Indonesia; **Mineral Premix: Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; biotin, 0.1 mg; choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu,12 mg; I,1 mg; Se, 0.2 mg, and adequate antioxidant.

Table 2 - Composition of nutrient content of treatment

Nutrient	100% of Commercial feed	70% commercial feed + 30% corn
Crude Protein (%)	23.50	22.01
Crude Fiber (%)	1.88	4.21
Crude Fat (%)	5.87	7.42
ME (Kcal/kg)	3,050	3,055

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Growth performance

Feed intake and BWG were recorded weekly throughout the experiment. Feed intake was corrected for body weight taking account of mortality if any. Feed intake was calculated as a difference between the amount of feed supplied to the birds and the amount of feed that remained at the end of each feeding period. BWG was calculated as a difference between the final and initial birds weight during each of the weighing periods. Feed intake and BWG were recorded at week 1 to week 10 and FCR was calculated as a ratio between feed intake and BWG for each period.

Analysis of plasma total cholesterol

The TCHO was determined with Microlab 300 (Vital Scientific, Netherland) as per the manufacturer's instructions. Samples were assayed together and in a random sequence for each sample.

Statistical analysis

Data obtained were analyzed by T test. Significant differences will be given in the symbol $p<0.05$. Data to be displayed was ± SEM which is processed by SPSS commercial software (2007). Before data processing was performed, all raw data was performed by the Thompson test to eliminate outlier data using the test level ($p<0.05$), then proceed with data analysis.

RESULT AND DISCUSSION

Feed Intake

Weekly feed intake of the birds is shown in Table 3. T test results of feed intake, did not show significant effect ($P>0.05$). Feed intake during the entire experimental period, ranging from 406.31 to 400.6 g/bird/week, respectively. This shows that substitution commercial feed with corn up to 30% did not affect feed intake. The result was consistent with previous work (Puspani et al., 2011) who revealed that substitution of commercial feed with corn up to 20% did not alter feed intake in broiler chickens. Similarly, Winarti and Wiranti (2013) who reported that substitution of broiler commercial feed with corn even up to 40% did not significantly change feed intake in native chickens. It seems that substitution of broiler commercial feed with corn did not alter feed intake thereby the composition of nutrients such as crude protein and energy metabolism in between two treatments given to KUB chickens still adequate to maintain their growth.

It is well know that level of protein and feed energy will affect the consumption of feed. Feeds that contain relatively similar protein and energy cause the same consumption of feed (Astuti, 2012). According to Parakkasi (1985) chickens consume rations mainly to meet their energy needs. Chickens cannot adjust to their rations precisely but consume more energy if their feed energy levels are low (Anggorodi, 1994). However, as shown at Table 2, the average feed intake of KUB chicken fed with substitution commercial feed with corn was 400.6 gram/bird/week higher than previous study (Munira et al., 2016) who found that the average of feed intake of KUB chickens was 307.80 grams /bird/week when fed a basal control diet prepared in 10-week. The reason for these discrepancies on feed intake due to KUB chickens is unknown. Such differences also might be attributed the size of the feed ingredient composition, feed formulation and feed pellet quality and management including environmental management, feed and water availability to the birds, disease control, and stocking density (Ferket and Gernat, 2006; Kuleile et al., 2020). These results also may imply that KUB chickens have a low nutrient requirement for maintenance and growth compared to broiler chickens. The trend of average weekly feed intake of KUB is shown in Figure 1. As shown in Figure 1 during the first 7 weeks, the average feed intake in chickens on both experimental rations increased gradually and showed similar trends. There was no increase in the feed intake of chickens during 8th and 10th.

Table 3 - Average of feed intake of KUB chickens provided with commercial feed or substitution commercial feed with corn (gram/bird/week)

Age (week)	Control (broiler commercial feed)	30% substitution
1	99.52	90.96
2	188.6	183.20
3	265.6	244.80
4	334.8	339.68
5	413.4	409.60
6	532.4	545.80
7	559.2	546.60
8	557	549.20
9	556.2	546.20
10	556.4	550.00
Average	406.312	400.604

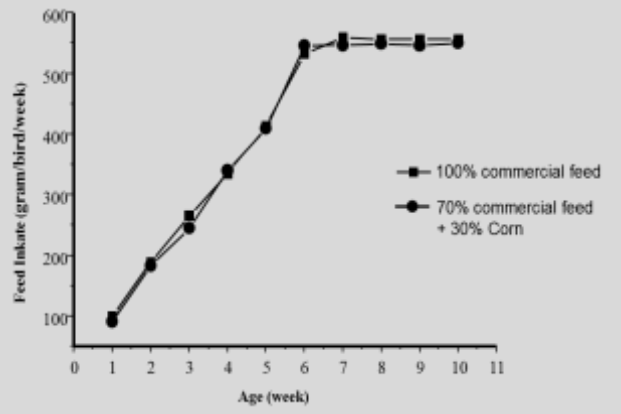

Figure 1 - Effects of experimental rations on the weekly feed intake of KUB chickens.

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Body weight gain

Results of BWG of birds fed with experimental diets are presented in Table 4. The results of data analysis showed that the substitution of commercial feed with 30% corn did not significantly ($p>0.05$) affect BW gain in KUB chickens. The average BW gain during 10 weeks old of was 487.0 and 420.5 g/head/week, control and substitution treatments, respectively. These results confirmed with previous work (Winarti and Wiranti, 2013) who found that reported that substitution of feed with corn up to 40% did not significantly alter BW gain in native chickens. This results might be attributed by feed intake were also similar of the two treatments. Visualization of the average weight gain of super native chickens during the study is shown in figure 2. As shown in Figure 2 that during the first 5 weeks, the average BW gain in chickens on both experimental rations increased gradually and showed similar trends. However, during 5th and 10th the average BW gain increased sharply. It seems that substitution broiler commercial feed had higher trend than control diet during 5th and 10th. This shows that it is advisable to carry on keeping the KUB chickens until 10th week as the chickens consumed more feed and gained gradually. These results indicate that the 5th week was the period of the beginning of gradually growth which then continuously growth in sharply trend up to 10th week.

Table 4 - Effect of partial replacement of broiler commercial feed with corn on BW gain in KUB chickens (gram/bird/week)

Age (week)	Control	Substitution
1	63.92	64.00
2	99.28	122.72
3	166.48	190.84
4	233.88	243.12
5	331.84	368.32
6	396.16	491.16
7	487	548.52
8	546.32	639.40
9	604.04	719.12
10	689.4	818.24
Average	487.00	420.54

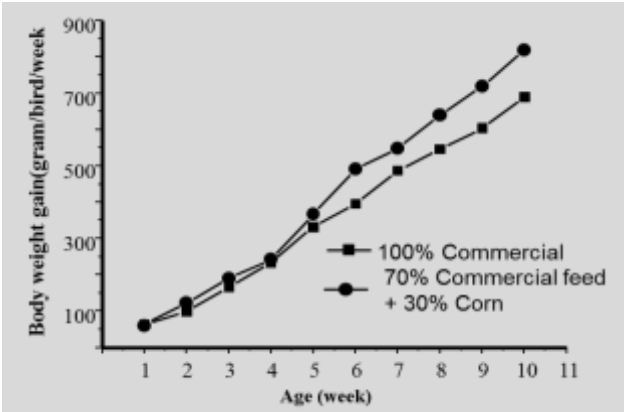

Figure 2. Average of BWG of KUB chickens in feeding substituted with corn

Feed conversion ratio

The average FCR for 10 weeks g /bird/week raised from lowest to highest respectively 4.78 and 5.0 as in Figure 3. The results of the data analysis show that the replacement of commercial rations with corn did not significant effect ($P>0.05$) on FCR. The results of this study were not much different from the results of the study of Munira et al. (2016) who demonstrated that the average FCR in super native chickens up to 10 weeks of age with substitution of fermentation rice bran was ranging from 4.1 to 4.9

Economics of production

Economic analysis as influenced by substitution commercial feed with 30% corn is shown in Table 5. Total input cost per bird was calculated on the basis of total feed cost and cost of chicks and cost management. As KUB chicken fed on substitution commercial feed with 30% corn the cost of experimental ration decrease. Net profits were obtained for the group compared to fed by 100% of commercial feed.

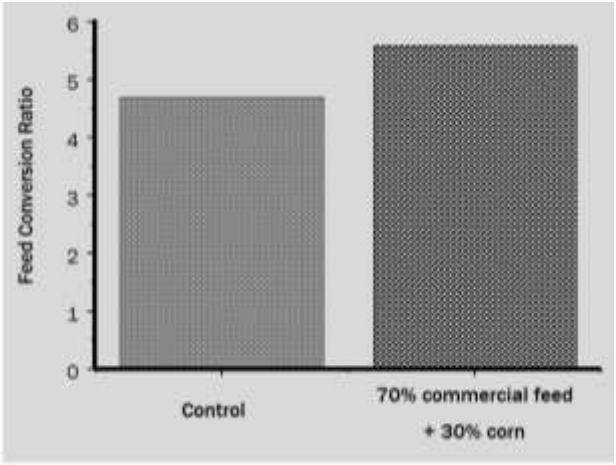

Figure 3 - Effects of experimental rations on the weekly feed conversion ratio of KUB chickens

Table 5 - Economic analysis of feeding commercial feed and substitution of commercial feed with corn to KUB Chickens (in Indonesian Rupiah)

Parameters/rations	Control (broiler commercial feed)	70% commercial fee + 30% corn
Cost* of feed	577,500	502,500
Cost of chicks	350,000	350,000
Cost of management	350,000	350,000
Total cost	1,277.500	1,202.500
Sale revenue	2,000000	2,000000
Average	722,500	797,500

*Based on Indonesian currency

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Total cholesterol

The results of the study of commercial feed substitution with corn feed ingredients on TCHO of KUB chicken plasma is shown in Figure 4. Based on statistical analysis, there is no effect of experimental rations TCHO levels ($p>0.05$). Study of plasma metabolites in bird enables metabolic change to be evaluated that are due to the effects of many factors, including pharmacological condition physiological state, age, husbandry condition, and genetic type (Meluzzi et al., 1991; Gayathri et al., 2004; Erwan et al., 2014, 2017, 2020). The average TCHO level in the control and substitution with 30% corn was 154.52 mg/dl and 166.42 mg/dl included in the normal range according to finding of Mangisah (2003) who explained that normal chicken blood cholesterol levels ranged from 125-200 mg/dl.

Partial substitution of commercial feed with corn feed ingredients did not affect TCHO. No differences TCHO levels presumably correlated to feed ingredients both treatments were similar. This result shows that substitution 30% commercial broiler feed with corn still could be tolerate on plasma metabolite especially TCHO in plasma. TCHO derived from feed plays an important role, because it is the main sterol in the body and the cell surface components and intracellular membranes. De novo cholesterol biosynthesis is much influenced by stress factors of super native chickens. Overal these results indicated that KUB chicken fed by substitution commercial feed with 30% corn did not adverse performance and plasma cholesterol .

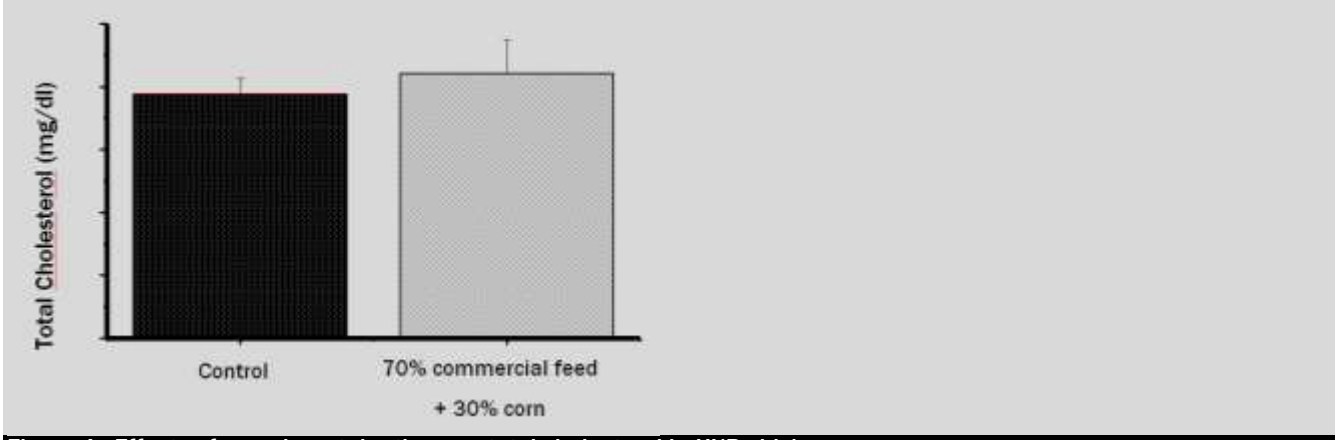

Figure 4 - Effects of experimental rations on total cholesterol in KUB chickens

CONCLUSION

It is concluded that corn could be used up to 30% to substitute commercial feed in diets of KUB breed chickens (local breed) could reduce cost of production without change growth performance and plasma cholesterol level in local breeds of broiler chickens. A future study will explore on the effect of using 30% to substitute commercial feed on performance and plasma metabolites in other poultry species.

DECLARATION

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Availability of data

The data can be availed to the journal upon request.

Consent to publish

Not applicable

Conflict of interest

The author declares they have no competing of interests.

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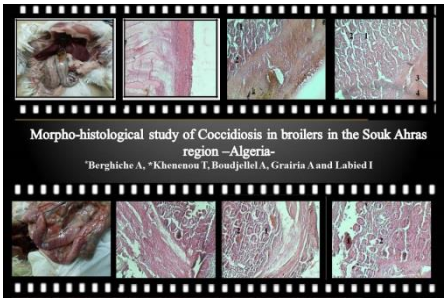

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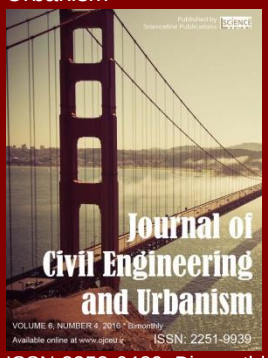

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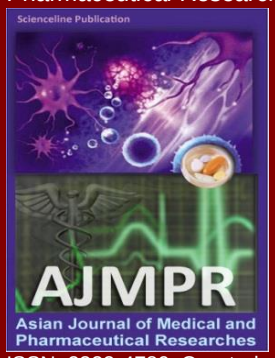

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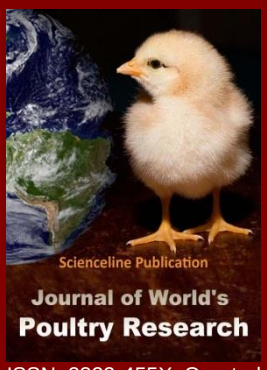

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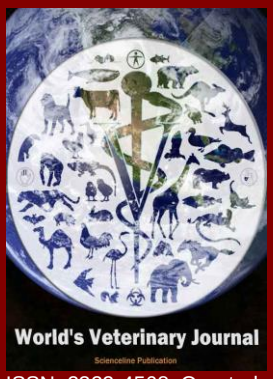

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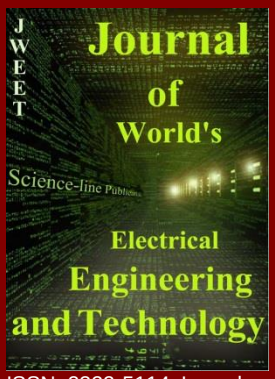

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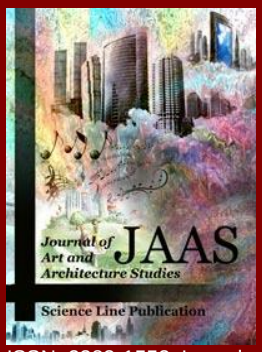

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