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Volume 11 (3); May 29, 2021

Research Paper

Chemical and microbiological properties of broiler litter kept at different altitudes.

Utama CS and Christiyanto M.

Online J. Anim. Feed Res., 11(3): 88-94, 2021; pii: S222877012100015-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.15

Abstract
The research objective was to assess the chemical and microbiological properties of broiler litter raised in broiler closed house at different altitudes. The design used was a completely randomized design with 3 treatments and 5 replications. The treatments consisted of T1 (broiler closed house at an altitude of ≤100 meters above sea level (masl), T2 (broiler closed house at an altitude of 100 - 300 masl) and T3 (broiler closed house at an altitude of 300 - 500 masl). The results showed that the difference in the altitude where broilers were raised had no significant effect on moisture, ash, extract ether (EE), crude fibre (CF), nitrogen free extract (NFE), total digestible nutrients (TDN), cuprum (Cu), lead (Pb), mercury (Ag), lactic acid bacteria (LAB), *Salmonella, E. coli, Clostridium sp.,* antibiotic contamination (below the threshold) and predominance of gram-positive bacteria. The results of water content 22.71-24.65%, crude protein 13.13-13.47%, Pb 35.15-47.80 ppm, Cu 102.46-136.96 ppm, LAB 3.48 - 7.00 × 10^6 cfu/g. In conclusion, the differences in the altitude did not affect the chemical and microbiological properties of broiler litter.
Keywords: Altitude, Bacteria, Chemical properties, Litter, Poultry.

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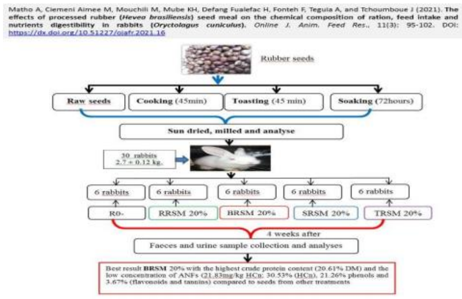
Research Paper

The effects of processed rubber (*Hevea brasiliensis*) seed meal on the chemical composition of ration, feed intake and nutrients digestibility in rabbits (*Oryctolagus cuniculus*).



Matho A, Ciemeni Aimee M, Mouchili M, Mube KH, Defang Fualefac H, Fonteh F, Teguia A, and Tchoumboue J.

Online J. Anim. Feed Res., 11(3): 95-102, 2021; pii: S222877012100016-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.16

Abstract
This study aimed at evaluating the influence of processed rubber (*Hevea brasiliensis*) seed meal (RSM) on the chemical composition of feed, feed intake and nutrients digestibility in rabbits. For the purpose, 30 female (local breed) rabbits with an average weight of 2.7 ± 0.12 kg were randomly allotted to five dietary treatments (6 rabbits in 1 treatment). The feeding trial lasted for four weeks and the various rations were: negative control ration, not containing rubber seeds (R0-) and 4 different rations each containing 20% of the rubber seed meal (raw, toasted, soaked, and boiled respectively for R0+, R1, R2, and R3 rations). The rubber seed meal, faeces and feed sample were sun dried to constant weight, urine was also collected and a sample was used for chemical analysis. The results showed that the boiled seeds RSM exhibited the highest crude protein content (20.61% DM) and the low concentration of anti-nutritional factors (ANFs) (21.83 mg/kg HCn; 30.53%; 21.26% and 3.67% respectively for hydrogen cyanide (HCn), phenols, flavonoids and tannins) compared to seeds from other treatments. Animals receiving the ration with boiled rubber seed meal (BRSM) at 100°C for 45 minutes (R3) showed better feed intake and best rate of digestive utilization coefficients of dry matter, organic matter, crude protein and crude fibres 32.24%; 38.55%; 61.03% and 80.58% respectively. The ration containing the boiled rubber seeds for 45 min resulted in better nutrient intake and the best digestive utilization coefficients in rabbits.
Keywords: Digestibility, *Hevea brasiliensis*, Nutritional value, Rabbit, Rubber seed.

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Research Paper

Effect of lime (*Citrus aurantifolia*) juice as source of vitamin c on performance and some blood parameters in Pelung chickens (*Gallus gallus domesticus*).

Krismiyanto L, Wahyuni HI and Suthama dN.

Online J. Anim. Feed Res., 11(3): 103-108, 2021; pii: S222877012100017-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.17

Abstract
Pelung chicken (*Gallus gallus domesticus*) is a typical Indonesian local breed originating from West Java. Pelung chicken breeding, especially male bird, was initially used as a hobby livestock because they have tunable voice. This study aimed to evaluate the effect of feeding lime (*Citrus aurantifolia*) juice as a source of vitamin C on blood parameter and performance of male pelung chickens. The experimental birds were 64 male pelung chicken aged 12 weeks, divided into 4 weight groups namely group I: 740 – 910 g, group II: 910 – 1,080 g, group III: 1,080 – 1,250 g, and group IV: 1,250 – 1,420. The treatments applied were T0: formulated diet; FD, T1: FD+ lime juice 1%, T2: FD + lime juice 2%, and T3: FD + lime juice 3%. The present experiment was assigned in body weight-based randomized block design. Measured parameters were heterophile, lymphocyte, heterophile-lymphocyte ratio (H/L), total plasma protein, the relative weight of lymphoid organs (spleen and bursa of fabricius) and performance (feed intake, daily body weight gain and feed conversion ratio) of birds. The results showed that the feeding diet added with 1-3% lime juice significant on heterophile, H/L ratio, the relative weight of spleen, total plasma protein, daily body weight gain and feed conversion ratio, but not significant on lymphocyte, the relative weight of bursa of fabricius and feed intake. Male pelung chicken fed diet added with 3% lime juice (T3) indicates better blood parameter stability (H/L ratio and lymphoid organs), total plasma protein and performance.

Keywords: Blood parameter, Lime, Pelung chicken, Performance, Vitamin C.

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Phone: +90 538 770 8824 (TURKEY); +1 209 732 4265 (CANADA)
Homepage: www.science-line.com
Emails: administrator@science-line.com ; saeid.azar@atauni.edu.tr

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FORAGE PARTICLE SIZE: IT'S IMPLICATIONS ON BEHAVIOR, PERFORMANCE, HEALTH AND WELFARE OF DAIRY COWS

Emran HOSSAIN

Professor and Head, Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Zakir Hossain Road, Khulshi, Chattogram-4225, Bangladesh

Email: emran@cvasu.ac.bd; ORCID: 0000-0002-1750-7284

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ABSTRACT: Forage particle size has long been recognized as the key intricate factor determining ration contents of physically effective neutral detergent fibre, which in recent years has become the most important consideration in advanced dairy feed formulation for the rumen health, milk yield and welfare of the dairy cows. Fine chopping reduces particle size, resulting in reduced forage dry matter intake, lower retention period of digesta, decreased digestibility of fiber, inconsistent quality of ruminal mat, decreased milk yield, depression of milk fat, and health issues secondary to sub-acute ruminal acidosis. Similarly, excessive coarse forage particles may be lead to reduced total nutrient intake, poor digestibility of organic matter, reduced milk yield and quality, and compromised overall performance. The rumen is a constant fermentation vessel, containing vast amounts of hydrogen ions that can only be stabilized by a proper salivary buffer balance obtained through intake of sufficient effective neutral detergent fibre and its optimal duration. The overall impacts of particle size, however, depend on forage type, forage to concentrate ratio and, fermentability characteristics of the organic matter in the formulated ration. In general, 8-19 mm particle size irrespective of forage type measured on Penn State Particle Separator may be considered optimum for practical dairy feed formulation.

Keywords: Dairy cattle, Forage particle size, Health, Milk yield, Performance.

INTRODUCTION

Forage particle size (FPS) or chop length (CL) has long been recognized as one of the principal factors influencing feed intake (Haselmann et al., 2019), feed sorting behavior (Jiang et al., 2018), digestibility of feed (Zhao et al., 2020), rumination (Deswysen et al., 1978), turnover kinetics of rumen metabolites (Storm and Kristensen, 2010), rumen pH (Kmicikewycz and Heinrichs, 2015), microbial protein synthesis (Rodríguez-Prado et al., 2004), milk yield (Havekes et al., 2020), milk fat content (Sharifi et al., 2012), profile of milk fatty acid (Thomson et al., 2017), milk protein percent (Nasrollahi et al., 2015) and overall, cow health (Havekes et al., (2020). The ruminant diet is dominant in crude fibre which is inevitable for their health, productivity and welfare. The rumen appears to be a continuous fermentation vat that produces large quantities of hydrogen ions (60,000 mEq/day or more) (Allen, 1997). Thus, upkeep of stable ruminal pH is intricate. The ruminal pH is in equilibrium by two key factors: an appropriate balance of slowly and rapidly fermentable carbohydrates and adequate physical fiber to stimulate chewing activity and saliva production (Allen, 1997). Fine chopping reduces FPS and thus can decreases the physically effective neutral detergent fibre (peNDF) contents of diet (Stojanović et al., 2013). The peNDF contents of diet, thus, virtually results from the interaction between the contents of chemical fiber and forage chop size in the diet (Gümüş and Bayram, 2020). The shorter than the optimum FPS may result in low dry-matter intake, decreased fiber digestibility, decreased milk yield, milk fat depression, and health problems secondary to sub-acute ruminal acidosis. Similarly higher than recommended FPS may provoke feed sorting, spending excessive time for re-chewing, higher retention time of digesta and reduced fractional passage kinetics of particulates. This review, therefore, aims to highlight the link between FPS and peNDF contents of the rations and their subsequent buffering implications on production, health, efficiency of nutrient utilization, and welfare of the dairy animals and explains why not too long, not too short, but "just right" FPS is required for dairy cows.

BEHAVIOR

Feed sorting

Twenty Holstein bull calves were exposed in the total mixed rations to observe the effect of early introduction to rations varying in FPS on the progression of feed sorting in dairy calves (Miller-Cushon et al., 2013). Calves offered the low FPS diet consumed less neutral detergent fiber as a level of anticipated intakes and would in general consumed less acid detergent fiber and more non-fiber sugars, than the calves recently fed the high FPS diet. It showed that calves recently fed the low FPS diet were sorting for concentrate and this sorting behavior might have been influenced by their early involvement in the rations varying in FPS. Similarly, twelve multiparous lactating Holstein dairy animals were exposed in a

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replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement to decide the impacts of FPS on feed sorting behavior. As a rule, sorting activity decreased with reduced forage particle size. In any case, the dairy animals fed high quality forage had a lower sorting activity and higher production performance than those who fed low-quality forage (Jiang et al., 2018). In another study, larger FPS forages increased sorting behavior (Figure 1); however, had no impact on rumen fermentation or chewing behavior of the animal (Suarez-Mena et al., 2013). In another experiment (Leonardi et al., 2005), the consequences for feed sorting of various amounts, characteristics, and lengths of alfalfa feed were tried, without changing the extent of concentrate in the eating regimens. These investigators found that the dairy animals increased their sorting activity with more hay and with longer hay, though the quality of feed had no impact. Similar impacts of particle size have been demonstrated by other investigators (Kononoff and Heinrichs, 2003).

Despite the fact that few scientists have indicated that sorting against long particles and NDF can be decreased by changing the quality of forages inside a TMR (Kononoff and Heinrichs, 2003) nobody has clearly explained how do the extent of forage in the eating regimen impacts sorting behavior. It was demonstrated that animals effectively opposed long particles, NDF, and peNDF, and chose for short particles when taken care of a long forage diet. This finding negates the speculation that animals would sort more with increased forage in the eating regimens. It was accepted that animals would be exceptionally energetic to sort for the concentrate part of their TMR, particularly when accessibility of concentrate was restricted. Accordingly, it was accepted that decreasing the concentrate divided in the TMR would propel dairy cows to sort for the concentrate and against forage (Voelker et al., 2002). The increased sorting of the long forage diet demonstrated, in any case, that a ration with higher extent of concentrate might be all the more effectively sorted, essentially in light of the fact that the concentrate content is progressively available than the roughages.

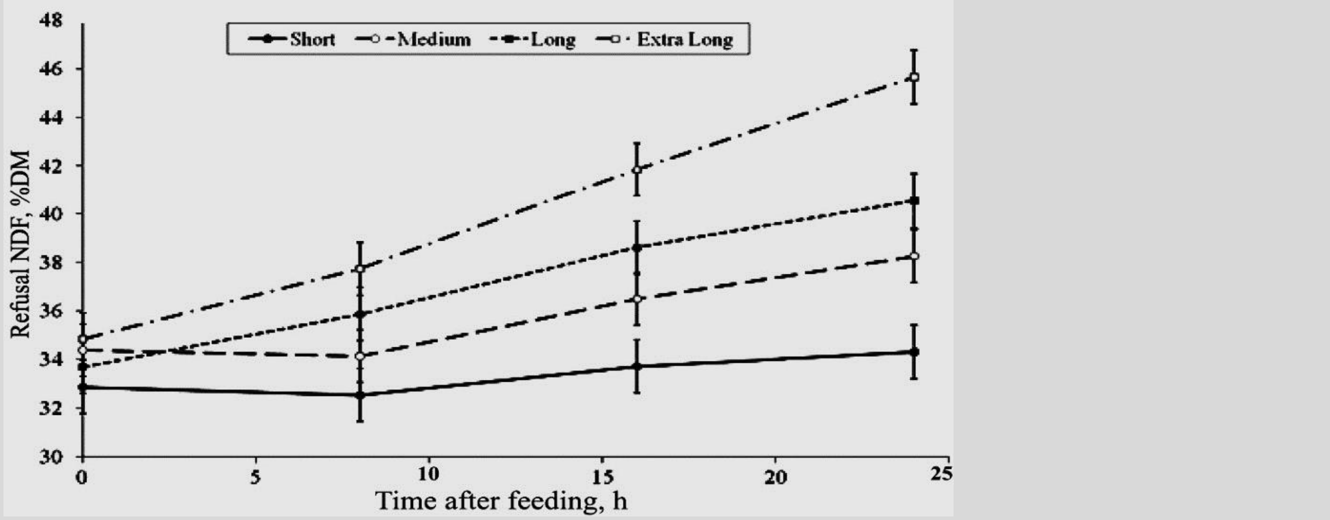

Figure 1 - Effects of forage particle size on feed sorting in dairy cows (Maulfair et al., 2010). The TMR contains short (1.5 mm), medium (6.5 mm), long (8.6 mm), and extra-long (11.7% 26.9 mm) particles.

Chewing and rumination

Forage size affects both eating and chewing time (Table 1; Figure 2). Rumination time decreased from 504 to 400 min/d for cows expending short particle size contrasted with long particle size. Similarly, chewing was decreased from 702 to 570 min/d when dairy animals consumed short particle size (Ramirez Ramirez et al., 2016). In a different study, forty-eight Holstein calves were arbitrarily distributed in a 2 × 2 factorial plan to examine the impacts of FPS on sorting behavior of dairy calves fed texturized concentrates (Omidi-Mirzaei et al., 2018). Calves fed forage with long FPS invested more energy for rumination, eating forage, and invested less time lying and non-nutritive oral practices than medium particle size. Essentially, the cows decreased eating and ruminating time by 4.8 and 1.9 min, respectively per kilogram of DMI and demonstrated lower rumination endeavors while fed low FPS diet (Haselmann et al., 2019). Thus, increased chewing and rumination because of elevated FPS is predictable.

Table 1 - Influence of forage physical form on chewing activity

Item \ Form of hay	Long	Chopped	Pelleted
Eating, min/d	196[a]	174[a]	128[b]
Ruminating			
min/d	383[a]	398a	61[b]
min/kg NDF intake	64.1[a]	64.4[a]	10.2[b]
Total chewing			
min/d	579[a]	572[a]	189[b]
min/kg DM intake	29.2[a]	28.6[a]	9.8[b]

[a-b]Means in the same row within measures with different superscripts differ (*P*<0.001).

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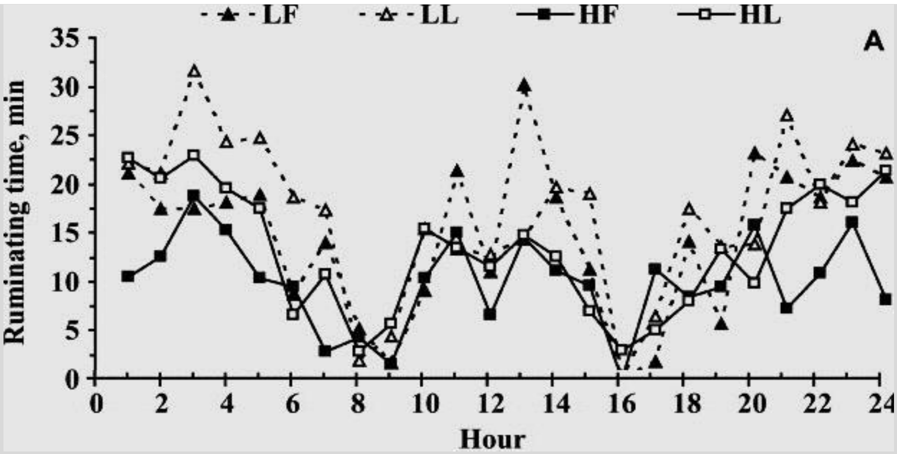

Figure 2 - Relationship between forage particle size and ruminal pH (Zebeli et al., 2007). LF = low concentrate level and fine hay; LL = low concentrate level and long hay; HF = high concentrate level and fine hay; HL = high concentrate level and long hay.

Ruminal mat consistency

Particle size legitimately influences the rumen mat, which is shaped by little particles holding adherence of the longer-stem forages that float in the rumen. This connection permits the smaller particles to stay suspended in the rumen to be appropriately fermented. Without adequate fiber, particles may sink into the less-desirable sites of the rumen where they cannot be digested properly. Thus, the benefits of high FPS is likely for better mat consistency.

Retention time of digesta

Rumen fill can physically constrain the retention time of digesta in dairy cows in forage-based eating regimens with high FPS (Shaver et al., 1988). Feed residues normally do not get away from the rumen either by a decrease or by the restricted entry of additional intake. In spite of the fact that the impacts of the rate of particle size decrease on ruminal retention time is not consistent, nevertheless, it was recommended that the rumen comprises of a rumination pool of larger particles that cannot go through the reticulo-omasal orifice until arrive at a smaller particle size (Ternouth, 1968). In light of this rate restricting particle size decrease hypothesis for particulate passage, it was recommended that the higher FPS will lengthen retention time (Figure 3 and 4) and the other way around (Poppi et al., 1980). Detailed information is minimal about the distribution of particle size at different locations within the reticulo-rumen, abomasum and intestines. However, coarse particles were more abundant in the dorsal than ventral rumen, which decreased with time after eating, it was observed (Evans et al., 1973). This variable distribution of the small particle pool coupled with small variations in high and low FPS chewing behavior may raise the question as to the role of reducing particle size in the rumen particle passage. For low and high fiber forages, the large proportion of small particles in the lumen suggests that the rate of escape of small particles from the rumen is an important factor determining the retention time of the rumen. In relation to the longer retention time of the large particle itself, the effect that larger particles have greater retention time in the rumen may be due to their effect on the forming of the rumen mat and eventual trapping of small particles (Shaver et al., 1988).

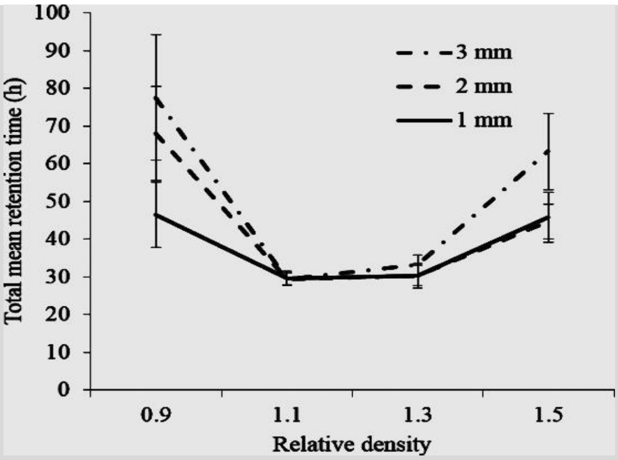

Figure 3 - Influence of relative density (0.9–1.5) and diameter (1–3 mm) on total mean retention time in the entire digestive tract (Dufreneix et al., 2019).

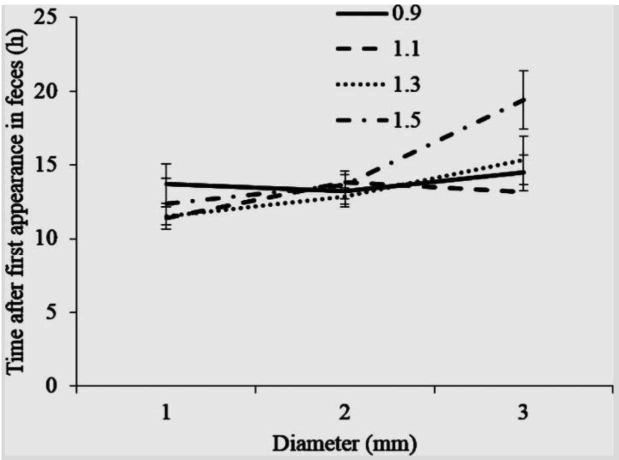

Figure 4 - Influence of relative density (0.9–1.5) and diameter (1–3 mm) of plastic particles on the time until they first appear in the feces (Dufreneix et al., 2019).

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PERFORMANCE

Dry matter intake

Overall, type and nature of the feed, body condition score, health, sex, age, equality of animal, milk yield, milk composition, environmental temperature, and humidity are the most dependable indicators influencing dry matter intake (DMI; Table 1) in dairy animals (Méndez et al., 2020). Explicit impacts of FPS on DMI are scant. The impact of FPS on DMI in dairy animals was assessed utilizing a meta-analysis with 46 papers and 28-91 preliminaries of published information from the literature (1998-2014). It was obvious that DMI improved because of bringing down the impact of FPS containing silage however not hay (Nasrollahi et al., 2015). In another study, the peNDF substance of dairy cattle diets was changed by differing levels of FPS of alfalfa silage (Li et al., 2020). Expanding peNDF substance of diets by expanding FPS decreased DMI. Therefore, the expansion in DMI because of decreased FPS was noteworthy in animals taken care of wild-rye feed diets (Jiang et al., 2018). In another study, the effects of physical form and stage of maturity at harvest of whole-crop barley silage on feed intake in dairy steers were evaluated in a 4 × 4 Latin square design (Rustas et al., 2010). It was assumed that, chopping increased DMI when grain was collected at the dough stage however not at the peak phase of maturity (Rustas et al., 2010). Decreasing the corn silage chop length increased dry matter intake ranging 22.3-23.2 kg/d, at 4 to 5 h in the wake of intake or milk production (Bhandari et al., 2008). The decrease in FPS increased DM consumption from 19.4 to 20.1 kg/d at the elevated level of concentrate and from 16.9 to 17.7 kg/d at the low degree of concentrate (Einarson et al., 2004).

Despite the fact that, sorting practices because of inconsistencies in FPS typically hinder the eating rate, yet again inverse evidence was demonstrated (DeVries et al., 2007). It was accounted for that, dairy animals consuming the long forage diet consumed at a more prominent rate and in a shorter time span. Johnson and Combs (1992) additionally found that cows consuming a half forage diet invested less energy eating than those fed a 74% forage diet. Conversely, Voelker et al. (2002) revealed comparable intake times for cows fed a 67 or 44% forage diet. These last researchers proposed that such inconsistencies in intake times might be the aftereffect of contrasts in dietary particle size. However,, neither of these past studies revealed dietary particle size. Strikingly, Voelker et al. (2002) reported that the eating time per kilogram of DMI was lesser for the animals taken care of a 44% forage diet, showing that these dairy animals consumed their DM quicker, like the more noteworthy intake rates on the long forage diet found in the current study. Allen (2000) expressed that the dietary factors that expansion eating time could decrease the time accessible for ruminating, in this way expanding the filling impact of the diet. Similarly, in this study, the high forage diet spent more time to be consumed, likely due to its high NDF substance and longer particle size. These variables may have added to an increased filling impact, representing the lower DMI on the high forage diet. Johnson and Combs (1992) noticed DMI as lower on their higher forage diets.

Forage degradability

Digestibility of dry matter or organic matter is improved when long forage particles are chopped but not ground (Tables 2 and 3). The impacts of FPS on degradability of individual amino acid (AA) in the digestive tract of lactating dairy cows with ruminal and duodenal cannulas were estimated in a Latin square design (Zhao et al., 2020). Degradability of most individual AA in the rumen was not influenced by FPS. In another study, the low FPS diet altogether increased total tract digestibility of the supplements (Haselmann et al., 2019). Chopping the dough stage silage decreased the extent of grain in defecation from 97 to 43 g/kg DM demonstrating higher starch digestibility (Rustas et al., 2010). However, expanding FPS had no impact on AA supply however the digestibility of individual AA in the digestive tract changed significantly (Li et al., 2012). Total tract absorption of dietary NDF was decreased for fine-handled corn silage contrasted and control corn silage and coarse-prepared corn silage (28.4% versus 33.9 and 33.7%, respectively). Processing corn silage improved starch digestibility (Bal et al., 2000). It was accounted for that changing the forage particle size from 6 to 30 mm in a low-concentrate diet substantially increased the rumination time and ruminal mat consistency without influencing ruminal fermentation and passage. Further, particle breakdown and consistency of mat in the rumen increased, and *in situ* feed dry matter degradability improved, which thus demonstrated a higher capacity of ruminal digesta to degrade fiber (Zebeli et al., 2008).

Fractional passage kinetics

Long particle size feeding lowered the rate of dry matter passage from 3.38 to 2.89±0.42 percent/h; mean retention time rose concomitantly from 31.7 to 38.4±5.36h for long particle size diets (Ramirez Ramirez et al., 2016).

Feed efficiency

Twelve multiparous lactating Holstein animals were exposed in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement to decide the impacts of forage source and size of particle size on feed sorting, milk production and supplement digestibility in lactating dairy cows. The experiment featured that, feed efficiency (4% fat-adjusted milk/DMI) improved from 1.18 to 1.11 when FPS decreased independent of forage source (Jiang et al., 2018).

Milk yield

An increased DMI is associated with increased milk yield (Table 5). Thus, increasing peNDF content of diets by increasing F:C ratio may decrease milk yield due to decreased DMI (Li et al., 2020). In an exhaustive meta-study, milk production reliably increased (0.541 kg/d; heterogeneity = 19%) and milk protein production increased (0.02 kg/d) as

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FPS decreased, however FCM was not influenced by FPS (Nasrollahi et al., 2015). FPS influences chewing activities and production of milk fat precursors in the rumen and modified milk fat substance and yield of fat-corrected milk (Lu, 1987).

Table 2 - Intake and digestion of organic matter and acid detergent fiber (Firkins et al., 1986).

Parameter / Forage size	Long	Chopped	Ground	SEM
Organic matter intake (kg/day)	13.2	12.6	13.1	0.11
Apparent digestion (% of intake) in total tract)	53.7	54.3	54.7	2.11
Apparent digestion (% of intake) in rumen				
Apparent	28.1[a]	31.2[a]	20.8[b]	2.62
True	57.7	56.0	55.3	4.32
Percent of apparent digestion in the rumen	52.3[a]	57.4[a]	38.0[b]	2.92
Acid detergent fiber intake (kg/day)	4.8	5.0	4.3	0.32
Apparent digestion (% in take) in total tract	36.7[c]	39.7[c]	24.0[d]	2.67
Apparent digestion (% intake) in rumen	36.0[c]	35.6[c]	16.2[d]	3.10
Percent of total digestion occurring in the rumen	98.0[a]	89.7[a]	67.5[b]	3.21

[a-b]Treatment means with different superscripts are significantly different (P<0.05); [c-e]Treatment means with different superscripts are significantly different (P<0.10).

Table 3 - Intake and digestion of nitrogen and partition of duodenal N flow.

Parameter / Forage size	Long	Chopped	Ground	SEM
N intake (g/day)	422a	322[b]	409[a]	9.3
Non-ammonia nitrogen flow at duodenum				
g/day	405[c]	337[d]	462[e]	27.0
% of N intake	96[c]	103[cd]	112[d]	4.67
Feed and endogenous N	37.2[c]	37.5[c]	46.8[d]	3.46
g/day	157[cd]	124[c]	194[d]	14.0
% of N intake	37.2[c]	37.5[c]	46.8[d]	3.46
Bacterial N at duodenum				
g/day	248[cd]	212[c]	269[d]	13.6
g/kg OM TROMD*	32.5[c]	30.7[c]	37.5[d]	2.39
Post-ruminal N digestion g/day	246[a]	203[a]	334[b]	25.7
Apparent N digestion in total tract, % of N intake	5.9[c]	58.3[c]	68.5[d]	2.39

*True rumen organic matter digestion; [a-b]Treatment means followed by different letters are different (P<0.05); [c-e]Treatment means followed by different letters are different (P<0.10).

Table 4 - Ruminal kinetics in steers fed chopped or ground hay.

Item / Treatment	Chopped	Ground
Ruminal particulate dilution rate, %/h	4.73	4.72
Ruminal fluid dilution rate, %/h	1034	10.64
Duodenal fluid flow, liters/d[c]	78.8	76.5
Ruminal fluid volume, liters[c]	78.1	67.7
Ruminal NH_3, mg/dl[d]	14.0	15.6
Total ruminal volatile fatty acid concentration, mM	76.1	77.5
Acetate, mol/100 mol[c]	71.5	71.2
Propionate, mol/100 mol	16.9	16.6
Buryrate, mol/100 mol[c]	9.5	9.8

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Table 5 - Relationship between forage particle size and milk yield (Grant et al., 1990).

Diet	Ruminal pH	A:P ratio	DMI (lb/d)	4% FCM (lb/d)	Milk fat (%)
Diet A-'Coarse'	6.0	2.9	48.8	64.9	3.9
Diet B-'Medium'	5.9	2.3	48.4	66.6	3.6
Diet C-'Fine'	5.3	1.9	49.3	60.5	3.0

Diet A-'Coarse' = 45% concentrate + 55% 0.38 inch TLC alfalfa silage; Diet B-'Medium' = 45% concentrate + 55% mixed length silage; Diet C-'Fine' = 45% concentrate + 55% 0.19 inch TLC alfalfa silage; A:P = Acetate: propionate; DMI = Dry matter intake; FCM = Fat corrected milk; TLC = Theoretical length of cut.

Feeding maize silage processed at adequate (6 mm; FCS) or abrasive (23 mm; CCS) FPS were fed to 22 lactating Holstein cows had no effect on milk yield (Couderc et al., 2006). These results are partially in alignment with previous studies in previous experiments where reduced FPS did not influence milk yield in advanced lactating dairy cows (Armentano et al., 1988). It was hypothesized that, variation in total tract digestibility and variable retention time may partially compensate for the differences in nutrient intake and mask the lack of effects on milk yield (Couderc et al., 2006). Therefore, because the quality of the fat-corrected milk yield and the particulate passage rate measured were not impaired by the procedure, moderate genetic merit and high variability among the cows used in these studies may restrict the capacity of the cows to react to minor changes in nutritional intake or failure of the statistical model to detect variations in the experiment. Similarly, 16 mid-lactation Holstein dairy animals designated in a 4 × 4 Latin square design with a 2 × 2 arrangement were fed two distinctive chop lengths (shorter = 10 mm or longer = 19 mm) of alfalfa silage and corn silage for a time of 21 days where treatments had no impact on milk yield (Bhandari et al., 2007). The absence of an effect of the FPS of alfalfa silage and corn silage on milk yield resembles earlier studies (Krause et al., 2002b) where alfalfa silage and corn silage exhibited no effect on milk yield. Similarly, corn silage at one-half milk-line stage of maturity and at 0.95-cm theoretical length of cut without processing (control) or 0.95-, 1.45-, or 1.90-cm theoretical length of cut with processing at a 1-mm roll clearance had no effect on milk yield (Bal et al., 2000).

Microbial protein synthesis

Increasing peNDF content of diets decreased microbial protein synthesis (Li et al., 2020). Two principal factors impact ruminal digestion of forages, i.e., FPS and level of feed consumption (Firkins et al., 1986). Processing forages usually decreases ruminal fluid-phase dilution rate (D) but elevates particulate D (Weston and Hogan, 1967) and decreases extent of ruminal digestion (Blaxter et al., 1956; Hogan & Weston, 1967). Accelerating feed consumption also results in quicker D (Grovum and Williams, 1977). Accumulative D of particulate or fluid digesta may aggravate efficiency of microbial protein synthesis (MPS) (Bergen et al., 1980). Consequently, FPS and, feed consumption may also affect efficiency of MPS. Studies evaluating effects of feed intake and forage processing in the same experiment (Alwash and Thomas, 1974; Beever et al., 1972) have indicated depressions in ruminal digestion of organic matter (OM) and fiber because of decreased ruminal retention times related with increased feed intake or smaller forage particle size. For ground hay, the digestibility of ADF in the rumen was lower but was partly accounted for by improved digestion of ADF in the hindgut. The duodenum feed flow plus endogenous nitrogen (N) was 37Va and 47Vo on N intake, respectively, with long and field hay. When field hay replaced long hay, MPS efficiency increased by 75Va and post-ruminal N digestion increased by 36Vo. MPS efficiency was positively proportional to the rate of turnover of ruminal solids and inversely related to the rate of liquid dilution. These observations indicate increases in MPS efficacy with either increasing quantities of forage in the diet or improved solid ruminal passage (Rode et al., 1985).

RUMEN PHYSIOLOGY

Rumen pH

Four rumen-fistulated cows were randomly assigned in iso-energetic and iso-nitrogenous diets to study the effects of FPS on rumen pH (Rustomo et al., 2006). Expanding forage particle size at the same time increased the most extreme pH for dairy animals which demonstrated that coarse forage particle size can constrict drops in ruminal pH (Figures 5 and 6). In another experiment, expanding FPS increased eating time and decreased eating rate thus even hardly increased FPS was useful to mitigate decline of ruminal pH while profoundly fermentable carbohydrates were offered (Nasrollahi et al., 2014). Decreasing the FPS of corn silage improved rumen pH at 4-5 h after feeding ranging 6.12 to 6.20 (Bhandari et al., 2008).

Ruminal fermentation

The impacts of, and associations between, dietary grain source and moderate changes in alfalfa hay (AH) particle size (PS) on digestive processes of dairy cows were assessed (Nasrollahi et al., 2012). The results demonstrated that the minor increment of size of AH delayed eating time and improved rumen fermentation, in particular, feeding regimen in barley cereal. In another study, the peNDF substance of dairy cow eating regimens was altered by differing the FPS of alfalfa silage and impacts on ruminal fermentation (Li et al., 2020). Expanding FPS decreased VFA concentration in rumen.

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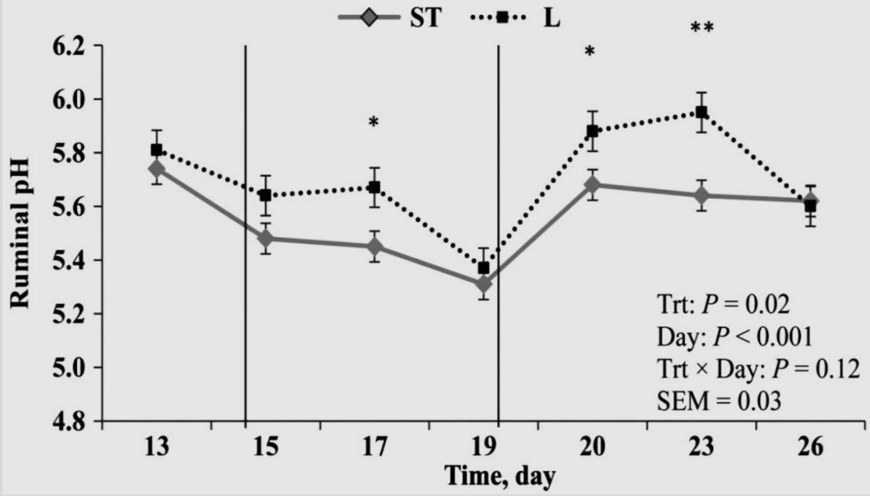

Figure 5 - Effect of rumen challenge on ruminal pH of dairy cows fed short (ST) or long (L) corn silage TMR (Kmicikewycz et al., 2015).

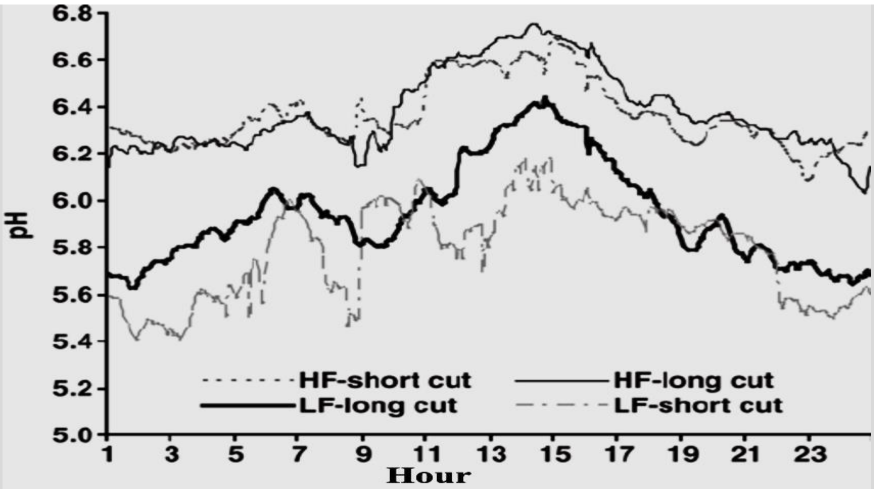

Figure 6 - Effects of forage particle length (short and long cut) on diurnal variation of ruminal pH. Feeding times were 0600, 1500, and 1800 h, and the pH values were recorded every 5 min over a 48-h period (Yang & Beauchemin, 2009).

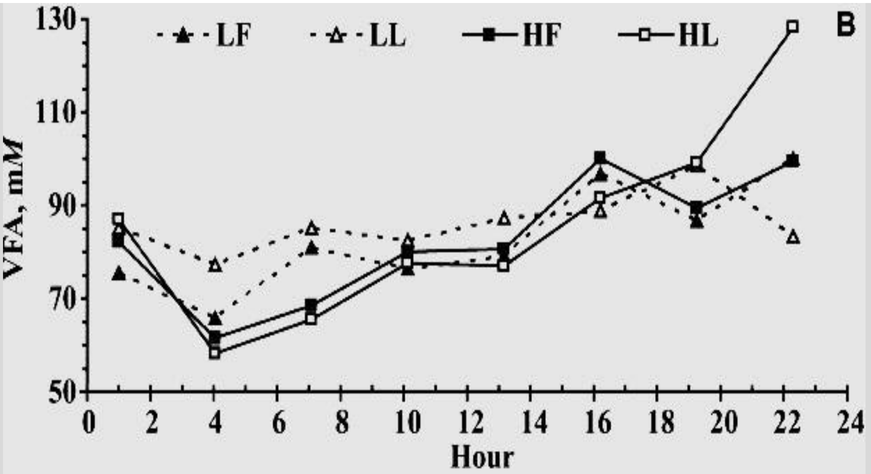

Figure 7 - Relationship between forage particle size and ruminal fermentation (Zebeli et al., 2007). LF = low concentrate level and fine hay; LL = low concentrate level and long hay; HF = high concentrate level and fine hay; HL = high concentrate level and long hay.

In alfalfa silage and corn silage based eating regimen, decreasing the chop length of alfalfa silage increased the concentrations of total VFA and the molar extent of acetic acid derivatives in rumen liquor yet did not influence the molar extents of propionate and butyrate and the acetic acid derivatives to propionate proportion (Bhandari et al., 2007). Indeed, the chop length of corn silage did not influence concentrations of total VFA, the molar extents of VFA, and the acetic acid derivatives to propionate proportion in the rumen. Maybe, lessening the FPS may have increased ruminal site of assimilation and VFA production because of increased surface area for microbial attack (Krause et al., 2002a). Likewise, lessening FPS can decrease saliva production and fluid passage rate (Krause et al., 2002a), in this manner

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increasing the concentrations of VFA in the rumen. Further, a decrease of FPS may likewise decrease VFA production (Figure 7) in the rumen because of increased particulate disappearance rate (Soita et al., 2003).

Like Krause et al. (2002b), Kononoff and Heinrichs (2003) found that a decrease of the FPS of hay silage increased the concentration of total VFA in rumen liquid. In any case, as opposed to this study, in these previous experiments, concentration of propionate increased more than that of acetic acid derivatives, which decreased the acetic acid derivatives to propionate proportion. Studies have detailed inconsistent outcomes on the impacts of the FPS of corn silage on rumen VFA. Similarly, it was found that decreasing the FPS of corn silage increased the concentration of total VFA in the rumen. Unlike VFA, decreasing the FPS of corn silage did not influence the rumen alkali concentration (Bhandari et al., 2007). Nevertheless, Kononoff and Heinrichs (2003) and Beauchemin and Yang (2005) did not find that a decrease of the FPS of alfalfa silage and corn silage influenced the outflow rate of fluid and particulate digesta from the rumen. Similarly, it was found that the FPS of corn silage did not influence rumen VFA (Kononoff & Heinrichs, 2003).

CONCLUSION

Optimum forage particle size ensures maximum dry matter intake, reduced feed sorting, extended gut chewing and rumination activities in dairy cows. It further balances rumen pH, ruminal fermentation, forage degradability, fractional passage kinetics of nutrients and feed effectiveness as a whole. Thus, microbial protein syntheses, body condition score of the host animal, milk yield and milk quality are accelerated. The overall impacts of particle size, however, depend on forage type, forage to concentrate ratio, and fermentability characteristics of the ration. In general, 8-19 mm particle size irrespective of forage type measured on Penn State Particle Separator may be considered optimum for practical dairy feed formulation.

DECLARATIONS

Corresponding Author

E-mail: emran@cvasu.ac.bd

Authors' Contribution

I am the sole contributor of the manuscript.

Conflict of interests

There is no any conflict of interest.

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Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Zakir Hossain Road, Khulshi, Chattogram-4225, Bangladesh.

Animal welfare statement

No moral endorsement was required as this is a review article with no unique exploration of information.

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HISTOLOGICAL FEATURES OF THE ATRIAL MYOCARDIUM AND SCALLOP MUSCLES OF ANGLO-NUBIAN GOAT (*Capra aegagrus hircus*)

Victor KHVATOV and Mikhail SHCHIPAKIN

Saint Petersburg State University of Veterinary Medicine, 5, Chernigovskaya st., St. Petersburg 196084, Russia

Email: shipakin@spsuvm.ru; ORCID: 0000-0002-2960-3222

Supporting Information

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ABSTRACT: This study presents results about features of the structure of the atrial myocardium and scallop muscles of the Anglo-Nubian goat (*Capra aegagrus hircus*), and their morphometric parameters have been determined. Sixteen corpses of Anglo-Nubian goats at the age of twelve months were studied in the Department of Animal Anatomy of the St. Petersburg State University of Veterinary Medicine. The study was carried out by making histological preparations. The atrial myocardium of Anglo-Nubian goats consists of working cardiomyocytes, tightly adjacent to each other, between which capillary blood vessels and single adipocytes are revealed. It has been determined that atrial cardiomyocytes in Anglo-Nubian goats are characterized by heterogeneity of their structure, and secretory granules and perinuclear clearances are found in cardiomyocytes less differentiated in terms of contractile activity. In the present study, the histological structure of the atrial scallop muscles of the Anglo-Nubian goat was identified. Also, the atrial myocardium's working cardiomyocytes' morphometric parameters and its scallop muscles had discovered. The thickness of the endocardium in these sections was measured by determining the diameters of its nuclei. The obtained data indicate that the histological structure of the atrial myocardium and its scallop muscles in Anglo-Nubian goats has possibly similar features in compared with small ruminants from commercial breeds (sheep and goat). These results expand the field of knowledge on pedigree anatomy and histology of the heart in farm animals, particularly in small ruminants.

Keywords: Atrium, Goat, Histology, Myocardium, Scallop muscles.

INTRODUCTION

The myocardium is the heart's main shell, from the functional state of which its contractile ability consists (DiVincenti et al., 2014; Emam and Abugherin, 2019). Knowledge of its macro-anatomy and histological structure is essential and imperative for any practicing veterinarian. Almost any heart disease is accompanied in one way or another by a change in the biochemical structure of the myocardium, thereby causing its pathology (Frandson et al., 2009).

Studies aimed at evaluating the histostructure of the heart myocardium in animals in terms of species and also species expand the area of knowledge of veterinary specialists, which allows the most accurate and individual surgical and therapeutic treatment of animals with cardiac pathologies, which is more important issue in farm animal veterinary (Buczinski et al., 2010).

Anglo-Nubian goats (*Capra aegagrus hircus*) are distinguished by their high body weight characteristics, have an excellent milk yield level, and are also valued for the quality of dairy and meat products in Russian Federation (Kuga, 2012). The atrial myocardium in farm animals includes two layers - superficial and deep, connected by tight muscle anastomoses (Kuga, 2012). According to König and Libich (2004), the atrial myocardium's superficial layer in small and in cattle it is circular and common to both the right and left atria, and the circular layer is located from one ear to the other. According to Kozlovskaya, (2014), Ateş et al. (2017), and Emam and Abugherin (2019), the second layer of the atrial myocardium is deep, and its muscle fibers have an oblique-longitudinal direction. This layer is individual for each atrium and starts from the fibrous rings in the atrioventricular opening area and ends in the superficial layer's muscle fibers.

The atria in ruminants from the inside have the appearance of a sponge due to the scallop muscles, which contribute to the complete squeezing of blood during the chamber's contraction. The scallop muscles are absent in the interatrial septum (Maximilian Buja, 2007). According to Huxley and Haselgrove (1977) his number of scallop muscles on the inner surface of the heart of animals and humans is relatively constant, and the intervals between them decrease in the direction from the base of the ear to its apex.

The scallop muscles in domestic animals are divided into muscles of the first and second order, the first of which has a large diameter and are located perpendicularly and slightly obliquely to the ear's longitudinal axis (Hooper and Thuma, 2005). The second ones depart at an angle from the first order's muscles and are significantly inferior in diameter. In cattle, the scallop muscles are of moderate severity (Vansyatskaya and Kirpaneva, 2014).

In the studied literature, there was not any completive and strength information on the atrial myocardium's histological structure and their scallop muscles in small ruminants, namely, in Anglo-Nubian goats. Thus, this study's

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purpose was to investigate and evaluate the histological structure peculiarities of the atrial myocardium and their scallop muscles of an Anglo-Nubian goat and establish the morphometric data of the histological structures of the scallop muscles and cardiac muscle of the atria.

MATERIALS AND METHODS

There were used dated materials for the study obtaining during slaughter from the farm "Gzhel Podvorie" in the Russian Federation's Moscow region. The dated material was presented by sixteen corpses of Anglo-Nubian goats older than one year.

The material was fixed in a 10% solution of neutral formalin for 24 hours (Gushchin and Muzhikyan, 2014), after which it was embedded in paraffin according to the generally accepted technique (Muzhikyan et al., 2014). Then sections with a thickness of 3-5 μm were made, stained with hematoxylin and eosin. According to McManus, some of the sections were stained with Trichrome according to Masson, toluidine blue, and Schiff-iodic acid. The histological preparations analysis was carried out using a Carl Zeiss Axioskop 2 Plus light-optical microscope (Germany) at a magnification of 40, 100, 400, 1000. Microphotography was performed using a digital camera (CarlZeiss Axio Cam ERc5s, Germany) and the AxioVision 4.8 software. Morphometric measurements were performed manually using the AxioVision 4.8 software (Image J, Germany).

Statistical analysis

The variational-statistical processing of the research results was carried out on IBMPC / AT and "Pentium IV" in Windows 2000, using the data analysis package in the program "Excel Windows Office XP" and "Statistika 6.0" (Statsoft, USA) with the calculation of the arithmetic mean and its standard error (M ± m). In the statistical analysis of the data obtained, the Student's t-test was used for independent samples, while the differences were considered significant at $p<0.05$. All anatomical and histological terms correspond to the "International Veterinary Anatomical Nomenclature," fifth edition, translation and Russian terminology of Zelenevsky (2013) and International histological nomenclature edited by Semchenko et al. (1999).

Ethical regulation of study

This research was conducted in accordance with tin accordance with ethical standards for the use of animals for scientific purposes, referred to Federal Law of 24.04.1995 N 52-FL (as amended on 08.12.2020) "On the animal world". The main research technique was chosen to study the microstructure of the atrial myocardium and scallop muscles - the production of histological preparations for sampling the material under study on fresh hearts of Anglo-Nubian goats. The material was taken by the method of fine anatomical preparation (Minutes No. 134 dated 09/15/2020).

RESULTS AND DISCUSSION

The atrial myocardium of an Anglo-Nubian goat is formed by tightly adjacent contractile (working) cardiomyocytes, forming long multidirectional strands (Figures 1 and 2). A more tortuous course of muscle fibers and wider gaps between them are noted in the studied animals than in the interventricular septum. The contractile cardiomyocytes in this section are elongated. The cell thickness varies in the range of 16-20 microns and averages 17.6 ± 1.5 microns. The area of cells on a cross-section averages 294.5±31.6 $μm^2$. In general, in this part of the heart, cardiomyocytes are characterized by structural heterogeneity; in cells less differentiated in terms of contractile activity, perinuclear clearances and secretory granules are determined, which are not found in cardiomyocytes of the interventricular septum (Figure 3). The nuclei of cells are oval, slightly elongated, normochromic, with a uniform distribution of small-lumpy chromatin. In some of the cells, the nuclei are elongated, flattened, moderately hyperchromic. The average values for a large core diameter are 10.1 ± 1.1 μm, for a small one, 4.3 ± 0.4 μm. The average area of the nuclei of contractile cardiomyocytes varies within 40-48 $μm^2$ and averages 42.7 ± 3.2 $μm^2$.

According to Masson, staining with trichrome between the muscle elements of the myocardium, layers of loose, small-cell, unformed connective tissue of different thickness, containing capillary-type blood vessels and single small groups of adipocytes, are revealed (Figures 4 and 5). Along the periphery, thin cords of collagen fibers pass into a loose, unformed connective tissue of the endocardium, covered with one layer of flattened endothelial cells (Figure 6). The endocardium thickness in this section varies within 13-25 microns and averages 17.2 ± 2.2 microns. The nuclei of endothelial cells are predominantly oval and elongated. The large and small diameter of the endothelial cell nucleus averages 7.8 ± 1.5 and 4.8 ± 0.6 microns.

The scallop muscle in the Anglo-Nubian goat is formed by tightly adjacent contractile (working) cardiomyocytes, forming long multidirectional cords (Figures 7 and 8). The contractile cardiomyocytes in this section are elongated. The cell thickness varies within 12-24 microns and averages 18.9 ± 1.6 microns. The area of cells on a cross-section averages 278.3 ± 24.1 $μm^2$. The nuclei of cells are oval, slightly elongated, normochromic, with a uniform distribution of small-lumpy chromatin. In some of the cells, the nuclei are elongated, flattened, moderately hyperchromic. The average values for the large diameter of the nucleus are 12.7 ± 1.3 μm, for the small diameter - 4.2 ± 0.4 μm. The average area of the nuclei of contractile cardiomyocytes varies within 40-45 $μm^2$ and averages 42.9 ± 3.2 $μm^2$.

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Table 1 - Morphometric data of the histological structures of the atrial myocardium and their scallop muscles in Anglo-Nubian goats, μm.

Heart histostructure / Localization	Atrial myocardium	Atrial comb muscles
Thickness of working cardiomyocytes	17.6±1.5	18.9±1.6
Average area of working cardiomyocytes, μm^2	294.5±31.6	278.3±24.1
Average area of nuclei of working cardiomyocytes, μm^2	42.7±3.2	42.9±3.2
Large diameter of the nuclei of working cardiomyocytes	10.1±1.1	12.7±1.3
Small diameter of the nuclei of working cardiomyocytes	4.3±0.4	4.2±0.4

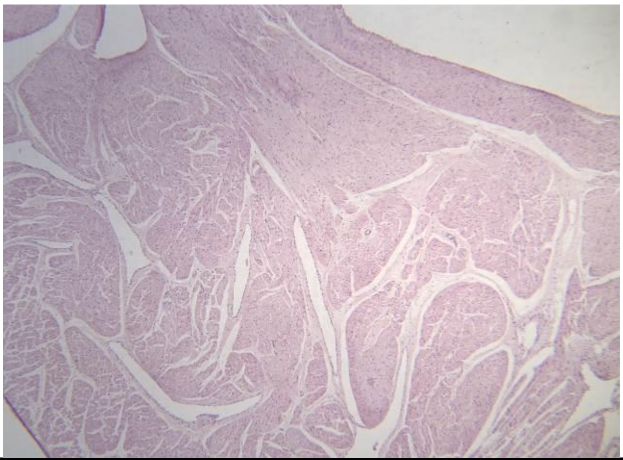

Figure 1 - Atrial myocardium. Hematoxylin-eosin staining. Magnification 40.

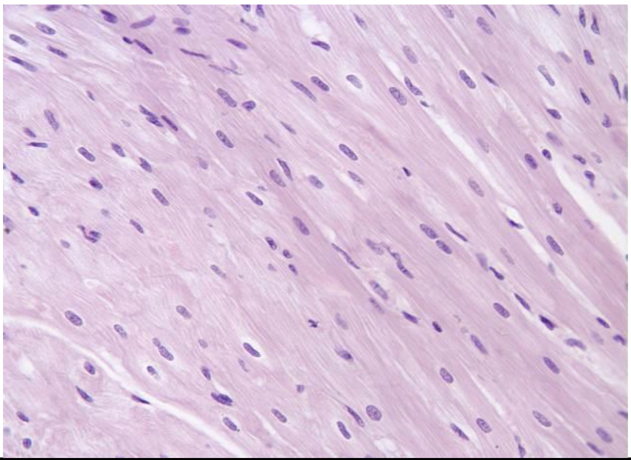

Figure 2 - Atrial myocardium. Cords of contractile (working) cardiomyocytes of typical structure are determined. Hematoxylin-eosin staining. Magnification 400.

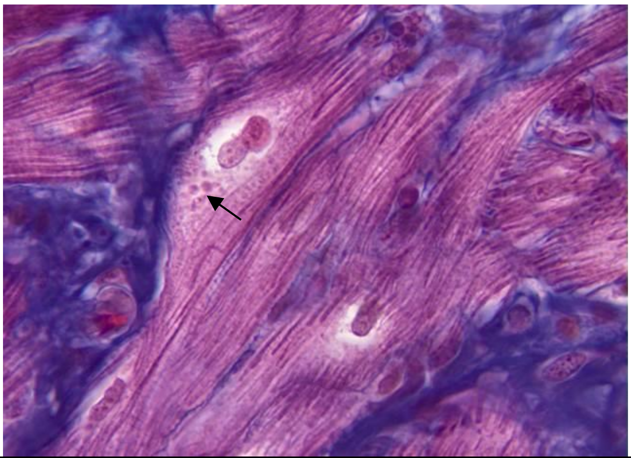

Figure 3 - Atrial myocardium. Poorly differentiated binucleated cardiomyocytes containing characteristic secretory granules (arrow) are revealed. Masson's trichrome staining. Magnification 400.

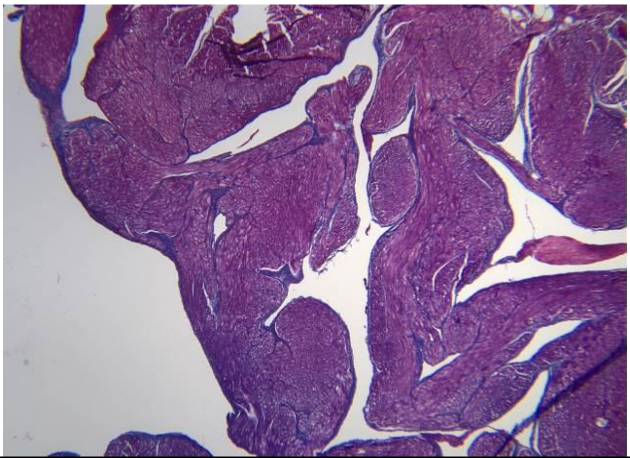

Figure 4 - Atrial myocardium. Masson's trichrome staining. Magnification 40.

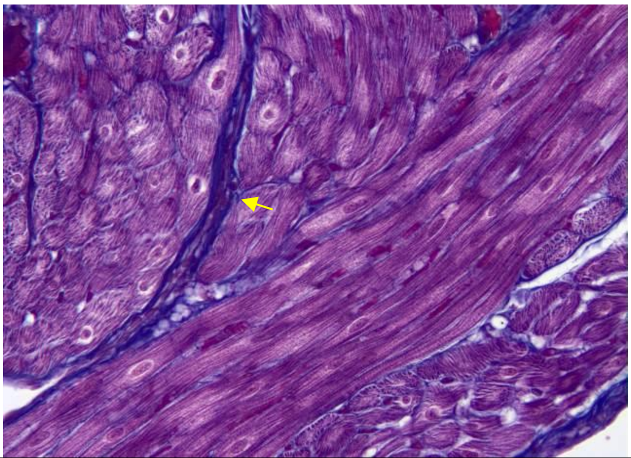

Figure 5 - Atrial myocardium. Cords of cardiomyocytes are separated by connective tissue layers stained with a blue dye (arrow). Masson's trichrome staining. Magnification 400.

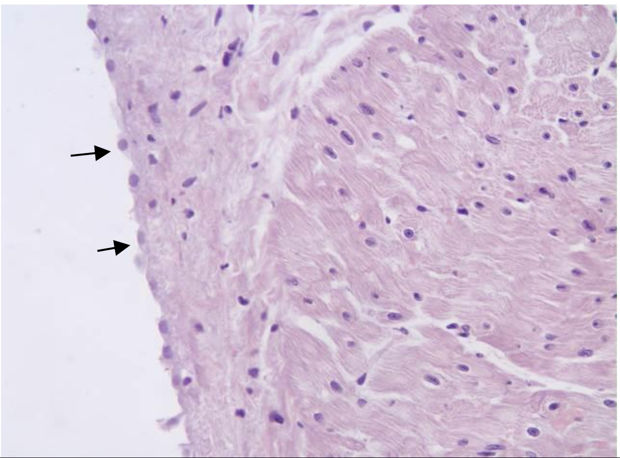

Figure 6 - Atrial myocardium. The strands of contractile (workers) are determined. The endocardium is lined with one layer of endothelial cells (arrows). Hematoxylin-eosin staining. Magnification 400.

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When staining with Masson's trichrome, between the muscle elements of the scallop muscle's myocardium, layers of loose, small-celled unformed connective tissue of varying thickness, containing capillary-type blood vessels and single small groups of adipocytes, are revealed (Figures 9 and 10). Along the periphery, thin cords of collagen fibers pass into a loose, unformed connective tissue of the endocardium, covered with one layer of flattened endothelial cells. The endocardium thickness in this section varies within 15-30 microns and averages 21.6 ± 3.5 microns. The nuclei of endothelial cells are predominantly oval and elongated. The large and small diameter of the endothelial cell nucleus averages 7.3 ± 0.5 μm and 3.1 ± 0.3 μm (Figures 11 and 12).

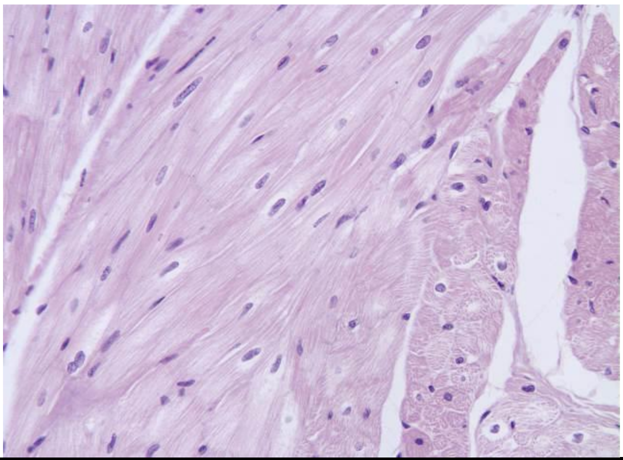

Figure 7 - The scallop muscle. The multidirectional strands of contractile (working) cardiomyocytes are determined. Hematoxylin-eosin staining. Magnification 400.

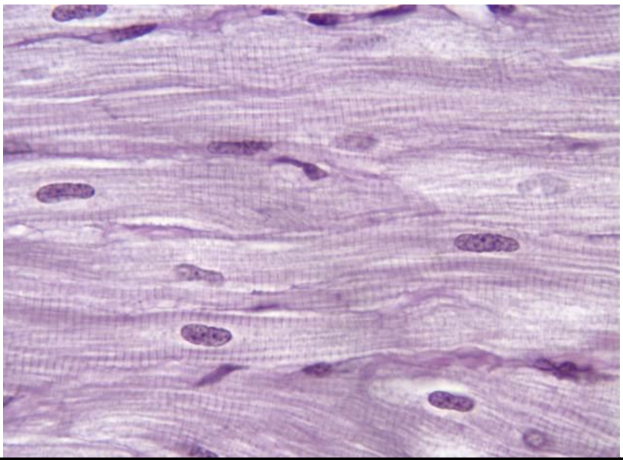

Figure 8 - The scallop muscle. Multidirectional strands of contractile (working) cardiomyocytes with a clearly expressed transverse striation are determined. Staining with Schiff-iodic acid according to McManus. Magnification 1000.

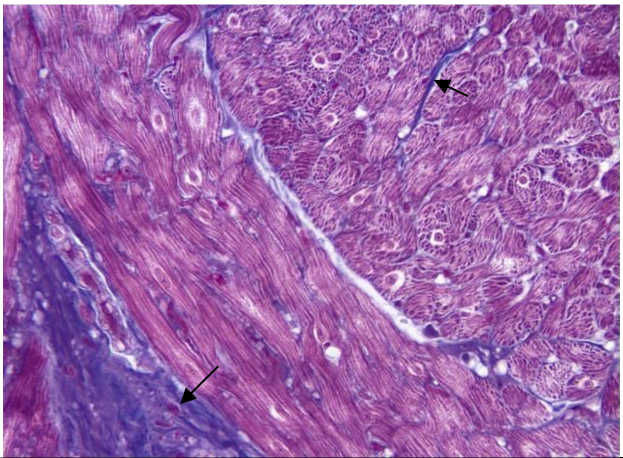

Figure 9 - The scallop muscle. Cords of cardiomyocytes are separated by connective tissue layers stained with a blue dye (arrow). Masson's trichrome staining. Magnification 400.

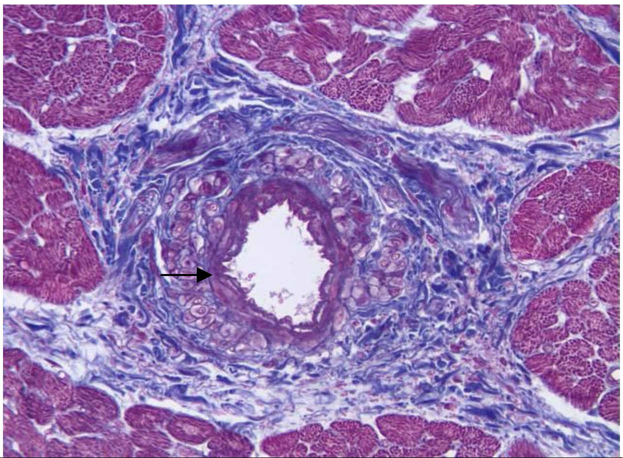

Figure 10 - The scallop muscle. In the thickness of the myocardium, a blood vessel (artery) of the muscle type (arrow) is determined, surrounded by loose connective tissue. Masson's trichrome staining. Magnification 400.

Figure 11 - The scallop muscle. Designations: endocardium (1), myocardium (2). Hematoxylin-eosin staining. Magnification 100.

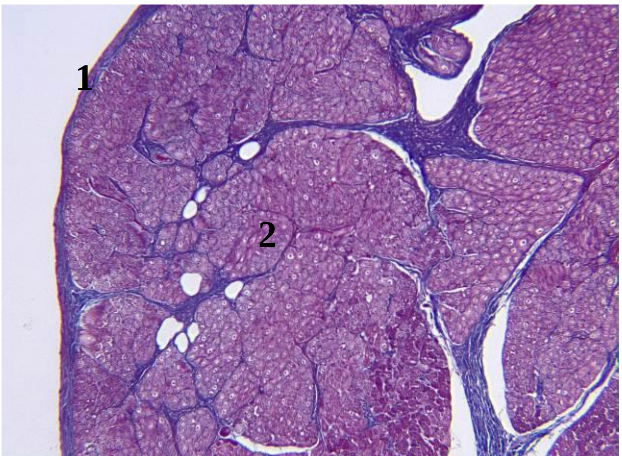

Figure 12 - The scallop muscle. Designations: endocardium (1), myocardium (2). Masson's trichrome staining. Magnification 100.

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In some of related studies (Crick et al., 1980; Frandson et al., 2009; Emam and Abugherin, 2019), there was no contradict the data on the histology of the atrial myocardium in the report, and this study confirms these results. Findings of present study was in agreement with Endo et al. (1995) and Calkins et al. (2008) which identified the anatomy and histology of the atria of domestic and wild animals' hearts, and had same results on the scallop muscles' histology.

CONCLUSION

It was found that the atrial myocardium of Anglo-Nubian goats consists of working cardiomyocytes, tightly adjacent to each other, between which capillary-type blood vessels and single adipocytes are detected. As well as, atrial cardiomyocytes in Anglo-Nubian goats are characterized by heterogeneity of their structure. In less differentiated, in terms of contractile activity, cardiomyocytes, secretory granules, and perinuclear clearances are identified and reported. The histological structure of the scallop muscles was identified. It was determined that these heart structures consist of working cardiomyocytes, which in their structure are similar to the atrial myocardium. The morphometric parameters of the working cardiomyocytes of the atrial myocardium and its scallop muscles were identified in Anglo-Nubian goats. The data obtained indicate that possibly the histological structure of the atrial myocardium and its scallop muscles of Anglo-Nubian goats has similar features inherent in goat or sheep species (there in no breed-specific variations). These results expand the field of knowledge on pedigree anatomy and histology of the heart in farm animals, particularly in small ruminants. The established morphometric parameters of the atrial myocardium and scallop muscles can be used as a theoretical basis by veterinary histologists, embryologists, and pathologists in the course of diagnosing heart pathologies in Anglo-Nubian goats.

DECLARATIONS

Corresponding Author

Email: shipakin@spsuvm.ru

Authors' Contribution

V.Kh. conceived of the presented idea and developed the theory and performed the computations. M.Sh. verified the analytical methods to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF BROILER LITTER KEPT AT DIFFERENT ALTITUDES

Cahya Setya UTAMA* and Marry CHRISTIYANTO

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro. Jl. Prof. H. Soedarto, S.H, Semarang City, Central Java 50275, Indonesia

Email: cahyasetyautama@gmail.com; ORCID: 0000-0002-96729579

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ABSTRACT: The research objective was to assess the chemical and microbiological properties of broiler litter raised in broiler closed house at different altitudes. The design used was a completely randomized design with 3 treatments and 5 replications. The treatments consisted of T1 (broiler closed house at an altitude of ≤100 meters above sea level (masl), T2 (broiler closed house at an altitude of 100 - 300 masl) and T3 (broiler closed house at an altitude of 300 - 500 masl). The results showed that the difference in the altitude where broilers were raised had no significant effect on moisture, ash, extract ether (EE), crude fibre (CF), nitrogen free extract (NFE), total digestible nutrients (TDN), cuprum (Cu), lead (Pb), mercury (Ag), lactic acid bacteria (LAB), *Salmonella, E. coli, Clostridium sp.,* antibiotic contamination (below the threshold) and predominance of gram-positive bacteria. The results of water content 22.71-24.65%, crude protein 13.13-13.47%, Pb 35.15-47.80 ppm, Cu 102.46-136.96 ppm, LAB 3.48 - 7.00 × 10^6 cfu/g. In conclusion, the differences in the altitude did not affect the chemical and microbiological properties of broiler litter.

Keywords: Altitude, Bacteria, Chemical properties, Litter, Poultry.

INTRODUCTION

The increase in demand for broiler chicken meat is in line with the increase in livestock business. The increase in demand for broiler chicken meat in Central Java, Indonesia in 2019 was around 13,000 tons (Statistics of Central Java, 2019). Broiler chicken farming industry produces waste in the form of litter and can be a cause of environmental pollution (Dunlop et al., 2016; Seidavi et al., 2019). Efforts to reduce poultry waste can be done by processing the waste into materials with higher economic values (Sahoo et al., 2017).

The macroclimate and microclimate conditions of broiler house are one of the determining factors of success in the world of animal husbandry (Kic, 2016). The macroclimate and microclimate conditions of broiler house are affected by the altitude of the region where the broiler farm is built (Nazareno et al., 2016; Dróżdż et al., 2020). Extreme temperatures can be stressful for livestock and therefore affecting livestock productivity (Henry et al., 2018).

In general, the optimum temperature for the growth of broiler chickens ranges from 18-21 °C, and those highland areas have lower temperatures than lowland areas (Vilchis et al., 2012). Farm management is the key to successful production of broiler chickens; one of the efforts to improve management is the use of pedestals and chicken warmers in the form of litter (Wang et al., 2016). Traditionally, the litter can be prepared from rice straw, husks, or sawdust (Garcia, 2007). The constituent composition of litter is manure, chaff and limestone. Litter contains 9-11% protein, 91-94% dry ingredients, 11-50% crude fiber and 1-3% crude fat (Rahimi et al., 2018). Good quality litter will not be the growth medium for parasite development (Najibulloh et al., 2020). Good litter has the characteristic that the water content is 20-25 percent, capable of well absorbing water, does not contain harmful materials and is dust-free (Petek et al., 2014).

The study aimed to examine the chemical and microbiological qualities of broiler litter raised in broiler closed house at different altitudes. It is expected that the study results will provide preliminary information on the quality of broiler litter obtained at different altitudes so that it can be used as the basis for the processing of litter into alternative feed materials.

MATERIALS AND METHODS

Materials

The material used was litter of 15 broiler closed house of PT. Citra Unggas Lestari located in Demak Regency (altitude ≤ 100 meters above sea), Semarang city (altitude 100-300 meters above sea) and Kendal Regency (altitude 300-500 meters above sea level).

Methods

The research began with litter sampling of broiler chickens from 15 partnership cages PT Citra Unggas Lestari. Sampling is done purposive random sampling that can represent the percentage of area and capacity of the cage. The

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research design used is complete randomized design (CRD) with 3 treatments and 5 repeats. The treatment given is T1 (closed house cage at an altitude of ≤100 meters above sea level), T2 (closed house cage at an altitude of 100-300 meters above sea level) and T3 (closed house cage at an altitude of 300-500 meters above sea level).

Research parameter testing

Proximate analysis

Proximate analysis includes testing water content, ash, crude protein, crude fat, crude fiber, calculation of extract materials without nitrogen and TDN (AOAC, 2005). Water content analysis is done using drying method with oven. Analysis of ash content is done by the method of smoking using *muffle furnace* (AOAC, 2005). Analysis of crude protein levels using the Kjeldahl method (AOAC, 2005). Crude Fat level analysis is done by ether extraction method (AOAC, 2005). Analysis of crude fiber levels using a method of boiling with a solution of weak acids and weak bases (AOAC, 2005). NFE levels are calculated using a formula (Alagbe et al., 2020) namely:

NFE = 100 - (Ash + Crude Fat + Crude Fiber + Crude Protein)

The calculation of total digestible nutrients is calculated by formula (AOAC, 2005) namely:

TDN = % Crude Fiber digestible + % NFE + % Crude Protein + 2.25% Crude Fat

Metal content testing Pb

Testing of pb metal content in the sample was done using Atomic Absorption Spectophotometer (AAS) method with different wavelength principle for each type of metal (Oliviera et al., 2017). Absorbance measured at a wavelength of 283 nm using AAS variant AA240 Australia and calculated the concentration of lead metal (Pb) using lead metal concentration equation (Pb) as follows (AOAC, 2005).

(μg/g) = "C x VC x V" / "W W"

Description:

C = concentration of mg/L (ppm) measurement results converted into units of μg/L (ppb)

V = total sample volume (mL) converted to liter unit (L)

W = sample weight (g)

Cu content testing

Testing of Cu content in litter samples is done by AAS method (Gu et al., 2020). The pre-prepared sample was measured with an AAS at a wavelength (λ) of 324.8 nm to determine its concentration.

Mercury testing

Mercury test was conducted using qualitative and quantitative analysis of mercury using atomic absorption spectrophotometer method (Pasinszki et al., 2020). The tools used are mercury analyzer.

Antibiotic contamination analysis

Antibiotic contamination test is done using the method of screening tester bioassay (Jonkers et al., 2020). Testing using special tools such as HPLC (high pressure liquid chromatography), TLC (thin layer chromatography) and GC *(Gas Chromatography)* to find out the type of antibiotic compounds quantitatively (Mahmood et al., 2019).

Total lactic acid bacteria analysis

Determination of the number of total lactic acid bacteria (LAB) colonies of each litter sample is measured using the total plate count (TPC) method (Kwak et al., 2008). Colonies that grow stem-shaped, have positive grams, not form spores, are non-motile, are anaerobic, catalase negative and oxidation positive. Then the LAB population is calculated as follows:

LAB Population (cfu/g) = Number of Colonies x Dilution

Gram positive and negative bacterial analysis

Testing gram positive and gram negative is done by pour method (pour plate) followed by gram staining method (gram staining method) (Rajivgandhi et al., 2018). Observations are carried out under a microscope with a magnification of 1000x, observed the shape of the cell and its color. If the bacteria is pink belongs to the gram negative group.

Salmonella content testing

Salmonella content testing using carter method (Andoh et al., 2017).

Escherichia Coli content testing

Identification of *E. Coli content* is done by Most Probable Number (MPN) method (Ahmed et al., 2015).

Clostridium sp content testing

Clostridium sp content testing is done by Total Aerobic Count method (Angelotti et al., 1961).

Statistical analysis

The data obtained was analyzed using analysis of variance (ANOVA) test to test data diversity and if there is any real influence, it will be continued with Duncan's multiple range test (DMRT) at a 5% confidence level.

Ethical approval

The *in vitro* experiment did not need ethical approval from the animal ethics committee.

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RESULTS AND DISCUSSION

Proximate composition and TDN of litter from broiler chickens raised at different altitudes

The results of the analysis indicate that the altitude of the various cage locations, namely T1 (altitude of 0-100 meters above sea level), T2 (altitude of 100-300 meters above sea level) and T3 (altitude of 300-500 meters above sea level)) did not have a real impact on the content of litter water (P>0.05).In the broiler litter, the total water content was 9.2 %. Owen al. (2008) noted that in broiler house with a height of 120 meters above sea level, litter in the broiler chicken had a water content of 12-25%. Qian et al. (2018) documented that broiler house at low altitudes had high temperatures that cause the chickens to consume more water so that the excreta released was moist. As the litter in all broiler houses came from the same substance, namely husks, the moisture content of the litter was not largely different. Ritz et al. (2017) stated that a suitable litter has a characteristic that the water content ranges from 20-25%, able to absorb water well, does not contain harmful materials and is free from dust. The effect of altitude also does not affect the water content of litter because the type of litter source cage is the same, namely closed house cages. *Closed house* has a mechanism to maintain litter quality in order to remove excess moisture in the cage. This is thought to result in the litter water content produced in this study, which is not influenced by the altitude of the location. Kaukonen et al. (2016) stated that a good litter condition can be measured through the water content parameter; the optimal litter water content indicates that the litter condition is good.

The findings of the present study revealed that different altitudes of broiler house had no significant (P>0.05) effects on the ash content of litter. In broiler chicken litter, the total ash content was 24.11%. Barnes et al. (1997) reported that in broiler litter the average ash content value is 20-25 percent. In general, there are several factors affecting the ash content in broiler litter, which are the type of litter material as well *as* the altitudes of broiler house located. Pappas et al. (2010) stated that litters prepared from the materials such as wood shavings have higher absorption and have more organic content. The altitude of the location did not affect the litter ash content, presumably because there was no difference in maintenance management from each study cage. The ash content in the litter is influenced by the mineral composition of the excreta and husks, so that no differences in the mineral content have an impact on the ash content of the litter which is not different either. Owen et al. (2008) stated that the mineral content in raw materials is influenced by mineral content, because the ash content describes the amount of mineral substances that do not burn into steam.

It was shown in this study that the differences in the altitudes of location of broiler houses showed no substantial effect on the content of crude protein litter (P>0.05). Crude protein content in litter has an average value of 13.31%. Cross (1995) stated that the crude protein content in poultry chicken litter is 11-15%. The high protein content in litter is caused by ammonia. Ferguson et al., (1998) states that the protein content in litter is still relatively high due to the presence of ammonia formed from the decomposition of nitrogen by microbes and the amount of nitrogen that is not absorbed by chickens so that it is released along with substances that are not absorbed by the body, namely excreta. This is also the reason why the elevation of the location does not affect the protein litter levels. The relatively same cage management makes no difference in ammonia in litter, so that the height of the location does not affect the litter protein content.

In this study, the altitudes of location of broiler houses had no effect on the content of Crude fiber of broiler litter (P>0.05). The average crude fiber content in broiler litter was 23.34%. Stephenson et al. (1990) stated that the fiber content in poultry manure is 18-69%. The factor that causes high crude fibers in litter is the constituent component of fibers, namely lignin. Knudsen (2014) states that high fiber content is caused by lignin content as the main component of plant tissue formation. The altitude of the location does not affect the litter crude fiber content produced. The main fiber content of litter comes from rice husks, so that the fiber measured in litter is a large contribution from rice husk fiber. The cages for the source of research litter used the same base, namely rice husks, so that there was no effect of height differences on litter fiber content. The altitudes of location of broiler houses had no substantial effect on crude fat content of broiler litter. The altitude of the location does not affect the litter fat content. This can happen presumably because the litter water content is not significantly different from the location height treatment. Pappas et al. (2010) stated that the high and low levels of fat are influenced by the moisture content of the material, the amount of fat is calculated to be greater in the proximate analysis, it can occur if the water content that comes out of the material is higher. The average content of Crude fat in chicken litter was 1.72%.

The results of the analysis showed that the altitude of different cage locations, namely T1 (cage altitude 0-100 meters above sea), T2 (cage altitude 100-300 meters above sea level) and T3 (altitude 300-500 meter above sea) had no real effect on the content of NFE Digestibility litter (P>0.05). The absence of the influence of the location height treatment on the crude protein and crude fiber, the cause of the NFE litter value is also not affected by the location height treatment. The average content of NFE Digestibility litter chicken is 37.54. Caswell et al. (1978) stated that NFE digestibility content in chicken litter is 26.1%. The value of nitrogen free extract ingredients is determined by other nutrient levels. Alam et al. (2008) stated that NFE digestibility value is influenced by the ups and downs of other levels such as crude fiber, crude protein on the material.

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The results of the analysis showed that the different altitudes of broiler house locations, namely T1 (cage altitude 0-100 meters above sea), T2 (cage altitude 100-300 meters above sea) and T3 (altitude 300-500 meters above sea level) showed no real effect on the content of crude fat litter (P>0.05). TDN was not different because other proximate contents such as moisture content, ash content, crude fiber, crude protein, crude fat and NFE were not affected by the altitude of the location. This results in the altitude of the location not giving different results to the TDN litter. Alam et al. (2008) stated that TDN is digestible energy derived from crude protein, crude fiber, crude fat, and NFE. The average content of chicken litter TDN was 44.86%. Bagley et al. (1996) stated that the content of chicken litter TDN is 60%. The TDN value of chicken litter is influenced by the fiber content of the feed. Utama et al., (2019) states that the higher the crude fiber feed, the smaller the digestibility of the feed.

Contamination of Pb, Cu, Mercury and antibiotics of litter from broiler chickens raised at different altitudes

Table 2 points to the results that the average Pb content in chicken litter was 42.14 ppm. Uchimiya et al. (2012) states that the average Pb content in the chicken litter was 38 ppm, which indicated that the level was still in normal condition. T1, T2, and T3 treatments showed no real different results. The factor that affects Pb levels in the cage is the altitudes of the cage area. Uchimiya et al. (2010) states that the use of litter in cages is determined by moisture an area, if the area has a high moisture then it can be ascertained the addition of litter so that the soil element in litter more and more.

Table 2 also shows that the average content of Cu in broiler chicken litter was 118.66 ppm. Codling et al. (2008) stated that in broiler chicken manure has an average content of Cu of 332 ppm which is still classified as normal and when consumed ruminant livestock will not dissolve in the bloodstream and do not settle on the organs of livestock. The T1, T2 and T3 treatments showed no significant different results. The factor that affects the value of Cu is the humidity of the excreta. According to Hoeven (2014) that moisture in excreta can affect the content of Cu in broiler litter. High humidity in the high altitude can cause excreta to get wet. According to Santos et al. (2020), the height of the altitudes, which is a part of the macro environment, is related to the conditions of the broiler house microclimate. When the land level of the cage is low, the humidity in the cage is high. Environmental variables are another aspect that influences levels of Cu. In contrast to regions with higher altitudes caused by soil elements in high areas, dissolved and settled in low areas.

Based on Table 2, that mercury content in chicken litter is low. The value of mercury in the litter of broiler chickens used ranges from 0-0.3 ppm. Malone and Chaloupka (1983) argues that mercury levels range at least 0.03-0.79 ppm, while at 0.3-0.5 ppm mercury content begins to be high. According to SNI: 7387 (2009), mercury content threshold is 0.005 ppm. Mercury levels can be overcome by some microorganisms such as *pseudomonas fluorescens* bacteria (Gupta and Kelly, 1992). Table 2 shows that no antibiotic residues were found in the sample chicken litter. This shows that farmers already understand the importance of discontinuing antibiotic administration and administering antibiotics according to dosage of Sun et al. (2014). According to SNI No: 01 – 6366 (2000), tetracycline antibiotic content should not exceed 0.1 Mg/Kg.

Table 1 - Proximate composition and TDN litter broiler chickens kept at different altitudes.

Parameters	Treatment (%)		
	T1	T2	T3
Water content	22.71±0.64	24.65±0.80	22.85±0.22
Ash content	25.55±3.51	22.79±1.36	23.98±1.23
Crude protein	13.13±1.69	13.33±2.47	13.47±1.16
Crude fibers	24.31±5.28	24.94±8.03	20.77±1.81
Crude fat	2.47±1.95	1.28±0.81	1.41±0.74
Nitrogen free extract (NFE)	34.54±3.92	37.65±6.29	40.37±3.39
Total digestible nutrients (TDN)	43.25±6.75	43.76±10.02	47.57±2.81

Table 2 - Content of Pb, Cu, Mercury and antibiotics of litter from broiler chickens raised at different altitudes.

Parameters	Treatment (ppm)		
	T1	T2	T3
Pb	43.49±16.67	35.15±9.62	47.80±19.61
Cu	102.46±21.03	136.96±38.06	116.58±24.35
Mercury	0.001	0.001	0.001
Antibiotics	0.001	0.001	0.001

Non-significant mean (P<0.05) on all treatments.

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Table 3 - Microbiological quality of broiler chicken litter maintained at different altitudes.

Parameters	Treatment (cfu/g)		
	T1	T2	T3
Total LAB	7.00×10^6	3.48×10^6	5.46×10^6
Gram +	Stem, Solitary	Stem, Solitary	Stem, Solitary
Gram -	Not Found	Not Found	Not Found
Salmonella	0	0	0
E. Coli	0	0	0
Clostridium sp	Negative	Negative	Negative

Microbiological quality of broiler chicken litter maintained at different altitudes

The population of lactic acid bacteria in litter is an indicator of the microbiological quality of the material. The results of the variety analysis showed that the height of different places had no real effect on the total LAB litter. Total LAB observations ranged from 3.48×106 – 7.00×106 cfu/g. The environment suitable for LAB life includes temperature, potential hydrogen (pH) and nutrient content. Too high temperatures will damage the proteins that support bacterial life and cause bacteria to die (Kwak et al., 2008). Too low temperature will result in LAB dormant and not growing. Lactic acid bacteria have an optimal temperature range of 40– 45°C and can live at pH 4 - 6.5 (Adamberg et al., 2003).

The results of identification of the presence of gram-positive bacteria are rod-shaped and solitary. One type of bacteria that grows in litter is a type of gram-positive bacteria that is *Lactobacillus Sp* which is LAB. Lactic Acid bacteria is a bacterium that produces lactic acid that is able to inhibit the growth of gram-negative bacteria. Kwak et al., (2008) states that gram-negative bacterial permeability can be weakened by lactic acid by damaging the outer membrane of gram-negative bacteria.

Test results on litter samples showed no *salmonella* found results on litter. *Salmonella* is a type of bacteria that is pathogenic (Wiedemann et al., 2015), and according to SNI 7388 (2009) the safe limit of *Salmonella* content is less than 25 g. Litter may contain *Salmonella* derived from chicken excreta infected with *Salmonella* then mixed with litter. The impact of *Salmonella* bacterial infection on livestock can lead to Salmonellosis disease. One way to reduce *salmonella* content in an ingredient is by heating at a temperature of about 80^0C (Kim et al., 2012). Investigation on the litter sample obtained no results of *Escherichia coli* found on the sample. *E. coli* are bacteria that include pathogens for humans and livestock. According to SNI 7388 (2009) the threshold of *Escherichia coli* infection is 10 g. *E. coli* in litter comes from excreta infected with *E. coli*. The presence of *E. coli* bacteria in livestock that are often found in the gastrointestinal tract that cause colibacillosis disease will have an impact on the inhibition of nutrient absorption until the death of livestock (Hinton et al., 2000). The provision of antibacterial ingredients can inhibit the growth of *E. coli*. Khan et al. (2018) states that antibacterial compounds such as essential oils, triterpenoids, saponins, flavonoids, and tannins can inhibit the development of bacteria *E. coli*.

Testing the presence of *Clostridium sp* on litter samples showed negative results in all treatments. *Clostridium sp* includes bacteria that are often found in livestock products and belong to the category of pathogenic bacteria (Lepp et al., 2021). According to SNI 7388 (2009) the content limit of *Clostridium sp* is 1×10^2 colonies / g. Infection from *Clostridium sp* can cause Clostridial Necrotizing Enteritis (CNE) disease in poultry, both diseases can result in death (Mwangi et al., 2019). Efforts can be made to inhibit the growth of *Clostridium sp,* namely by heating the indicated material *Clostridium sp* and administering vaccines to livestock.

CONCLUSION

In conclusion, the difference in the altitudes of the region does not affect the chemical quality, contamination and microbiology of litter broiler chickens that are kept at different altitudes. The chemical quality of litter that is not affected by the height of the area is moisture, ash, crude protein, crude fibre, crude fat, NFE and TDN. The Pb content in broiler chicken litter was 35.15-43.49 ppm, Cu content was 102.46-116.58 ppm, mercury content was 0.001 ppm and no antibiotic content was found. Total litter bacteria ranged from $3.48 - 5.46 \times 10^6$, gram-positive bacteria that grew on solitary and rod-shaped litter, gram-negative bacteria, bacteria, *Salmonella*, *E. coli*, *Clostridium sp* in broiler litter were not found.

DECLARATION

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Conflict of interests

The authors declare that they have no competing interest.

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THE EFFECTS OF PROCESSED RUBBER (*Hevea brasiliensis*) SEED MEAL ON THE CHEMICAL COMPOSITION OF RATION, FEED INTAKE AND NUTRIENTS DIGESTIBILITY IN RABBITS (*Oryctolagus cuniculus*)

Amandine MATHO[1], Michelle CIEMENI AIMEE[1], Mama MOUCHILI [1], Kuietche Hervé MUBE[1], Henry DEFANG FUALEFAC[1,2], Florence FONTEH[1], Alexis TEGUIA[1], and Joseph TCHOUMBOUE[1]

[1] *Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box: 222 Dschang, Cameroon.*
[2] *University of Buea, Higher Technical Teachers Training Collage (HTTTC), Kumba, Cameroon.*

Email: amandine_matho@yahoo.com; ORCID: 0000-0002-2749-8115

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ABSTRACT: This study aimed at evaluating the influence of processed rubber (*Hevea brasiliensis*) seed meal (RSM) on the chemical composition of feed, feed intake and nutrients digestibility in rabbits. For the purpose, 30 female (local breed) rabbits with an average weight of 2.7 ± 0.12 kg were randomly allotted to five dietary treatments (6 rabbits in 1 treatment). The feeding trial lasted for four weeks and the various rations were: negative control ration, not containing rubber seeds (R0-) and 4 different rations each containing 20% of the rubber seed meal (raw, toasted, soaked, and boiled respectively for R0+, R1, R2, and R3 rations). The rubber seed meal, faeces and feed sample were sun dried to constant weight, urine was also collected and a sample was used for chemical analysis. The results showed that the boiled seeds RSM exhibited the highest crude protein content (20.61% DM) and the low concentration of anti-nutritional factors (ANFs) (21.83 mg/kg HCn; 30.53%; 21.26% and 3.67% respectively for hydrogen cyanide (HCn), phenols, flavonoids and tannins) compared to seeds from other treatments. Animals receiving the ration with boiled rubber seed meal (BRSM) at 100°C for 45 minutes (R3) showed better feed intake and best rate of digestive utilization coefficients of dry matter, organic matter, crude protein and crude fibres 32.24%; 38.55%; 61.03% and 80.58% respectively. The ration containing the boiled rubber seeds for 45 min resulted in better nutrient intake and the best digestive utilization coefficients in rabbits.

Keywords: Digestibility, *Hevea brasiliensis*, Nutritional value, Rabbit, Rubber seed.

Abbreviations: UDTRF: University of Dschang Teaching and Research Farm; ANPROLA: Animal Production and Nutrition Laboratory; SOCAPALM: Cameroon Company of Palm Groves; RRSM: raw rubber seed meal. DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, CF: Crude Fibre. T0: Ration not containing rubber seeds meal, RRSM: Control ration with 20% untreated rubber seed meal, BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes, SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours, TRSM: ration with 20% toasted rubber seed meal for 45 minutes; ANOVA: Analysis of variance; SPSS: Statistical Package for Social Sciences.

INTRODUCTION

Food remains the major challenge for successful animal breeding (Niba et al., 2012). It represents 60-70% of production costs in intensive farming (Defang et al., 2014). This could be explained by the regular increase in the price of conventional feedstuffs such as soybeans or soybean meal which is imported, and competition between human and livestock for this primary product. In this situation, some breeders in developing countries tend to reduce the incorporation rate of expensive and scarce ingredients in ration formulation, which could lead to an unbalanced feed and consequently to a poor expression of the genetic potential of animals. According to Akinmutimi (2007), the consequences of the above are the reduction in the animal protein production and then, insufficient animal protein consumption, due to the scarcity and high cost of animal products. The attention in animal nutrition in recent years has been centred mostly on the search for alternative feed ingredients which are cheaper, readily available, have potential to replace expensive conventional feedstuffs during period of shortage and has little or no competition with human dietary demand (Alao et al., 2017; Sugiharto et al., 2018).

Rubber seeds (*Hevea brasiliensis*) appear as one of the alternative non-conventional feedstuff of interest (Iyayi et al., 2008). In Cameroon, rubber is produced for exportation and seeds are usually discarded causing environmental hazard. Rubber seeds are produced by the rubber tree (*Hevea brasiliensis*), belonging to the family of Euphorbiaceous. It is known and used for its latex, but its seeds can be used in animal feeding. Rubber seed is a capital non-conventional feedstuff, which has received scant research attention in fish feeding (Sharma et al., 2014; Suprayudi et al., 2015; Deng et al., 2015), layers (Atchibri et al., 2008), pregnant and lactating pigs (Koné et al., 2016), chickens (Aguihe et al., 2017) and ducks (Amanidja et al., 2019). Researchers have revealed that dried seeds of *Hevea brasiliensis* contain between 17- 25% protein (Sharma et al., 2014), 50.2% fat, 6.5% crude fibre, 3.6% ash and 18.2% carbohydrate, as well as reasonable

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amounts of mineral trace elements (Udo et al., 2018). However, variability in nutrient content will depend on the variety, age at harvesting, soil type, processing methods and storage of the by-product. Despite the acceptable protein rate, rubber seed meal is characterized by the presence of anti-nutritional factors (ANFs), specially cyanogen glycosides, whose metabolic end product is hydrogen cyanide, that impacts negatively the physio-metabolic responses (Sharma et al., 2014; Deng et al., 2015). Freshly harvested rubber seeds contain anti-nutritional factors such as tannins (0.07%), oxalate (0.18%), saponins (0.76%) and phytate (0.51%) and a toxic element that can create disorders when they are immediately introduced into animal feed; also it contributes to gastrointestinal issues and reduce metabolic activity when ingested (Thangaraj, 2016). It is the hydrocyanic acid (Syahruddin et al., 2014; Ahaotu, 2018), whose rate is 24.89 % (Udo et al., 2018), similar to cassava cyanic acid (Udo et al., 2018). Hence, the processing of rubber seeds could reduce their anti-nutritional factors and consequently optimize their use in animal feed. It is with this in mind that this work was initiated in order to contribute to the valorisation of agricultural by-products in animal feeding. Specifically it was to evaluate the influence of processing rubber seeds on the anti-nutritional factor contents, nutritional values, chemical composition of the ration; feed intake and nutrients' digestibility in rabbits fed with rations containing seeds subjected to different treatments.

MATERIALS AND METHODS

Study site

The study was conducted at the rabbitry unit of the University of Dschang Teaching and Research Farm (UDTRF) and in the Animal Production and Nutrition Laboratory (ANPROLA) of the Dschang University, and under Ethical regulation of Dschang University for animal welfare in *in vivo* studies. The UDTRF is located between 5° 44'-05° 36 'and 5° 44'-05° 37 North 'latitude and 10° 06'-09° 94' and 10° 06'-09° 85 'East longitude, at an altitude of 1420 m from the West Cameroon Region. The mean annual rainfall is about 2000 mm, the wet season last from march to November and the dry season from late November to early March.

Experimental rabbits and housing and prophylaxis

A total of 30 female (local breed) rabbits aged 5 - 6 months were used for the trial. The rabbits were managed intensively and housed individually in specially constructed metabolic cages (Californian type) measuring 60 cm × 40 cm × 25 cm in dimension. The adaptation period lasted ten days, during which the rabbits were treated against parasitic infection with ivermectin® (0.2 ml / kg body weight) subcutaneous and multivitamin added to their water.

Experimental design and management

For the digestibility trial, 30 rabbits were randomly allotted to each of the 5 dietary treatments (6 rabbits per treatment). Each of the rabbit formed an experimental unit. The experiment lasted for seven days. The rabbits had free access to feed and potable water.

Plant material

The rubber seeds were harvested in the rubber plantations of SOCAPALM® (Cameroon Company of Palm Groves) in the Moungo Division, Littoral Region. They were decorticated, separated into 4 batches and processed differently. First batch was sun dried, second batch was cooked, third batch was toasted and the fourth batch was soaked. The processed rubber seeds were separately hammer-milled prior to experimental diet formulation to produce the respective meals as raw rubber seeds meal (RRSM), soaked (SRSM), boiled RSM (BSRM) and toasted RSM (TRSM).

Processing methods

Boiling/cooking

Raw decorticated rubber seeds were put into a cooking pot containing boiling water. The content was allowed to boil for 45 minutes before the seeds were descanted. The boiled seeds were sun dried, milled and stored for further use.

Toasting

Raw decorticated rubber seeds were toasted in a metallic frying pan for 45 min. After cooling, they were sun dried, milled and stored for further use.

Soaking

Raw decorticated rubber seeds were soaked in water at the ambient temperature, inside a closed inoxidable metal drum for 72 hours, followed by draining. The soaked seeds were sun dried, milled and stored for further use.

Chemical analyses

Chemical compositions of the processed rubber seed meals were analysed using the methods of AOAC (2000). The determinations of some minerals' contents were carried out according to the methods described by Pauwels et al. (1992). While the anti-nutritional factors were evaluated: the cyanide contents as described by ISO 6703-2 (1984); tannins content by the Folin-Ciocalteu method (Govindappa et al., 2011); the content of total phenols by the method described by (Ramdé-Tiendrébéogo et al., 2012); the total flavonoid content using the aluminium chloride colorimetric method (Chang et al., 2002).

Experimental diets

Five experimental diets were formulated to meet the nutritional requirements of the rabbits. T0 (negative control) diet was free of rubber seed meal, RRSM (positive control) contained 20 % untreated RSM, while, BRSM, SRSM and TRSM respectively containing 20% of treated rubber seeds meal as presented in Table 1.

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Table 1 - Percentage composition and proximate analysed experimental rations.

Ingredients (% MS) / Experimental rations (%)	T0 (0)	RRSM (20)	BRSM (20)	SRSM (20)	TRSM (20)
Maize	37	25	25	25	25
RRSM	0	20	0	0	0
Treated RSM	0	0	20	20	20
Wheat brand	20	20	20	20	20
Soybean meal	4	1	1	1	1
Fish meal	3	1	1	1	1
Palm kernel cake	11	11	11	11	11
Cotton seed cake	4	1	1	1	1
Trypsacum laxum	18	18	18	18	18
Sea shell	1	1	1	1	1
Palm oil	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5
Premix 0.5 *	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Analysed chemical compositions of rations					
Dry matter (%)	94.22	95.06	96.33	95.57	96.50
OM (% DM)	85.79	87.35	90.69	90.60	90.58
Ash (% DM)	8.64	7.71	5.71	4.85	5.99
CP (% DM)	16.89	16.70	17.42	16.90	16.65
ME (Kcal/Kg)	2659.12	2745.53	2773.96	2759.38	2753.45
CF (% MS)	12.14	12.67	13.71	13.38	12.95

* Composition of the 5% premix: Vit A: 3.000.000 IU, Vit D: 50.0000 IU, Vit E: 6.000 mg, Vit K: 600 mg, Vit B1: 600 mg, Vit B2: 800 mg, Vit B3: 1800 mg , Vit B6: 400 mg, Vit12: 6 mg, folic acid: 250 mg, Niacin: 600 mg, Cl: 86.500 mg, Fe: 12.000 mg, Cu: 1200 mg, manganese: 12.000 mg, Zn: 10.000 mg, I: 100 mg, Se: 40 mg, magnesium: 3397 mg, Na: 283 mg, CA: 215.166 mg, Methionine: 130.000 mg, lysine: 50.000 mg. RRSM: raw rubber seed meal. DM= Dry Matter, OM= Organic Matter, CP= Crude Protein, CF= Crude fiber. T0: Ration not containing rubber seeds meal, RRSM: Control ration with 20% untreated rubber seed meal, BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes, SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours, TRSM: ration with 20% toasted rubber seed meal for 45 minutes.

Data collection

Metabolic cage trial

During the experimental period, excreta and urine were collected daily for seven days. Faeces samples were pooled, weighed, dried, weighed again and ground. The nutrient composition of the faeces and urine samples was determined according to the procedures described by AOAC (2000). The resulting data were used to calculate the apparent nutrient digestibility coefficients.

Statistical analysis

Collected data were subjected to a one ways analysis of variance (ANOVA), using SPSS 20.0 software. Where there was a significant difference, Duncan's test at 5% significance was used to separate the means.

RESULTS

The effects of processing methods on anti-nutritional factors in raw and processed rubber seed meal (RSM) are presented on Table 2. Generally, processing had significant ($p<0.05$) effects on ANFs. The ANFs in the boiled RSM was significantly ($p<0.05$) lower compared to other treatments. However, flavonoids content (21.26%) for BRSM remained comparable ($p>0.05$) to that of TRSM (22.46%).

Effects of processing methods on proximate composition of raw and processed rubber seeds meals

Table 3 presents the effects of processing methods on proximate composition of raw and processed rubber seeds meals. It appears that with the exception of toasting RSM, the treatments significantly ($p<0.05$) improved the composition of the seeds meal. Boiled RSM had significantly ($p<0.05$) higher results but its fat content and energy was the lowest. Toasted RSM despite the fact that its content in other nutrient components was significantly ($p<0.05$) lower compared to untreated seeds, had the highest fat and energy contents.

Effects of processing methods on minerals composition of raw and processed rubber seeds meal

Table 4 shows the effects of processing methods on the minerals composition of raw and processed rubber seeds meal. Generally, the mineral contents in the processed RSM was significantly ($p<0.05$) influenced by processing methods compared to the raw RSM. For macro minerals (Ca, K and P) boiled seeds meal had significantly ($p<0.05$) higher values than that of the others treatment.

Effects of processing methods of rubber seeds meal on feed intake in rabbits

Table 5 presents the effects of rubber seeds treatments on rabbit feed intake. The ingestion of nutrients had significantly ($p<0.05$) increased with the addition of rubber seeds in the ration compared to the T0 ration (not containing rubber seeds). Nevertheless, feed ingestion of the dry matter and organic matter in animals fed RRSM diet was significantly lower ($p<0.05$) than that of batch T0. The BRSM showed the significantly ($p<0.05$) higher values.

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Table 2 - Effects of processing methods on anti-nutritional factors in rubber seeds meal.

Anti-nutritional factors (ANFs) / Processed rubber seeds' meals	RRSM	TRSM	SRSM	BRSM	SEM	P
HCn(mg/kg HCn)	87.34±0.00[a]	44.39±1.02[b]	30.57±2.06[c]	21.83±0.00[d]	2.12	0.001
Phenol (%)	90.71±0.07[a]	68.29±0.61[b]	43.53±0.10[c]	30.53±0.03[d]	6.97	0.01
Flavonoids (%)	45.47±0.15[a]	22.46±0.12[c]	43.14±1.10[b]	21.26±0.03[c]	3.39	0.001
Tanins (%)	16.04±0.04[a]	10.55±0.04[b]	5.63±0.07[c]	3.67±0.02[d]	1.44	0.001

[a, b, c] Mean values on the same row with different superscripts are significantly different (P<0.05) SEM: standard error on mean; p: probability. RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal; HCn: hydrogen cyanide.

Table 3 - Effects processing methods on analysed proximate composition of raw and treated rubber seed meal (RSM).

Parameters / Processed rubber seeds' meals	RRSM	TRSM	SRSM	BRSM	SEM	P
DM (%)	92.20±0.20[b]	89.20±0.20[c]	96.43±0.20[a]	92.00±0.01[b]	0.79	0.001
OM (%)	96.50±0.01[b]	95.68±0.09[c]	96.17±0.02[b]	97.53±0.07[a]	0.23	0.001
CP (%)	19.53±0.20[b]	18.39±0.20[c]	19.80±0.09[b]	20.61±0.15[a]	0.26	0.001
CF (%)	11.24±0.02[d]	14.99±0.04[c]	17.32±0.02[b]	18.90±0.05[a]	0.87	0.001
Fat (%)	37.04±0.08[c]	48.94±0.10[a]	42.17±0.03[b]	27.50±0.05[d]	2.36	0.001
Ash (%)	3.50±0.01[c]	2.31±0.09[d]	3.80±0.05[b]	4.43±0.07[a]	0.23	0.001
ME (Kcal)	4526.61±1.90[c]	4835.57±1.84[a]	4826.29±0.05[b]	3939.44±0.61[d]	1.78	0.001

[a, b, c, d, e]: the means bearing the same letters in the same row are not significantly different at the threshold of 5; SEM: standard error on mean; p: probability; DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fibre; RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal.

Table 4 - Effects of processing methods on the mineral composition of raw and processed rubber seeds meal.

Minerals (%) / Processed rubber seeds' meals	RRSM	TRSM	SRSM	BRSM	SEM	P
Ca^{2+}	0.09±0.01[b]	0.04±0.01[c]	0.02±0.00[d]	0.11±0.01[a]	0.011	0.001
Mg^{2+}	0.29±0.03[a]	0.04±0.01[c]	0.27±0.02[a]	0.18±0.04[b]	0.030	0.001
P	0.24±0.01[b]	0.08±0.00[d]	0.20±0.01[c]	0.32±0.01[a]	0.025	0.001
K^{+}	0.23±0.00[b]	0.15±0.01[c]	0.24±0.01[b]	0.29±0.01[a]	0.015	0.001
Na^{2+}	0.02±0.00[a]	0.01±0.00[b]	0.02±0.00[a]	0.02±0.00[a]	0.001	0.001

[a, b, c, d, e]: the means bearing the same letters in the same row are not significantly different at the threshold of 5%. RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal; SEM: standard error on mean; p: probability; Ca^{2+}: calcium, Mg^{2+}: magnesium, P: phosphorus, K^{+}: potassium, Na^{2+}: sodium.

Table 5 - Effects of processed rubber seed meal on rabbit feed intake.

Feed intake	T0	Processed rubber seeds' meals					
		RRSM	TRSM	SRSM	BRSM	SEM	P
DM (g)	114.90±0.29[d]	110.35±0.67[e]	116.67±0.12[c]	118.53±0.36[b]	125.17±0.39[a]	1.30	0.001
OM (g)	108.46±0.26[c]	102.85±0.69[d]	109.77±0.31[b]	110.27±0.34[b]	121.64±0.41[a]	1.63	0.001
CP (g)	19.24±0.31[d]	20.33±0.29[c]	20.58±0.70[c]	21.10±0.59[b]	22.01±0.13[a]	0.24	0.001
CF (g)	16.33±0.20[c]	18.10±0.19[b]	18.90±0.37[a]	18.98±0.19[a]	19.06±0.30[a]	0.28	0.001

[a, b, c, d, e]: the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; DM: dry matter; OM: organic matter; PB: crude protein; CB: cellulose brute. T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

Effects of processing methods of rubber seeds' meal on the apparent digestibility of nutrients in rabbits

Table 6 summarises the effects of processed rubber seeds meal on the nutrients digestibility in rabbits. In general, there were significant ($p<0.05$) differences in the apparent digestibility coefficient (ADC) of DM, OM, CP and CF on the control diets. Additionally, the apparent digestibility coefficient of DM, MO, CP significantly ($p<0.05$) increased for the rations containing processed RSM (TRSM, SRSM and BRSM) compared to the control ration (T0) and that containing untreated rubber seeds (RRSM). However, the apparent digestibility coefficient of all nutrients decreased significantly ($p<0.05$) for the RRSM ration except that of crude protein which remained comparable to that of the T0 ration.

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Effects of processing methods of rubber seeds on nitrogen balance

Table 7 shows the effects of processing rubber seeds meal on the apparent digestibility of nutrients and nitrogen balance. Processing significantly ($p<0.05$) influenced ingestion and digestibility of nitrogen. Ration containing the boiled RSM had the better results however the nitrogen content ingested was comparable among rations. The apparent digestibility of nitrogen was better with the ration containing boiled rubber seeds (30.29%).

Table 6 - Effects of processed rubber seed meal on the apparent digestibility of nutrients in rabbits.

ADC (%)	T0	Processed rubber seeds' meals					
		RRSM	TRSM	SRSM	BRSM	SEM	P
DM	21.16±0.14[c]	18.90±0.67[d]	26.79±0.26[b]	28.80±0.36[b]	32.24±2.47[a]	1.33	0.001
OM	18.15±1.33[c]	14.68±0.26[d]	31.10±0.73[b]	32.34±0.45[b]	38.55±1.93[a]	2.42	0.001
CP	55.02±2.48[b]	56.07±0.21[b]	55.46±0.80[b]	60.30±1.07[a]	61.03±1.81[a]	0.75	0.001
CF	64.86±1.83[b]	53.84±1.51[d]	57.63±2.71[c]	80.57±0.11[a]	80.58±0.67[a]	3.02	0.001

[a, b, c, d, e]: the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; ADC: apparent digestibility coefficient; DM: dry matter; OM: organic matter; CP: crude protein; CF: Crude fiber. T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

Table 7- Effect of processed rubber seeds meal on the digestive system of nitrogen.

Nitrogen (N) balance (g/j)	T0	Processed rubber seeds' meals					
		RRSM	TRSM	SRSM	BRSM	SEM	P
Intake nitrogen	2.51±0.13[a]	2.45±0.17[a]	2.52±0.05[a]	2.60±0.53[a]	2.62±0.03[a]	0.02	0.34
Faecal nitrogen	1.28±0.02[c]	1.05±0.19[d]	1.52±0.08[b]	1.59±0.07[ab]	1.77±0.11[a]	0.07	0.001
Urinary nitrogen	0.41±0.02[c]	0.43±0.01[c]	0.53±0.02[b]	0.38±0.05[c]	0.64±0.06[a]	0.02	0.001
Retention nitrogen	0.73±0.05[a]	0.36±0.01[d]	0.46±0.02[c]	0.62±0.01[b]	0.74±0.04[a]	0.04	0.001
ADC (%)	29.25±1.65[a]	13.16±0.31[d]	18.20±0.54[c]	24.07±0.31[b]	30.29±0.72[a]	1.69	0.001

[a, b, c, d, e]: the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

DISCUSSION

The HCn content of the fresh RSM (87.34 mg/kg) was lower than 186; 415.10 and 315.89 mg/kg values reported by Eka et al. (2010), Sharma et al. (2014) and Aguihe et al. (2017) respectively. This result supports the report of (JECFA, 1993) that genetic and environmental factors, location, season, and soil factors are reasons for the wide variations observed in the ANFs. Moreover, reduction in the level of ANFs was effective with the adopted processing methods and this revealed that cyanide, tannins, phenol and flavonoids level in the raw seeds decreased for soaked, toasted, and boiled processing techniques. The highest level of reduction was obtained in the boiled seeds followed by soaked seeds and this is in agreement with previous researchers (Sharma et al., 2014) who stated that heat treatments and fermentation tends to reduce the concentration of ANFs in RRSM and makes them nutritionally less active. The finding corresponds with the description that anti-nutritional factors are heat liable (Eka et al., 2010; Aguihe et al., 2017; Udo et al., 2018) factors, this could be explained by the fact that the heat would have destroyed part of the ANFs and the water cooking would have washed out other ANFs. This result corroborates with that of Ogundipe et al. (2008), which showed that 71.91% of tannin is destroyed after 30 minutes of boiling the seeds by Mucuna. Similarly Chakam (2006) concluded that cooking is the best treatment for detoxify cowpea seeds. Soaked seeds take second place for this low content, then follow the toasted seeds. This could be explained by the unique action of heat on the one hand (toasting) and water on the other hand (soaking) or in the treatment by boiling the two factors interact.

The crude protein (CP) value of the raw rubber seed (19.53% DM) obtained in this study was in agreement with the results of Lalabe (2017) but higher than 17.41 % as reported by Eka et al. (2010). The crude fibre content (11.24%) obtained in this trial is in agreement with that of Nwokolo (1988) (10.3% DM), but significantly higher than that of Aguihe et al. (2017) and Udo et al. (2018) who obtained 4.40; 5.61; 5.88% DM respectively. The variations observed could be attributed to the differences in variety of rubber trees, soil composition, climatic conditions as well as agricultural practices or plan mechanism meant for absorption of nitrogenous nutrients and inadequate fertilizer application of the rubber plantation (Chanjula et al., 2010). Also it can be explained by the processing and analysis methods used by these authors. The boiling of rubber seeds exhibited the greatest crude protein content while that the toasting presented the smallest value, this corroborates the results of Udo et al. (2018) who reported an increase in crude protein content when

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rubber seeds were boiled and a reduction in this content when toasted. The differences could be due to the effect of heat, which denatured part of the proteins during the two treatments.

The minerals contents of the raw seed were lower than that of Udo et al. (2018) with 1740 and 2066 mg/kg for calcium and magnesium respectively. Likewise, the minerals content of raw rubber seeds meal (RRSM) is lower than that obtained by Far et al. (2019). These differences may be attributed to genetic variations, as well as climate, environmental and geographical factors. The increase in minerals after boiling would be due to the fact that the minerals trapped by the anti-nutritional factors were released by heat. This result is in agreement with that of Udo et al. (2018) and that of Far et al. (2019) who both noted an increase in the minerals when boiling and a reduction in these when toasting the rubber seeds. The best results were registered with the boiled RSM with regard to the calcium, potassium, sodium and phosphorus contents and with the treatments SRSM and RRSM for magnesium.

Intake of nutrients were significantly higher for rabbits fed boiled RSM this could be explained by the fact that the boiling time was an effective treatment to reduce trypsin inhibitors, cyanide, saponins, and tannins (Chakam, 2006). The result of this trial is in agreement with the findings of Aguihe et al. (2017) and Far et al. (2019) who noted a better intake of the ration containing boiled rubber seeds meal.

The apparent digestibility coefficient of dry matter (DM) and organic matter (OM) were significantly higher for rabbits fed diet containing boiled rubber seed meal compared to the rest of the treatments. These results are lower than those of Lounaouci-Ouyed (2014), who obtained a load digestive factor from dry matter (79.8%; 75.8%; 78.2%) and organic matter (79.2%; 75.6% and 78.0%), when rabbits were fed soybeans, peas and faba bean as sources of protein respectively. Likewise Cunha (1993) obtained a digestive utilization coefficient of the dry matter of 60.9% and an apparent digestibility coefficient of the organic matter of 61.4% when soybean was replaced at 20% by *Vicia benchalensis* in the rabbit ration. Indeed, these results could be due to the effects of the chemical composition of the ration, sex, age and breed, since digestibility depends on these factors. In fact, Cunha (1993) worked with animals of 3 months, of New Zealand breed. While in this study the animals used were 5 to 6 months. The apparent digestibility coefficient of crude protein (CP) and crude fibre (CF) were comparable for rabbits fed with RRSM soaked in water at room temperature for 72 hours but significantly higher compared with the rest of the treatment. The results obtained within these framework were lower than those obtained by Aguihe et al. (2017) (72.54%) when broilers were fed a ration containing boiled rubber seeds; but comparable to those of Wafar et al. (2017), who obtained a digestibility coefficient of (60.45%) when rabbits were fed raw kapot tree seeds. The best coefficient of digestibility of crude cellulose was obtained with the ration containing the boiled rubber seeds meal (BRSM), a result comparable to that of Wafar et al. (2017) (58.14%), but higher than that of Aguihe et al. (2017) (43.47%). This difference could be due to the chemical composition of the ration and the animal species, which agrees with Riviére's assertion (Rivière, 1991), which showed that feed and animal species strongly influence digestibility of nutrients.

This apparent digestibility coefficient of nitrogen was comparable to that of ration R0- not containing rubber seeds (29.25%); but higher than that of the ration containing untreated seeds (13.16%). This could be explained by the presence of anti-nutritional factors in the latter that would have interfered with digestive use nitrogen.

CONCLUSION

The results of the present research showed that boiling rubber seed is effective in reducing anti- nutrients factors in rubber seed. Also, the contents of dry matter, organic matter, proteins, crude fibre, ash and energy increased with treatment boiling rubber seed meal. The best results of ingestion and digestibility coefficient of the components of the ration were obtained with the ration containing the boiled RSM. The processing treatments used in this work caused reduction at different rates in the level of anti-nutrients present in the raw seed of *Hevea brasiliensis* to tolerable level safe for consumption.

DECLARATIONS

Corresponding author's Email: amandine_matho@yaho.com

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Authors' contribution

MA conceived the study, designed the study, collected data, contributed in data analysis, and writing the manuscript, coordinated the inputs of all the other authors; DFH performed critical reviewing of the manuscript and supervision of the study; FF, TA and TJ contributed in conception of the study and performed critical reviewing of the manuscript; CMA contributed in design of study, data collection and writing the manuscript; MM contributed in data analysis and performed critical reviewing of the manuscript; KMH contributed in conception of the study and critical reviewing of the manuscript.

Competing interest

The authors declare they have no conflict of interest.

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EFFECT OF LIME (*Citrus aurantifolia*) JUICE AS SOURCE OF VITAMIN C ON PERFORMANCE AND SOME BLOOD PARAMETERS IN PELUNG CHICKENS (*Gallus gallus domesticus*)

Lilik KRISMIYANTO, Hanny Indrat WAHYUNI and dan Nyoman SUTHAMA

Department of Animal Science, Faculty of Animal and Agricultural Science Universitas Diponegoro, Semarang City 50275, Indonesia

Email: lilikkrismiyanto@lecturer.undip.ac.id; ORCID: 0000-0001-5634-6965

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ABSTRACT: Pelung chicken (*Gallus gallus domesticus*) is a typical Indonesian local breed originating from West Java. Pelung chicken breeding, especially male bird, was initially used as a hobby livestock because they have tunable voice. This study aimed to evaluate the effect of feeding lime (*Citrus aurantifolia*) juice as a source of vitamin C on blood parameter and performance of male pelung chickens. The experimental birds were 64 male pelung chicken aged 12 weeks, divided into 4 weight groups namely group 1: 740 – 910 g, group 2: 910 – 1,080 g, group 3: 1,080 – 1,250 g, and group 4: 1,250 – 1,420. The treatments applied were T0: formulated diet; FD, T1: FD+ lime juice 1%, T2: FD + lime juice 2%, and T3: FD + lime juice 3%. The present experiment was assigned in body weight-based randomized block design. Measured parameters were heterophile, lymphocyte, heterophile-lymphocyte ratio (H/L), total plasma protein, the relative weight of lymphoid organs (spleen and bursa of fabricius) and performance (feed intake, daily body weight gain and feed conversion ratio) of birds. The results showed that the feeding diet added with 1-3% lime juice significant on heterophile, H/L ratio, the relative weight of spleen, total plasma protein, daily body weight gain and feed conversion ratio, but not significant on lymphocyte, the relative weight of bursa of fabricius and feed intake. Male pelung chicken fed diet added with 3% lime juice (T3) indicates better blood parameter stability (H/L ratio and lymphoid organs), total plasma protein and performance.

Keywords: Blood parameter, Lime, Pelung chicken, Performance, Vitamin C.

INTRODUCTION

The are many kinds of local chickens in Indonesia throughout the country, such as Kedu, Sentul, Nunukan, Pelung, Sumatra, Bekisar chickens and others. The local chicken population in the last 10 years has increased significantly between 249,963,499 to 310,959,951 birds (Directorate General of Livestock and Animal Health, 2020). Pelung chicken are local Indonesian poultry originated from Cianjur, West Java, which has existed since 1850 (Asmara et al., 2019). This type of chicken is well known as a fancy bird and the body weight of the ready-to-cut at 12 weeks old can achieve between 700 – 800 g (Hidayat and Asmarasari, 2015). Indonesian local poultry, including pelung chickens, are generally known to be adaptive well to the tropical environment (Sumantri et al., 2020). However, efforts to maintain body resistance in order to be more adaptive to the fluctuating tropical environment need providing natural additives are important. Body resistance is generally related to the ability of natural additives work as a source of antioxidants (Zhou et al., 2019; Righi et al., 2021).

The provision feeding diet with additives containing antioxidant and anti-stress substances are very relevant with the aim to increase immune-related blood parameter (Surai et al., 2019; Marimuthu et al., 2020). A suitable natural additive that is locally available as an antioxidant and anti-stress is lime juice which is rich in vitamin C (Elwan et al., 2019; Ali, 2020). Shrestha et al. (2012) reported that the content of vitamin C in lime juice is equal to 118.2 – 140.8 mg per 100 g. Vitamin C in particular, has been proven to be beneficial in over-coming cold and heat stress in tropical region (Kusnadi, 2009). Vitamin C supplementation is also reported to be effective in increasing heat resistance during maintenance at high temperatures (Sahin et al., 2003) and very efficient in regulating the activity of amylase enzyme and uric acid metabolisms in broilers (Imik et al., 2013). Amylase activity and uric acid metabolisms are both in related to the ability of glucose and blood protein regulations due to heat stress (Belhadj Slimen et al., 2016). Ferreira et al. (2015) showed that vitamin C can prevent the effect of heat on broilers reared at 39 °C which is characterized by the difference in body weight gain with the area of meat fiber specifically increase as compared to control.

Although chickens capable in producing their own endogenous vitamin C, but is not enough to overcome stress when reared in the tropical region (Abidin and Khatoon, 2013). Therefore, they require exogenous vitamin C supplementation. The use of lime juice as a source of vitamin C is very relevant for raising chickens in the tropical environments that possible for the adaptation process. Dietary addition of lime juice is expected to increase blood parameter of Pelung chickens, since vitamin C functions as an antioxidant. Although, free radical was not observed in the

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present study, vitamin C is known to be able. Vitamin C is able to donate electrons and hydrogen to the free radicals; consequently free radicals become relatively stable. Criteria for blood parameter in present study included ratio of heterophile-lymphocytes (H/L) and blood plasma protein. H/L ratio is the main stress indicator in poultry, because the higher the ratio number, the severe stress level is experienced, and vice versa (Kusnadi, 2009). Plasma protein is a secondary indicator of blood parameter besides the H/L ratio, because it is an indication of an adequacy supply of amino acids for antibody formation. The general phenomenon is that the higher plasma protein, the better blood parameter of chickens that support productivity.

MATERIALS AND METHODS

Experimental animal and diet

The experiment used 12-week old of 64 male birds obtained from *in situ* maintenance (native habitat) in Cianjur, West Java, divided into 4 groups based on body weight (group 1: 740–910 g, group 2: 910–1,080 g, group 3: 1,080–1,250 g, and group 4: 1,250–1,420 g) and reared for 6 weeks. Basal diet composed of rice bran, yellow corn, white bran, soybean meal, meat bone meal, and mix mineral which are prepared with a crude protein content of 14.57% and metabolizable energy (ME) of 2,892.03 kcal/kg (Table 1). Lime juice according to treatment level at 1, 2 and 3% were mixed with approximately 20 g of diet and given in the morning, to ensure all lime juice can be complete consumed, and followed by diet without lime juice to fulfill daily requirement. Experimental diets were provided from 13 week to 18 week old after condition period for one week (12-13 week).

Table 1 - Compositions and nutrient content for formulated diet.

Ingredients	Compositions (%)
Rice bran	20.00
Yellow corn	55.00
Wheat bran	10.00
Soybean meal	6.00
Meat bone meal	4.00
Mineral and vitamin mix[3]	5.00
Total	100.00
Metabolizable energy (kcal/kg)[2]	2,892.03
Crude Protein[1]	14.57
Crude Fiber[1]	7.37
Ether extract[1]	6.57
Calcium[1]	0.81
Total phosphor	0.55

Sources: [1]Based on chemical analysis at the Laboratory of Feed and Nutrition Science, Diponegoro University; [2]Based on Bolton (1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen free extract] + 2.5}; [3]Provided minerals per kg of the feed: calcium 32.5%, phosphor 1%, iron 6 g, mangan 4 g, iodine 0,075 g, copper 0,3, zinc 3,75 g, vitamin B12 0,5 mg, vitamin D3 50,000 IU.

Experimental Parameters

Observed parameters were heterophile, lymphocytes, heterophile-lymphocytes ratio, plasma protein and lymphoid organs (spleen and bursa of fabricius) measured at fifth the last week of experimental rearing period. Blood samples were taken through the brachial vein with 3 mL syringe. Blood was inserted into tube containing anticoagulant as was used sample for heterophile-lymphocyte test and total plasma protein based on Enzyme-Linked Immunosorbent Assay (ELISA) method. Chickens were further decapitated and dissected to obtain lymphoid organs (spleen and bursa of fabricius) to measure relative weight by dividing with final body weight and multiplied by 100%.

Experimental design and statistical analysis

The study was arranged in a randomized block design (RBD) based on body weight and as follows:

K1 = body weight range 740 – 910 g with average 835.51 ± 49.48 g
K2 = body weight range > 910 – 1,080 g with average 985.69 ± 42.04 g
K3 = body weight range >1,080 – 1,250 g with average 1,160.63 ± 55.69 g
K4 = body weight range >1,250 – 1,420 g with average 1,369.06 ± 65.97 g

The study was designed in 4 treatments and 4 groups as replications (4 birds each). The treatment applied:

T0 = formulated diet
T1 = formulated diet + lime juice 1%
T2 = formulated diet + lime juice 2%
T3 = formulated diet + lime juice 3%

Data subjected to analysis of variance to determine the effect of treatment. If the treatment indicated significant effect, it then followed by Duncan's test at 5% probability (Gomez and Gomez, 1984).

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Ethical Approval

The in vivo study was supervised by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro and managed in accordance with the recommendations in the basic animal husbandry and health protocols referred to in Legislation of the Republic of Indonesia No. 18, 2009.

RESULTS AND DISCUSSION

Blood parameter and immune organ

Dietary addition of lime juice indicated significant effect on heterophile, heterophile-lymphocyte (H/L) ratio, total plasma protein, and spleen relative weight (p<0.05), but it did not affect lymphocytes and the weight of the bursa of fabricius (Table 2). Heterophile and H/L ratio in the treatment with the addition of 3% lime juice (T3) was the same as does the feeding of 2% lime juice (T2), but lower than the control/without lime juice (T0) and 1% lime juice treatment (T1). The total plasma protein due to with the feeding effect of 2% (T2) and 3% (T3) lime juice was higher than that in T0 and T1.

Growth performance

The dietary inclusion with lime juice had a significant effect (p <0.05) on daily body weight gain (DBWG) and feed conversion ratio (FCR), but it did not affect feed intake of diet had the same value. The addition of lime juice at 1% (T1), 2% (T2), and 3% (T3) significantly (p<0.05) increased on DBWG and higher compared to formulated diet (T0). Feed conversion ratio with lime juice addition at 2% (T2) and 3% (T3) were higher than that of T0, but between that of T2 and T3 treatments were not different from T1.

Table 2 - Feeding lime juice on blood parameters and immune organs in male pelung chicken.

Parameter	T0	T1	T2	T3	SE	P-value
Heterophile (%)	70.05^a	71.20^a	40.62^b	40.05^b	8.12	0.035*
Lymphocyte (%)	60.12	62.00	50.12	60.10	1.26	0.200^{ns}
H/L ratio	1.16^a	1.15^a	0.81^{ab}	0.66^b	0.24	0.050*
Total plasma protein (g/dl)	4.25^b	4.28^b	4.35^a	4.41^a	1.14	0.028*
Relative weight of spleen (%)	0.35^a	0.32^a	0.28^b	0.26^b	0.44	0.047*
Relative weight of bursa of fabricius (%)	0.12	0.10	0.08	0.09	0.06	0.080^{ns}

^{ab}means values without common letter are different at p<0.05; T0: formulated diet/FD, T1: FD + lime juice 1%, T2: FD + lime juice 2%, T3: FD + lime juice 3%; * = P<0.05; ns= non significant.

Table 3 - Feeding lime juice on growth performance of male pelung chicken.

Parameter	T0	T1	T2	T3	SE	P-value
Feed Intake (g/bird/day)	107.99	114.08	109.03	111.27	1.35	0.250^{ns}
DBWG (g/bird/day)	12.85^b	15.45^a	15.03^a	15.95^a	0.45	0.036*
FCR	8,44	7.41	7.27	7.12	0.22	0.080^{ns}

^{ab}means values without common letter are different at p<0.05. T0: formulated diet/FD, T1: FD + lime juice 1%, T2: FD + lime juice 2%, T3: FD + lime juice 3%. FCR: feed conversion ratio; DBWG: daily body weight gain; * = P<0.05; ns= non significant.

DISCUSSION

Blood parameter and immune organ

The decreased heterophile and H/L ratio in T3 (Table 2) gave an indication that the vitamin C of lime juice functions effectively and the chickens could cope with stress, due to the changes environmental, temperature, from *in situ* to *ex situ* conditions. The changes in environmental condition are greatly possible for the chickens suffer from the complexity of stressor. Vitamin C, or called as the chemical ascorbic acid, is a 6-carbon lactone that can be synthesized from glucose by poultry and some other animal species. Even though chicken can synthesis ascorbic acid (vitamin C), it is not enough to be able to overcome heat stress which is reported by Ahmadu et al. (2016). Therefore, chicken absolutely needs additional vitamin C from exogenous sources which in this study derived from lime juice. The H/L ratio in present study was consistent with the report by Kusnadi et al. (2005) that dietary addition of vitamin C at 500 ppm produced lower H/L ratio with the average 0.61 than control (0.93). The decreased H/L ratio was supported by lower heterophile even though lymphocytes did not change much (Table 2). This condition means that more vitamin C supplementation (from lime juice) increases body resistance. The increased some blood parameters impacted to the chicken's life more comfortable because it connected with the function of vitamin C that could reduce heat production due to low body protein catabolism (Kusnadi, 2006).

In contrast, endogenous vitamin C synthesis in the group of chickens given diet without lime juice supplementation (T0) and also those fed diet with low vitamin C supplementation of lime juice (T1) were not able or not enough to cope

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with environmental influences. When environmental condition change, especially in the tropic region with open house rearing system, show that it is great possible the adaptation mechanisms is occurred in relation to the decrease in body resistance (Dantzer and Mormède, 1983). Vitamin C in the condition of T0 treatment is absolutely needed to be function as an antidote or stress control for chickens. In the tropical country, heat stress could suppress blood parameter when the intake of micronutrients as antioxidants, especially vitamins C and E was low (Ahmadu et al., 2016) and negatively affect that greatly disrupted metabolic physiology, growth, and detrimental to the production performance in poultry (Attia et al., 2009; 2011; 2016).

The low effectiveness of vitamin C results in an increase in the H/L ratio that occurs in T0 and T1 treatments is in line with the increase in spleen weight (Table 2). The large size of the spleen indicates more and more antigens are accommodated so that free lymphocytes in the blood decreases because the spleen is tasked with taking antigens from the blood that have been bound to lymphocytes, ultimately increasing the H/L ratio (Jamillah et al 2013). As discussed earlier, the H/L ratio is an indicator of the comfort level of poultry life, in which the higher the ratio is, and then the higher the discomfort is experienced by poultry (Kusnadi, 2009). Therefore, an unfavorable condition can cause an increase in the number of heterophiles and the total white blood cell according to Table 2.

The results (Table 2) indicated the phenomenon as evidence that vitamin C is indeed as an antioxidant that can reduce the negative effects of environmental temperature changes. Gursu et al. (2003) reported that vitamin C can reduce the negative effects of new environments through the work of antioxidant systems that important to over-come. Vitamin C has 2 hydroxyl groups which are easily oxidized, therefore they can easily release electrons and hydrogen to be donated to free radicals so that free radicals were not reactive or became stable (Kusnadi, 2006). Vitamin C is powerful antioxidant with 2 mechanisms activity namely conversion to L-dehydroascorbic acid and the formation of ascorbic radicals (Ajakaiye et al., 2011). First, conversion to L-dehydroascorbic acid is a back and forth reaction, and molecular interchange can form a redox system physiologically back to its original vitamin C activity. Second, through the formation of ascorbate radicals that can destroy free radicals that formed by oxygen, such as hydroxyl (OH*), mono-oxygen (O*), and superoxide (O2*). The mechanism mentioned above is closely related to other advantages of vitamin C which is able to restore the tocopherol radicals to a stable alpha tocopherol (Tamzil, 2014). These mechanisms may be proved by the decrease in H/L ratio and relative weight spleen supported by increase total plasma protein (Table 2).

Growth performance

The effectiveness of vitamin C in overcoming stress and increasing the ability to adapt to the ex situ condition (Semarang) with higher ambient temperatures can also be seen from the increase in plasma protein which further affect the productivity (Table 3). Total blood plasma protein in the treatment in T2 (2% lime juice) and T3 (3% lime juice) was higher as compared to T0 (without lime juice) and T1 (1% lime juice) (Table 2). The addition of lime juice 2-3% can increase total plasma protein. Examples of the normal range of total plasma proteins in broilers was between 4.14-4.53 g/dL (Fattah et al., 2008). The increase in total plasma protein can be associated with a decrease in uric acid which is an indicator of the effect of giving vitamin C, and could increase the efficiency of protein use (Kusnadi, 2006), and decrease blood uric acid in laying hens (Saki et al., 2010). The use of protein was related to the effectiveness of controlling stressed by vitamin C which could further suppress the body's protein catabolism; thereby, reducing plasma protein 2.6 to 2.9 mg/dL in blood broilers (Gursu et al., 2003), and decrease plasma uric acid from 6.25 mg/dL (control) become 3.50 mg/dL (Saki et al., 2010). This phenomenon was supported by the data vitamin C as much as 250 mg/kg can increase plasma protein. The result of this study indicated that blood with increased supplementation level of lime juice. Heat stress, as indicated that Attia et al. (2017), that were significant decreased protein total (–14.1%), albumin (–29.2%), glucosa (–6.8%), and increased H/L ratio (+17.7). Vitamin C supplementation is very helpful in reducing the negative effects of heat stress on metabolic parameters by increasing blood parameter and productivity in broiler.

Vitamin C levels derived from lime juice did not directly affect the feed intake of however; vitamin C had more beneficial impact on body resistance (Table 2). Antioxidant compounds in lime juice could increase daily body weight gain, especially in T2 and T3 treatments. Antioxidant functions of vitamin C exert of its effect as free radical breaker and enzyme cofactor. The present result was supported by Bikrisima et al. (2014) that vitamin C derived from guava juice has been known function antioxidant functions as a free radical breaker and enzyme cofactor. Similarly, PokuJnr et al. (2018) reported that an addition of vitamin C at 10-30 mg/bird/day could increase feed intake, body weight gain, and FCR in Guinea fowl.

CONCLUSION

Supplemented vitamin C is the level of 3% lime juice (T3) was indicated blood stability, to increase growth performance (daily body weight gain) and feed conversion ratio. The use of 3% lime juice resulted in the best outcomes for male pelung chicken growth. Lime juice needs to be extracted to get the real vitamin C.

DECLARATIONS

Corresponding Author
E-mail: lilikkrismiyanto@lecturer.undip.ac.id; ORCID: 0000-0001-5634-6965

Authors' Contribution

All authors contributed in research and writing, equally.

Conflict of interests

The authors declare that they have no competing interests.

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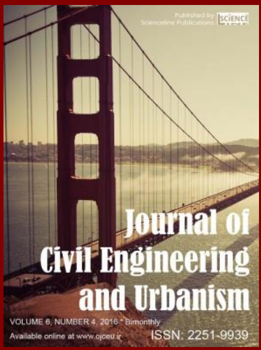

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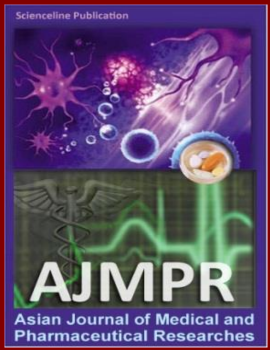

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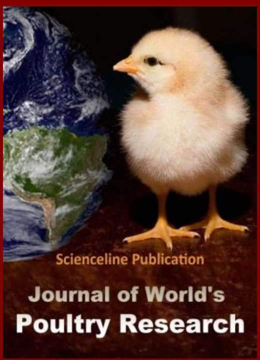

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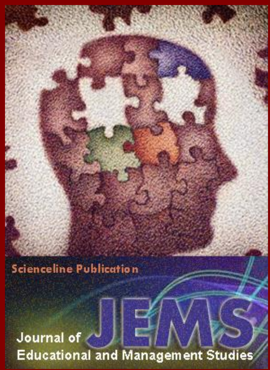

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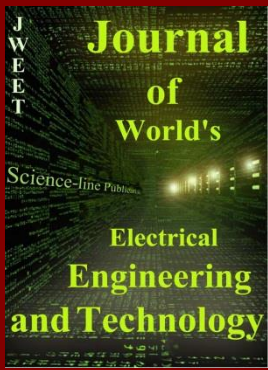

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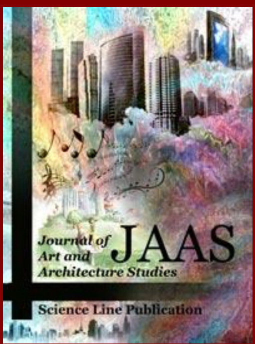

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