JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2024 Sep; 7(3): 546-555
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2024.d47
Published by www.bsmiab.org

Effects of Clove and Tulsi supplementation on the dynamics and cellularity of adipose tissue in the visceral and subcutaneous fat depots in Broiler

Rafiqul Islam[1], Sadia Islam Sinza[2], Nasrin Sultana[1,*]

[1]Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Comparative Biomedical Science, Louisiana State University, Louisiana, USA

***Corresponding author**
Nasrin Sultana, PhD
Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh, Bangladesh.
Email: nasrin.sultana@bau.edu.bd

Academic editor
Md Jamal Uddin, PhD
ABEx Bio-Research Center, Dhaka, Bangladesh

Article info
Received: 28 May 2024
Accepted: 03 July 2024
Published: 09 July 2024

Keywords
Adipocyte, Broiler, Clove, Fat Depot, Tulsi.

ABSTRACT

The substantial genetic enhancement of broiler chickens, coupled with the use of growth-promoting additives in feed, has led to accelerated growth rates, and elevated fat deposition in broiler. Clove and Tulsi, well-known medicinal plants, have been shown to improve both the growth performance and intestinal health of broilers. This study aimed to investigate the impact of Clove and Tulsi supplementation on the composition and cellular makeup in visceral and subcutaneous fat depots of broiler chickens. Sixty Cobb-500 broiler chicks were randomly divided into four groups to receive different supplemental treatments via drinking water. The groups included S0 (control), receiving no additional supplements; S1, receiving 0.5% Clove and 2% Tulsi; S2, receiving 1% Clove and 3% Tulsi; and S3, receiving 1.5% Clove and 4% Tulsi from day 8 to 28. On days 14 and 28, five broilers from each group were euthanized, and their visceral and subcutaneous fat depots were collected, weighed, and subjected to histological analysis to assess adipocyte histomorphology and histomorphometry. The S1 and S2 groups exhibited higher relative percentages of visceral fat depot, larger adipocyte size, and a greater percentage of larger adipocytes compared to the control, while adipocyte density was lower. Conversely, the S3 group showed minimal deviation from the control. Notably, no significant histomorphological differences were observed among the experimental groups. These findings suggest that Clove and Tulsi supplementation may modulate adipose tissue dynamics and cellularity depending on the concentration of supplementation.

INTRODUCTION

Every year, nearly 23 billion chickens are raised globally, averaging around three chickens per person, which has surged fivefold in the last half-century [1,2]. Through its rapid production turnover and ability to convert diverse agricultural by-products and leftovers into nutritious meat and eggs suitable for human consumption, poultry plays a pivotal role in ensuring food security and nutrition by providing essential energy, protein, and crucial micronutrients, contributing significantly to meeting dietary needs [2]. Reports suggest that 152 Mt of chicken meat will be consumed globally by 2030, accounting for 52% of the growth in overall meat consumption [1]. Therefore, the poultry industry has experienced significant expansion, mostly due to the effective use of several growth-promoting agents, disease prevention strategies, and different control measures [3].

Over the past decades, intensive broiler farming has experienced significant advantages by incorporating antibiotics as growth promoters (AGP) into animal feeds, notably enhancing growth performance and feed efficiency. However, since 2006, the utilization of AGP has been banned in certain areas, such as the European Union, due to escalating worries regarding antimicrobial resistance [4]. Consequently, poultry producers are

exploring alternative options to AGPs in diets, such as organic acids, enzymes, pro-/prebiotics, medicinal plants, and spices like Clove and Tulsi [5].

The primary bioactive component of Clove (72%–90%) is a phenolic molecule called eugenol [6]. Clove has been traditionally utilized as a treatment for various digestive and excretory system conditions, including gastritis, nephritis, diarrhea, dysentery, dyspepsia, and similar conditions, typically administered as an infusion or liquid extract [6]. However, a few studies depict the alleged physiological effects of spices on the cellular physiology of animals [7]. There is supporting evidence indicating that including Clove powder in diets (at concentrations ranging from 0.1 to 2.5 g/kg) enhanced both growth rate and feed efficiency [5]. Tulsi (eugenol) is being used as a growth promoter as it has anticancer effects, lowers blood sugar and cholesterol levels, and strengthens the immune system. It improves dressing percentage, liver, spleen, and entire giblet weight while decreasing fat accumulation [8]. Inadvertently, the genetic breeding of broilers for accelerated growth has also increased their propensity to accumulate excess adipose tissue [9]. Excessive fat deposition in broilers is a major concern for the poultry industry because it diminishes dietary efficiency and carcass yield, complicates meat processing, and can lead to consumer rejection of the meat [10,11]. Therefore, minimizing fat accumulation has surfaced as a current challenge for poultry production scientists since a small gain in feed efficiency results in high production gain [10]. To achieve this goal, a proper feeding strategy is needed along with genetic upgradation of the broiler.

Adipose tissue has been the subject of considerable study for the past 20 years and is now considered a significant endocrine organ that maintains metabolic and organ homeostasis [12]. Adipose tissue in various anatomical regions of the body appears to have unique physiological, cellular, and metabolic characteristics [12]. Adipocytes respond differently to physiological stimuli or metabolic stresses by secreting hormones that influence a range of functions, including inflammation, insulin sensitivity, glucose homeostasis, energy expenditure, appetite regulation, and tissue repair [13]. The size and number of adipocytes aid cellular development [14]. Several studies have shown how dietary food content affects the growth of adipose tissue and lipid metabolism [12]. Studies focused on health issues related to human obesity indicate that the dietary fatty acid profile influences the growth and development of adipose tissue in ways that might be reduced through dietary manipulation [15]. In this context, morphometric analysis of adipocytes in different fat depots can offer an effective method of studying adipose tissue and address the rising concerns about excessive fat deposition in broilers considering its public health significance [15,16]. Hence, this study aimed to explore the morphological and morphometric changes in adipocytes within visceral and subcutaneous fat depots in broilers in response to combined Clove and Tulsi supplementation.

MATERIALS AND METHODS

Ethical statement

The "Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University" granted permission (Approval ID - AWEEC/BAU/2021 [05], Date: 15 April 2021) to carry out the experimental investigation.

Housing and management of broiler

37% formaldehyde (35 ml) and KMnO4 (17.5 g) were used to fumigate the experimental broiler shed. After disinfecting the shed and cages with 3% KMnO4, 1% bleached water was used to wash the feeding trays and drinkers.

The one-day-old broiler chicks (n = 60) were collected from the "Nourish Poultry & Hatchery Ltd., Bangladesh". Following a seven-day acclimatization period, the chicks were split into four groups of fifteen birds, each at random. Each group was housed in separate cages measuring 5 × 4 × 2.5 cubic feet and reared under standard conditions.

Clove bud powder and Tulsi extract preparation

Initially, the Clove buds (*Syzygium aromaticum*) were crushed into a fine powder after being adequately sun-dried. Freshly harvested Tulsi (*Ocimum sanctum*) leaves were used to prepare an aqueous extract. The aqueous extracts of 2%, 3%, and 4% were made by blending the leaves with water.

Experimental ration

The broilers were given fresh drinking water with three different combinations of Clove and Tulsi extract, depending on their concentrations, and a balanced commercial food (Table 1) that was bought from Nourish Feeds Limited, Mymensingh, Bangladesh [17]. The supplements (S) were categorized as follows: S0 - Control group, receiving no plant extract; S1 - 0.5% Clove and 2% Tulsi; S2 - 1% Clove and 3% Tulsi; S3 - 1.5% Clove and 4% Tulsi. After adding the necessary amounts of Tulsi and Clove powder to drinking water, homogenous aqueous extracts were prepared. The plant powders were provided for 21 days of the experiment (from day 8 to day 28).

Table 1. Composition of the ration.

Nutrients and conditions	Starter	Grower
Metabolizable energy (kcal/kg)	3000	3050
Crude protein (%)	20	19
Calcium (%)	0.95	0.9
Phosphorus (5)	0.45	0.42
Crude fiber (%)	5	4
Lysine (%)	1.05	1
Methionine (%)	0.45	0.42
Vitamin and mineral (%)	*Ad libitum*	*Ad Libitum*
Humidity (%)	12	12

Collection and processing of samples

Five seemingly healthy broilers from each group were sacrificed (by cervical dislocation technique) on the 14th and 28th days of the experiment to collect the visceral and subcutaneous fat depots. The collected fat depots were weighed immediately after collection. After fixing the tissue for 72 hours in a 10% buffer formalin solution (Merck, Darmstadt, Germany), the tissues were dehydrated using ascending graded ethanol (Merck, Darmstadt, Germany), cleared with xylene (Merck, Darmstadt, Germany), immersed in melted paraffin (58-62 grade Paraffin wax, Techno Pharmchem, Haryana, India) of three different grades (58°C, 60°C, and 62°C), and finally embedded in paraffin wax (62°C). Afterward, sections with a thickness of 5 micrometers (μm) were then prepared (CUT 4062, Manual Precision Microtome, SLEE medical GmbH,

Germany). The tissues were stained with routine hematoxylin-eosin (Merck, Darmstadt, Germany) stain for the histological investigation. Five tissue sections from each group were then investigated blindly under a light microscope. Photomicrographs were taken randomly from five microscopic fields for each tissue section (B-290 TB, Optika, Ponteranica, Italy) and analyzed using the "Image J freehand tool." Therefore, the average number of adipocytes per microscopic field, their size (μm²), and circularity were analyzed to identify any alterations in adipocyte shape, as well as the percentage of smaller and larger adipocytes in the visceral and subcutaneous fat depots. Adipocyte diameters below 25 μm were considered smaller adipocytes.

Statistical analysis

The current study's datasets were acquired using an entirely random design. Since pen replication was absent, the single bird functioned as the analytical unit. Using Levene's test, the datasets' normality was examined. One-way analysis of variance (ANOVA) and post hoc Duncan's Multiple Range Test (DMRT) were carried out to determine group differences. For every example, $P < 0.05$ was regarded as significant.

RESULTS

Impact of Clove and Tulsi supplementation on the relative weights of fat depots

Figure 1 illustrates the relative weights of the visceral and abdominal fat depots. On days 14 and 28, the S2 group had a significantly greater ($P<0.05$) proportion of the visceral fat depot than the S0, S1, and S3 groups. Conversely, the S0 group had the highest percentage of subcutaneous fat depot, but the S2 group's subcutaneous fat depot percentage was considerably lower ($P < 0.05$).

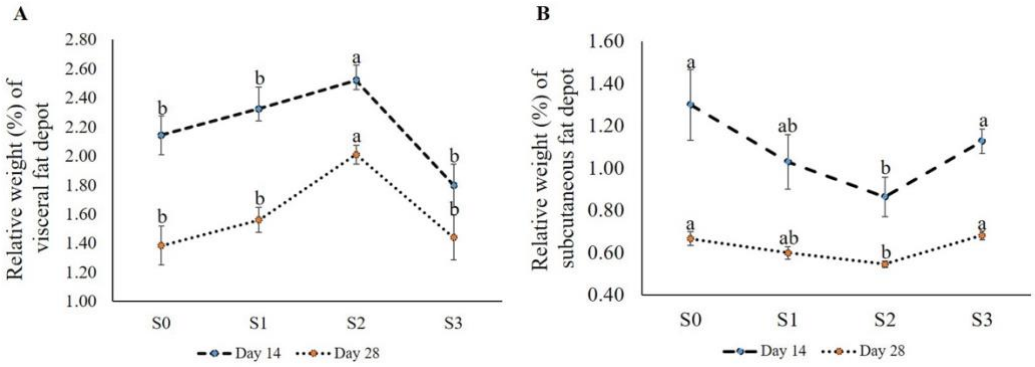

Figure 1. The relative weights (%) of the visceral (A) and subcutaneous (B) fat depots in broiler. Here, S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi. Data are presented as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) with the post hoc Duncan's Multiple Range Test (DMRT). Values with different alphabetic superscripts are significantly different from each other.

Impact of Clove and Tulsi supplementation on the histoarchitecture of adipocyte

The histoarchitectures of the fat depots are shown in Figure 2 and Figure 3, respectively. In each fat depot, there were round to oval-shaped adipocytes. No visible change in the histoarchitecture of the adipose tissue was noticed in the microscopic investigation. The size of the adipocytes was found to be comparatively larger in the supplement groups in the visual inspection under the microscope for both the fat depots.

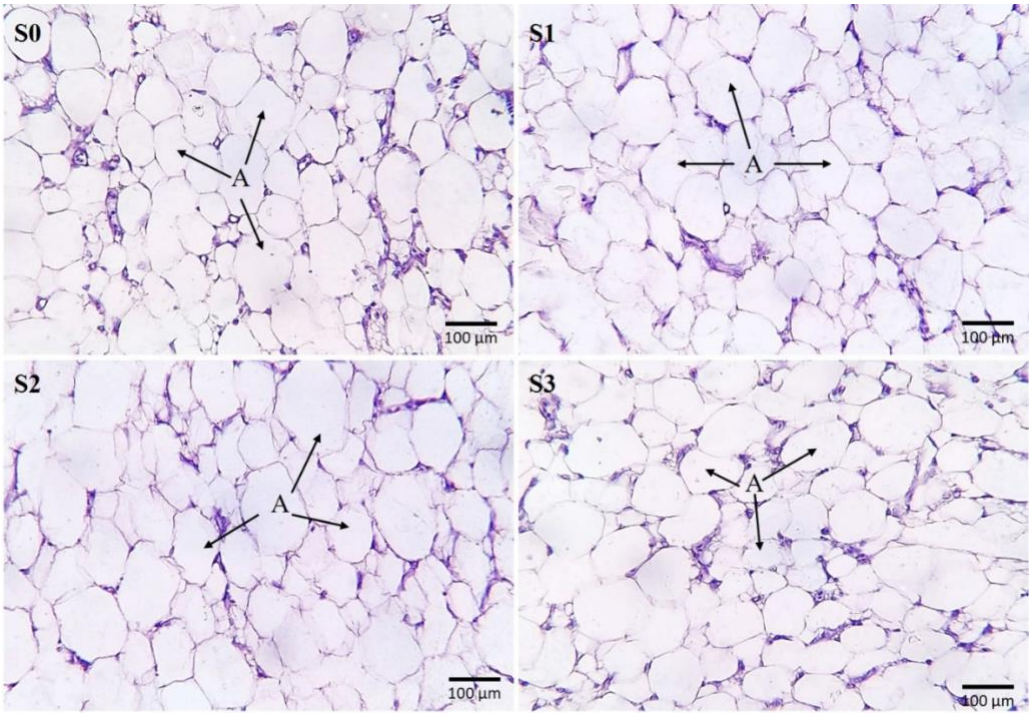

Figure 2. Histoarchitectures of the visceral adipose tissue of different groups of broilers. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi. Hematoxylin-eosin staining. A – Adipocytes. Magnification 400X; Scale bar = 100 µm.

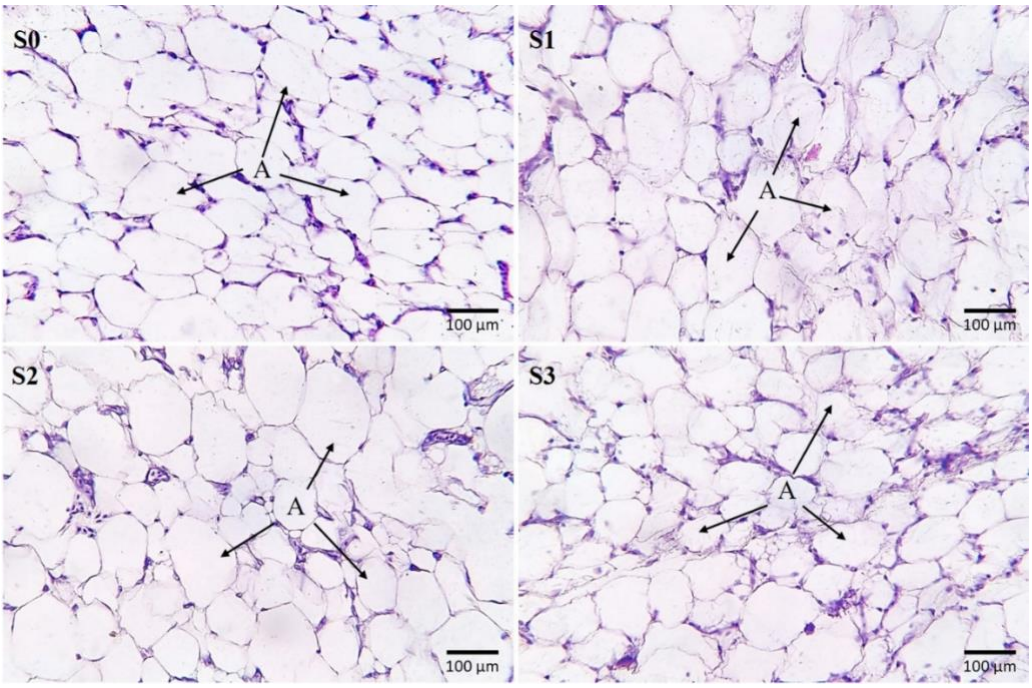

Figure 3. Histoarchitectures of the subcutaneous adipose tissue of different groups of broilers. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi. Hematoxylin-eosin staining. A – Adipocytes. Magnification 400X; Scale bar = 100 µm.

Impact of Clove and Tulsi supplementation on the density of adipocytes

The density of adipocytes (number of adipocytes per microscopic field) in the fat depots is presented in Figure 4. On days 14 and 28, the S1 and S2 groups' adipocyte density in

both fat depots were found to be comparatively lower ($P > 0.05$) than that of the S0 and S3 groups. The density of adipocytes decreased on day 28 compared to that of day 14, indicating the increased size of the adipocytes. On days 14 or 28, however, there was no discernible distinction ($P > 0.05$) between the groups.

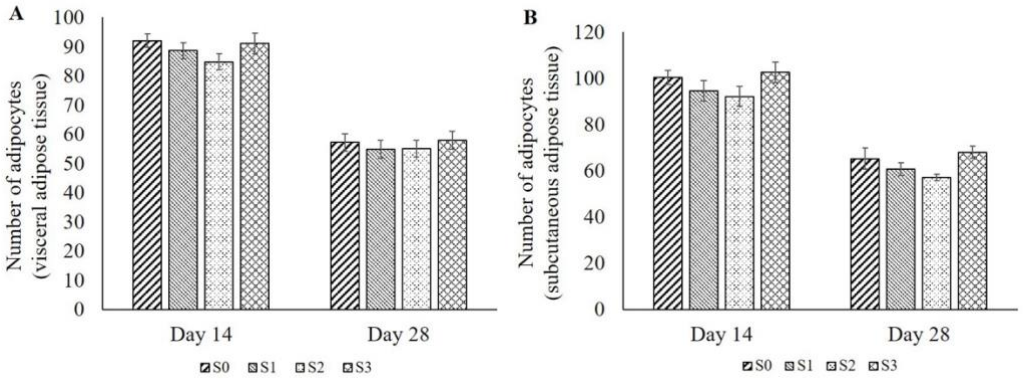

Figure 4. The density of adipocytes (number of adipocytes/microscopic field) within the visceral (A) and subcutaneous (B) fat depots. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi. The density of adipocytes numerically decreased on day 28 compared to that of day 14, indicating the increased size of the adipocytes. However, no significant difference was observed among the groups on day 14 or 28. Data are presented as mean ± SEM. Data were analyzed using one-way ANOVA with the post hoc DMRT.

Impact of Clove and Tulsi supplementation on the size of adipocytes

Figure 5 presents the size of the adipocytes in the fat depots. The size of the adipocytes in both fat depots increased on day 28 compared to day 14, which indicates a correlation between the age of broilers and adipocyte size. Despite the S1 and S2 groups' somewhat larger adipocytes, there was no statistically significant difference between the experimental groups ($P > 0.05$).

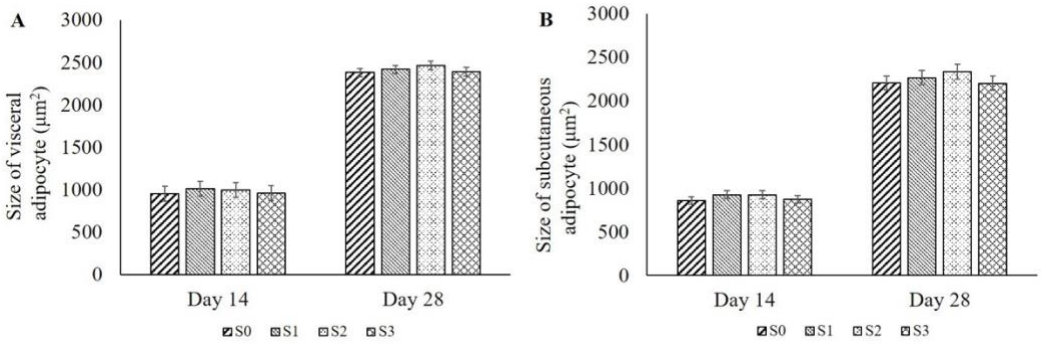

Figure 5. The size of adipocytes (μm^2) within the visceral (A) and subcutaneous (B) fat depots. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi. The size of the adipocytes increased on day 28 compared to day 14. However, the groups had no mentionable difference in size on days 14 or 28. Data are presented as mean ± SEM. Data were analyzed using one-way ANOVA with the post hoc DMRT.

Impact of Clove and Tulsi supplementation on the circularity index of the adipocytes

The circularity index of the visceral and subcutaneous adipocytes is given in Figure 6. The circularity index values of the adipocytes in both fat depots were mostly found

between 0.6-0.9, indicating rounded to oval-shaped adipocytes. The patterns of the adipocyte circularity index values showed no distinguishable difference ($P > 0.05$) in the visceral or subcutaneous fat depots.

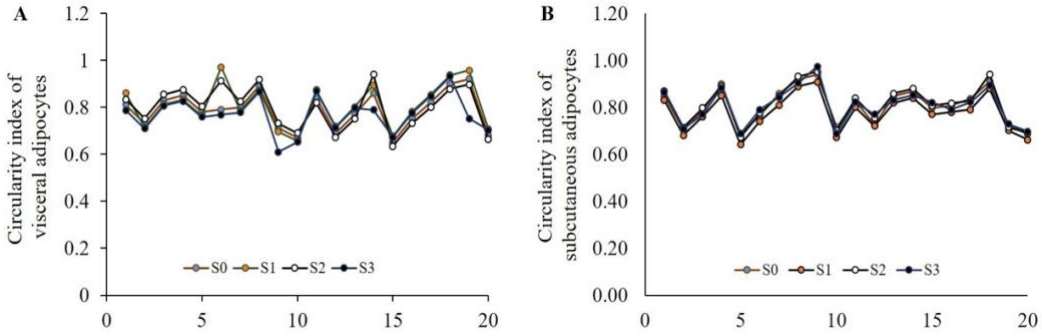

Figure 6. The circularity index of adipocytes (randomly selected 20 adipocytes) of the visceral (A) and subcutaneous (B) fat depots. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi.

Impact of Clove and Tulsi supplementation on the percentage of smaller and larger adipocytes

The percentages (%) of smaller and larger adipocytes in the fat depots are shown in Figure 7. On day 14, no larger adipocyte was found in the fat depots (the diameter of the adipocytes was below 25 μm). On the contrary, the percentage of larger adipocytes increased in the S1 and S2 groups on day 28 in both the fat depots.

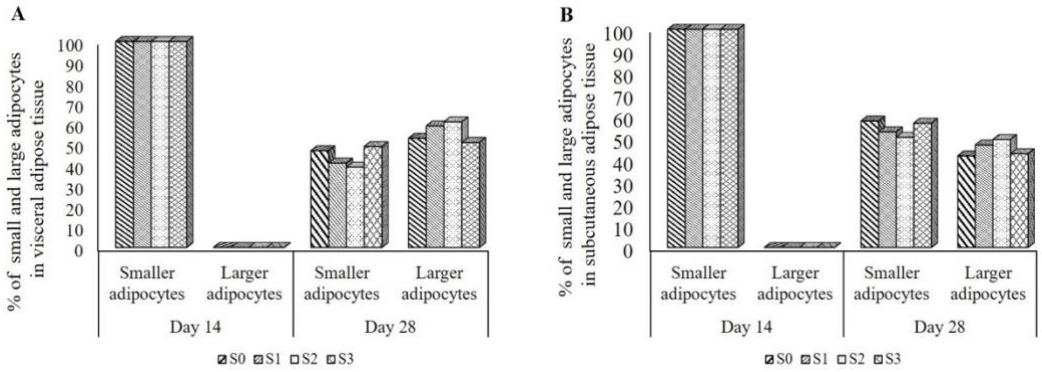

Figure 7. The percentage (%) of smaller and larger adipocytes in the visceral (A) and subcutaneous (B) fat depots. S0 – Control, S1 - 0.5% Clove and 2% Tulsi, S2 - 1% Clove and 3% Tulsi; and S3 - 1.5% Clove and 4% Tulsi.

DISCUSSION

Energy storage is the primary role of adipose tissue, which is functionally regarded as an endocrine organ [13]. In broilers, the major sites for fat deposition are the visceral and subcutaneous regions of the body [16]. The deposition of large amounts of fat results in economic losses as most of it is discarded before cooking or processing. Phytobiotic growth promoters like Clove and Tulsi reportedly promote growth performance [18]. However, the impacts of Clove and Tulsi on fat deposition and adipocyte cellularity are still unknown. Therefore, the present study intends to give a

clear picture of the role of these phytobiotics on fat deposition and the adipocytes' morphologic and morphometric characteristics.

The distribution and deposition of different fat depots are dependent on multiple factors and the current investigation focused on how the feeding of Clove and Tulsi affects the development of the visceral and subcutaneous fat depots in broilers. The present study results suggest that supplementation of 0.5-1% Clove and 2-3% Tulsi extract lowers the relative percentage of subcutaneous fat depot weight while increasing the amount of visceral fat (Figure 1). An earlier study on broiler reported a tendency for fat deposition in the visceral region compared to other body regions which corresponds to the current study findings [19]. According to another study report, 20% of the body fat is deposited in the abdomen, and 18% is in the subcutaneous region, though this proportion fluctuates based on the genetics, nutrition, sex, and age of the broiler chicken [20]. Dietary inclusion of steroid growth promoters like dexamethasone increases fat deposition in broilers by promoting de-novo hepatic lipogenesis [16,21]. The increase in visceral fat deposition may be related to increased feed intake resulting from the appetite-stimulating effects of Clove and Tulsi at 1% and 3%, respectively, and augmented triacylglycerol synthesis [10,18,19]. On the other hand, feeding 1.5% Clove and 4% Tulsi decreases appetite and feed intake in broilers [18], which might be linked to decreased visceral fat deposition in the higher dose group. Moreover, both Clove and Tulsi have strong anti-inflammatory and antioxidant properties. Therefore, higher doses may provide better protection against oxidative stress and inflammation and increase metabolic rate linked to decreased fat accumulation [6, 8, 23, 28]. This visceral fat accumulation is detrimental to broiler health as it is linked to different metabolic disorders and, therefore, needs to be controlled [22]. However, the underlying mechanism of lower amounts of subcutaneous fat deposition in response to Clove and Tulsi supplementation is still unclear and needs further investigation.

Adipocytes are the primary constituent of adipose tissue, and the size of the adipose depots is linked to the size and density of adipocytes [16,23]. The size of adipocytes depends on the amount of lipids they store and, therefore, possess the utmost significance in energy homeostasis [24]. Adipocytes of different sizes, designated as small and large adipocytes, are present in adipose tissue, which is a highly active tissue [25]. Smaller adipocytes support metabolic homeostasis, while larger adipocytes attract macrophages, support inflammation, and release several substances contributing to insulin resistance [26]. Chicken body weight and fat pad weight are positively connected with age-related changes in adipocyte size and number [27]. In the current study, the adipocyte sizes were increased while 0.5-1% Clove and 2-3% Tulsi extract were supplied to the broilers indicating a higher amount of lipid storage within the adipocytes (Figures 2, 3, and 7). The size of the adipocytes is important as they store the excess dietary energy in the form of lipid droplets and thus cause hypertrophy of the adipocytes [10]. Excessive deposition of lipid droplets results in cell wall breakdown and release of the lipid droplets in the circulation, resulting in inflammatory responses and metabolic disorders [10,23,26,28]. Furthermore, larger adipocytes have higher metabolic activity than smaller ones which may be more impacted by lipopolysaccharides [10,20]. Adipocyte numbers and size rise together with the increased accumulation of lipid particles, which causes adipose tissue to expand [10,16]. Therefore, the reduced density of adipocytes with their increase in size indicates a negative correlation between them (Figure 4). The number of larger adipocytes increased in the fat depots of the broiler while fed 0.5-1% Clove and 2-3% Tulsi in the present study. On the other hand, mean adipocyte sizes remained mostly unaffected compared to the control group while fed 1.5% and 4% of Clove and Tulsi (Figures 2,3

and 7). The current study findings and previous reports indicate that feeding 0.5-1% Clove and 2-3% Tulsi improves growth performance along with the augmentation of visceral fat deposition in broilers while feeding 1.5% Clove and 4% Tulsi lowers visceral fat deposition but compromises broiler growth.

CONCLUSION

Supplementation of Clove and Tulsi influences the size and density of adipocytes as well as the amount of fat deposited in the broiler. The age of the broilers and the size of the adipocytes were positively correlated, supporting the findings of the previous study [20]. In addition, supplementation of 0.5-1% Clove and 2-3% Tulsi increases relative visceral fat content and adipocyte size but decreases subcutaneous fat and adipocyte density, which might be associated with different metabolic disorders in the broiler. On the contrary, 1.5% Clove and 4% Tulsi supplementation lower visceral fat deposition without affecting adipocyte cellularity. However, the current study did not provide any information about the effect of high lipid profiles on the body metabolism of broilers as well as the molecular mechanism which needs to be investigated further.

ACKNOWLEDGMENT

We gratefully appreciate the laboratory facilities offered by Bangladesh Agricultural University's Department of Anatomy and Histology in Mymensingh, Bangladesh. We also acknowledge that the day-old chicks were provided by Nourish Poultry and Hatchery Ltd. in Bangladesh. The "Ministry of Science and Technology, Bangladesh" is acknowledged for funding this study (Grant no: BS-56/2021-22).

AUTHOR CONTRIBUTIONS

NS conceptualized and supervised the experiment. RI performed the experiment. SIS assisted in the sample collection and gross data recording. RI analyzed the data and interpreted the results. RI and SIS drafted the manuscript. NS critically revised and edited the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work, ensuring that questions relating to the accuracy or integrity of any part of the work.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] OECD-FAO Agricultural Outlook 2021-2030. OECD Agriculture Statistics (database) 2021. http://www.fao.org/3/cb5332en/cb5332en.pdf.

[2] Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. World's Poult Sci J. 2017; 73(2): 245-256.

[3] Kuldeep Dhama KD, Ruchi Tiwari RT, et al. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances-a review. Int J Pharmacol. 2014; 10: 129-159.

[4] Agostini PS, Sola-Oriol D, et al. Role of in-feed Clove supplementation on growth performance, intestinal microbiology, and morphology in broiler chicken. Livest Sci. 2012; 147(1-3): 113-118.

[5] Al-Mufarrej SI, Al-Baadani HH, et al. Effect of level of inclusion of Clove (*Syzygium aromaticum)* powder in the diet on growth and histological changes in the intestines and livers of broiler chickens. S Afr J Anim Sci. 2019; 49(1): 166-175.
[6] El-Saber Batiha G, Alkazmi LM, et al. *Syzygium aromaticum* L.(Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 2020; 10(2): 202.
[7] Kreydiyyeh SI, Usta J, et al. Effect of cinnamon, Clove and some of their constituents on the Na+-K+-ATPase activity and alanine absorption in the rat jejunum. Food Chem Toxicol. 2000; 38(9): 755-762.
[8] Eevuri TR, Putturu R. Use of certain herbal preparations in broiler feeds-A review. Vet World. 2013; 6(3): 172-179.
[9] Zuidhof MJ, Schneider BL, et al. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult Sci. 2014; 93(12): 2970-2982.
[10] Wang G, Kim WK, et al. Factors affecting adipose tissue development in chickens: A review. Poult Sci. 2017; 96(10): 3687-3699.
[11] Wen C, Chen Y, et al. Dietary betaine improves meat quality and oxidative status of broilers under heat stress. J Sci Food Agric. 2019; 99(2): 620-623.
[12] Mir NA, Rafiq A, et al. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol. 2017; 54: 2997-3009.
[13] Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. Nat Rev Endocrinol. 2019; 15: 507-524.
[14] Symonds ME, Budge H, et al. Adipose tissue development–impact of the early life environment. Prog Biophys Mol Biol. 2011; 106(1): 300-306.
[15] Kim M, Voy BH. Fighting fat with fat: N-3 polyunsaturated fatty acids and adipose deposition in broiler chickens. Front Physiol. 2021; 12: 755317.
[16] Sultana N, Islam R. Modulation of the dynamics and cellularity of adipose tissues in different fat depots in broilers by dietary dexamethasone. J Adv Vet Anim Res. 2022; 9(4): 583-590.
[17] Islam R, Sultana N, et al. Dose and time-dependent effects of glucocorticoid: A morphologic and morphometric study in the broiler lung. Turk J Vet Anim Sci. 2022; 46(4): 617-628.
[18] Islam R, Sultana N, et al. Modulation of growth performance, gut morphometry, and cecal microbiota in broilers by Clove (*Syzygium aromaticum*) and Tulsi (*Ocimum sanctum*) supplementation. Poult Sci. 2023; 102(1): 102266.
[19] Bai S, Wang G, et al. Broiler chicken adipose tissue dynamics during the first two weeks post-hatch. Comp Biochem Physiol Mol Integr Physiol. 2015; 189: 115-123.
[20] Tůmová E, Teimouri A. Fat deposition in the broiler chicken. Sci Agric Bohem. 2010; 41: 121-129.
[21] Cai Y, Song Z, et al. Increased de novo lipogenesis in liver contributes to the augmented fat deposition in dexamethasone exposed broiler chickens (*Gallus gallus domesticus*). Comp Biochem Physiol Part - C: Toxicol Pharmacol. 2009; 150(2): 164-169.
[22] Pascot A, Despres JP, et al. Contribution of visceral obesity to the deterioration of the metabolic risk profile in men with impaired glucose tolerance. Diabetologia. 2000; 43: 1126-1135.
[23] Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm. 2010; 2010: 289645.
[24] Goss AM, Gower B, et al. Effects of weight loss during a very low carbohydrate diet on specific adipose tissue depots and insulin sensitivity in older adults with obesity: a randomized clinical trial. Nutr Metab. 2020; 17(1): 1-2.
[25] Stenkula KG, Erlanson-Albertsson C. Adipose cell size: importance in health and disease. Am J Physiol - Regul Integr Comp Physiol. 2018; 315(2): R284-R295.
[26] Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr. 2006; 83(2): 461S-465S.
[27] Hersoug LG, Møller P, et al. Role of microbiota-derived lipopolysaccharide in adipose tissue inflammation, adipocyte size and pyroptosis during obesity. Nutr Res Rev. 2018; 31(2): 153-163.
[28] Luo L, Liu M. Adipose tissue in control of metabolism. J Endocrinol.2016; 231(3): R77-R99.