

**EFFECTS OF SUPPLEMENTING LEMON JUICE AND MOLASSES ON PRODUCTIVE PERFORMANCE, CARCASS CHARACTERISTICS AND HEMATOBIOCHEMICAL PARAMETERS OF**

**COMMERCIAL BROILER**

**Saidur Rahman**

Examination Roll No: 116/08; Registration No: 291

Semester: January-June 2016

A thesis submitted in partial of the requirements for the fulfillment of the degree of Master of Science in Animal and Poultry Nutrition

Department of Animal Science and Nutrition

Faculty of Veterinary Medicine

Chittagong Veterinary and Animal Sciences University

Khulshi, Chittagong-4225, Bangladesh

**June 2018**

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This is to certify that we have examined the above Master’s thesis and have found that the thesis is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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| \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  (Md. Ahasanul Hoque, PhD)  **Co-supervisor**  Professor, Department of Medicine and Surgery, CVASU | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  (Md. Emran Hossain)  **Supervisor**  Professor, Department of Animal Science and Nutrition, CVASU |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Prof. Md. Manirul Islam, PhD)

**Chairman**

Examination Committee

Department of Animal Science and Nutrition

Chittagong Veterinary and Animal Sciences University

Khulshi, Chittagong-4225, Bangladesh

**Authorization**

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**The Author**

**June 2018**

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**List of Abbreviations**

|  |  |  |
| --- | --- | --- |
| ANOVA | - | Analysis of variance |
| BBS | - | Bangladesh Bureau of Statistics |
| BCRDV | - | Baby Chick Ranikhet Disease Vaccine |
| BMD | - | Bangladesh Meteorological Department |
| CF | - | Crude fibre |
| CP | - | Crude protein |
| DM | - | Dry matter |
| EE | - | Ether extract |
| FAO | - | Food and agriculture organization |
| FCR | - | Feed conversion ratio |
| g | - | Gram |
| IBD | - | Infectious Bursal Disease |
| IBD | - | Infectious Bronchitis Disease |
| Kg | - | Kilogram |
| LW | - | Live weight |
| ME | - | Metabolizable energy |
| NFE | - | Nitrogen free extract |
| NS | - | Non-significant |
| SEM | - | Standard error of mean |
| SGOT | - | Serum Glutamic Oxaloacetic Transaminase |
| SGPT | - | Serum Glutamic Pyruvic Transaminase |
| MMT | - | Million metric ton |
| % | - | Percentage |
| et al | - | And his associates |
| LDL | - | Low Density Lipoprotein |
| HDL | - | High Density Lipoprotein |
| GDP | - | Gross Domestic Products |
| g/l | - | Gram per liter |
| v/v | - | Volume by Volume |
| w/v | - | Weight by volume |
| **°**C | - | Degree celsius |
| e.g | - | Example |
| i.e. | - | That is |
| ˂ | - | Less than |
| ˃ | - | Greater than |
| etc | - | Et cetera |

**Abstract**

Two hundred forty Cobb 500™ unsexed broiler chicks were used in a 28-day trial in a poultry farm at Guimara, Khagrachari Hill district to study the effects of supplementing lemon juice and molasses on productive performance, carcass characteristics and hemato-biochemical parameters of commercial broiler. Lemon juice and molasses were added with drinking water and fed in addition to regular pellet diet. The experiment was carried out in a 2×4 factorial arrangement with 2 levels of lemon juice (0 and 1 ml/L of drinking water) and 4 levels of molasses (0, 0.5, 1 and 1.5% of drinking water from 1-2 wks and 0, 2.0, 2.5 and 3.0% of drinking water from 3-4 wks). Birds were randomly distributed into 24 floor pens where 10 birds were allocated per pen. All birds had free access to ad-libitum feeds and water. Results indicated that, live weight significantly (p<0.01) increased in 3rd and 4th weeks, weight gain increased (p<0.01) in 1st and 3rd weeks, feed intake improved (p<0.001) in 1st and 3rd weeks and FCR improved (p<0.05) only in the 1st week as the main effects of supplementing lemon juice in the drinking water of the commercial broiler birds. On average, during 0-3 weeks, lemon juice exerted positive effects (p<0.01) on feed intake and weight gain. However, only feed intake improved (p<0.01) during 0-4 weeks. Similar to lemon juice, molasses improved (p<0.01) live weight in 2nd, 3rd and 4th weeks, weight gain increased (p<0.001) in 1st and 3rd weeks and feed intake improved (p<0.05) in 1st, 2nd and 3rd weeks and FCR improved (p<0.05) in 2nd, 3rd and 4th weeks as the main effect. Overall, feed intake (p<0.001), weight gain (p<0.001) and FCR (p<0.05) were improved during 0-3 weeks and only feed intake improved (p<0.001) during 0-4 weeks. Unlike main effects, combined effects of lemon juice and molasses were significant only for weight gain in 3rd week (p<0.05) and FCR in 1st and 4th weeks (p<0.01). Main effect of supplementing lemon juice was positive for wing (p<0.05), drumstick (p<0.001), neck (p<0.001), skin (p<0.05), intestine (p<0.001), breast muscle (p<0.001), liver (p<0.05), head (p<0.001), abdominal fat (p<0.001) and feather weight (p<0.001). Similarly, main effect of supplementing molasses was positive for drumstick (p<0.001), neck (p<0.001), intestine (p<0.05), breast muscle (p<0.001), shank (p<0.05), liver (p<0.05), head (p<0.05) and abdominal fat weight (p<0.01). Combined effects were positive for dressing percent (p<0.05), wing weight (p<0.05), drumstick weight (p<0.05), breast muscle weight (p<0.001), thigh muscle weight (p<0.05), gizzard weight (p<0.05) and abdominal fat weight (p<0.05). Main effect of supplementing both lemon juice and molasses were significant (p<0.01) for glucose, albumin, total protein, LDL, HDL and cholesterol. However, combined effects were significant (p<0.01) only for glucose, albumin, LDL and HDL. There was a positive relationship between cholesterol and glucose (r=0.70; p<0.01) and a negative relationship between total protein and glucose (r=-0.80; p<0.001). Inclusion of 1 ml/litre lemon juice and 3% molasses in drinking water in addition to regular pellet diet is recommended for better growth, optimum FCR and desirable carcass characteristics in commercial broiler.

**Keywords:** Carcass characteristics, hemato-biochemical parameters, lemon juice, molasses, productive performance

**Chapter I: Introduction**

The poultry sub-sector is an important avenue in fostering agricultural growth and reducing malnutrition for the people in Bangladesh. It is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity including support services for about 6 million people (**Ansarey, 2012)**. This sub-sector has been proven as an attractive economic activity, thereby, indicating its` importance for the entire economy. The sector accounts for 14% of the total value of livestock output and is growing rapidly **(Raihan and Mahmud, 2008).** It is reported that, poultry meat alone contributes 37% of the total meat production in Bangladesh. Poultry contributes about 22-27% of the total animal protein supply in the country **(Prabakaran, 2003)**. Thus, development of poultry has generated considerable employment opportunities through the production and marketing of poultry and poultry products in Bangladesh (**Ansarey, 2012).**

In the tropical regions like Bangladesh, the high ambient temperature is a major concern that adversely influences the physiological status and hence reduces the performance of broiler chickens. Heat stress is one of the major concerns in the poultry industry since it causes high mortality and low productivity, especially in summer season. It has been reported that heat stress reduces feed intake, weight gain, feed efficiency and immunity of broiler chickens (**Siegel, 1995; Borges *et al*., 2003; Lin *et al*., 2006**). Maximum performance was demonstrated in broiler chicken at temperature ranging from 18 to 240C while low performance observed in broiler chicken raised above 320C **(Alleman and Leclerq,1997).** The heat stress can be minimized by improving taste and quality of drinking water.

Bangladesh has the highest density of population in the world which accommodates about 157 million people covering 148460 km2. To meet the huge protein demand for a large population we must increase our poultry production even under adverse condition. Therefore, innovative ideas and sustainable technologies are developed for production of broiler in high ambient temperature.

Several studies have reported that administration of vitamin C alleviates the deleterious effects of heat stress on performance and metabolism of broiler chickens **(Kutlu and Forbes, 1993; Sahin *et al*., 2002; Gursu *et al*., 2004)**. In fact, vitamin C is the first line of defense against reactive oxygen species in the body (**McDowell, 1989).** In birds, vitamin C is generally synthesized in kidneys, but its quantity is not enough during heat stress condition since the rate of its usage for scavenging of the free radicals is increased. The ascorbic acid content in lemon (*Citrus limon*) juice obtained by fruit squeezing is reported to be 54.74 mg/100 mL **(Pisoschi *et al*., 2011)**. The active anti-oxidant compounds in lemon are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins, also some compounds found in natural foods such as vitamins C (**Prior, 2003).**

It was reported that, plasma concentration of antioxidants and vitamins such as vitamin C and E reduced the oxidative damage in birds during heat stress condition (**Sahin *et al*., 2002**) and addition of lemon juice to drinking water improved immunity of broiler chickens under heat stress condition (**Kadam *et al*., 2009).**

Molasses is a useful effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane or sugar beets **(Curtin, 1983).** Cane molasses is a viscous and dark colored liquid which is rich in soluble carbohydrate, vitamins, minerals and other nutrients (**Olbrich, 1963; Reddy *et al.,* 1998; Ndelekwute *et al.,* 2010**). Some of the mineral contents are iron, zinc, copper, manganese, potassium, sodium and calcium. The vitamins belong to vitamin B complex including: thiamine, riboflavin, niacin, panthothenic acid, biotin and choline. Molasses is a good quality pellet binder and appetizer for poultry feed except it lacks vitamin C and has very low content of phosphorus (**Curtin, 1983).** Despite many advantages, literature related to supplementation of lemon juice and molasses in drinking water in addition to regular pellet diet is scarce in Web of science, Pubmed and Google scholar. Therefore, following research hypothesis, general objective and specific objectives were developed for the study.

**1.1 Research hypothesis**

Supplementation of lemon juice and molasses in drinking water in addition to basal diet may improve productive performance, carcass characteristics and hemato-biochemical parameters in commercial broiler.

**1.2 General objective**

Assess the feasibility of using lemon juice and molasses in the drinking water of commercial broiler birds in addition to regular diets.

**1.3 Specific objectives**

1. To measure the effects of different levels of lemon juice and molasses added with drinking water on productive performance of commercial broiler.
2. To estimate the effects lemon juice and molasses on carcass characteristics of commercial broiler.
3. To determine the changes in serological parameters of commercial broiler fed diets supplemented with lemon juice and molasses.

**Chapter II: Review of Literature**

**2.1. Background**

According to the national health strategy, an adult people need 120 g of meat every day and 104 pieces of eggs per year. However, presently the availability is only 67.17 and 63.65%, respectively**(DLS, 2015)**. Although meat production has been increasing over time in the country but the per capita availability is far below the minimum requirement**(Begum, 2008).** Recently, the demand for poultry and livestock products, in general has been increased significantly that leads most to poultry-related development interventions promoting intensification of traditional poultry systems**(FAO, 2009)**. Under these circumstances to meet up the deficiency of meat and egg, the government and private organizations should put efforts together to enhance the present meat and egg production status.

The government is getting interested in this sector and is encouraging both urban and rural people to work here and enhance capacity. People in rural areas are getting attracted to this sector and taking it up as a business**(Ansarey, 2012).** Besides government, there are many private enterprises, like Aftab Poultry, Aman Poultry, Bangladesh Rural Advancement Committee, CP (Bangladesh) Co., Ltd., Kazi Poultry, Nourish Poultry; Paragon Poultry etc. are working for poultry and poultry products development in the country. Moreover, good management practices should improve for the development of poultry industry. Experiences from South Asian countries show that adoption of good practices of poultry management can significantly contribute to an improvement of farmer’s livelihoods **(Huque et al., 2011).**

**2.2. Contribution of livestock and poultry**

Livestock and poultry have playing an important role in the national economy, contributing significantly to agriculture and the gross national product. About 44% of human daily intake of animal protein comes from livestock products. Furthermore it plays a pivotal role in the rural socio economic system as maximum households directly involved in livestock. It has created job opportunity for more than 6 million people. The poultry industry has been engaging supply of quality protein to the Bangladesh population at the lowest price in the world. In the 90s total investment in this sector was only BDT 15 thousand million, but now it is more than BDT 150 thousand million. Investment in poultry sector should be doubled within the next decade and it will enhance the growth of this sector and contribute in the GDP and creates employment opportunity. The contribution of livestock and poultry in the national economy of Bangladesh is presented in Table 1.

**Table 2. 1** Contribution of livestock and poultry in the national economy of Bangladesh

|  |  |
| --- | --- |
| **Particulars** | **Contributions** |
| Contribution of livestock in Gross Domestic Product (GDP) | 2.50% |
| GDP Growth rate of livestock | 2.83% |
| GDP volume in million (Taka) | 132580 BDT |
| Share of livestock in agricultural GDP | 14.08% |
| Foreign exchange earnings (from hides and skin) | 4.31% |
| Nutition (combined with fisheries sector) | 80% |
| Employment (Directly) | 20% |
| Employment (Partly) | 50% |
| Cultivation of land | 50% |
| Animal draft power in agriculture | 50% |
| Animal draft power in transportation | 50% |
| Manure production | 80 MMT |
| Organic manure production | 10% of chemical fertilizer |
| Fuel supply | 25% |

**2.3. Growth of poultry in Bangladesh**

Poultry population in Bangladesh is estimated about 304.17 million where chicken population is about 255.31 million and duck population is about**(DLS, 2013)** 48.86. Changing pattern and growth of poultry since 2004-05 to 2013-14 is shown in Table 4 .The growth rate of chicken and duck for last 10 years was 3.75 and 3.05% respectively. Growth rate of poultry was about 6.21% during 2004-2007 but growth rate declined (2.70%) during the financial year 2007-08 due to incidence of bird flu (avian influenza). This situation improved during 2008-09 (4.20%) and again declined since 2009-10 about 2.89%.

**Table 2.2** Population and growth rate of chicken and duck in Bangladesh

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** | **Chicken** | |  | **Duck** | |  | **Total** | |
| **No. (million)** | **Growth(%)** |  | **No. (million)** | **Growth (%)** |  | **No. (million)** | **Growth (%)** |
| 2004-05 | 183.45 | - |  | 37.28 | - |  | 220.73 | - |
| 2005-06 | 194.82 | 6.21 |  | 38.17 | 2.39 |  | 232.99 | 5.55 |
| 2006-07 | 206.89 | 6.20 |  | 39.08 | 2.38 |  | 245.97 | 5.57 |
| 2007-08 | 212.47 | 2.70 |  | 39.84 | 1.95 |  | 252.31 | 2.58 |
| 2008-09 | 221.39 | 4.20 |  | 41.23 | 3.49 |  | 262.62 | 4.09 |
| 2009-10 | 228.04 | 3.00 |  | 42.68 | 3.52 |  | 270.72 | 3.08 |
| 2010-11 | 234.69 | 2.92 |  | 44.12 | 3.37 |  | 278.81 | 2.9 |
| 2011-12 | 242.87 | 3.49 |  | 45.70 | 3.58 |  | 288.57 | 3.5 |
| 2012-13 | 249.01 | 2.53 |  | 47.25 | 3.39 |  | 296.26 | 2.66 |
| 2013-14 | 255.31 | 2.53 |  | 48.86 | 3.41 |  | 304.17 | 2.67 |
| Av. (10 years ) | | 3.75 |  |  | 3.05 |  |  | 3.62 |

**2.4. History of lemon juice and vitamin C**

In Early 1700s, British Navy and seafarers who would spend months at sea fall in scurvy disease without supply of Vitamin C. In mid 1700s, Physicians had , however discovered that citrus fruits were an efficient cure for the disease and the late 1700s, all Royal Navy ships were required to serve lemon juice in Rations. Despite this recommendations, a lack of awareness of vitamin C content of lemons and limes, meant scurvy again become an issue in the early 1900s. when the royal navy began to start substituting lime juice for lemon juice ,as they could source these from within the british colonies,they did so under the assumption that the acidity of lemons was what warded off scurvy, and as limes were more acidic, it followed they would be equally effective. This had dire consequences, with several arctic expeditions succumbing to scurvy due to failure of lime juice to provide enough Vitamin C.

The confusion this caused was not fully resolved until the eventual isolation and discovery of vitamin C by the **Hungarian Albert Szent-Györgyi** in **1932**. Later awarded Nobel prizes with **Sir Walter Norman Haworth in** 1937. Vitamin C was actually named after the scurvy prevention abilities-the name ‘Ascorbic Acid ‘comes from ‘**Anti scorbutic**’ a term used to refer to substances preventing scurvy.

**2.5. Composition and Chemistry of Lemon Juice**

The main organic acids contained in the flesh of lemons are **citric acid** and **malic acid**. The sourness of citrus fruit is the taste of citric acid. Citric acid was first isolated in 1784 by the chemist Carl Wilhelm Scheele, who crystallized it from lemon juice .

|  |  |  |
| --- | --- | --- |
| C:\Users\Md. Emran Hossain\Desktop\download.png  Citric acid | Image result for formula of Ascorbic acid  Ascorbic acid | C:\Users\Md. Emran Hossain\Desktop\1200px-Äpfelsäure3.svg.png  Malic acid |

The amounts of each organic acid found in fruit juices were shown in below Table 3. It is clear that the. Previous researchers indicated the impact of species was significant on organic acid distribution of fruit juices **(Cunha et al., 2002)**, **citric acid** is the major organic acid found in fruit juices (7.1-85.2g/l), followed by malic acid and lactic acid. In general, oxalic, tartaric and **ascorbic acids** (0.911g/l) were present in minor quantities in citrus juices.

**Table 2.3** Percentage of different organic acids in different fruit source

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Fruit | Oxalic acid | Tartaric acid | Malic acid | Lactic acid | Citric acid | Ascorbic acid |
| Sweet orange | 0.119 | 0.409 | 1.156 | 1.789 | 12.989 | 0.526 |
| Red apple | 0.268 | 0.237 | 0.871 | 1.229 | 12.998 | 0.009 |
| White apple | 0.125 | 0.113 | 0.397 | 0.632 | 7.102 | 0.004 |
| Lime | 0.131 | 0.011 | 6.122 | 0.612 | 62.422 | 0.442 |
| Lemon | 0.084 | 0.086 | 0.989 | 1.322 | 85.256 | 0.911 |
| Pink grapefruit | 0.147 | 0.106 | 2.819 | 0.696 | 20.723 | 0.622 |
| White grapefruit | 0.117 | 0.156 | 1.802 | 0.725 | 23.022 | 0.741 |

**2.6. Beneficial effect of lemon juice**

Lemon juice is composed of several important organic acids which have much beneficial effect on immunity, feed intake and antimicrobial property. Organic acids have been approved by European legislation as an alternate source of antibiotic growth promoter in diets, because antibiotics are associated with problems due to potential residual effects via food animals developing resistant strains of pathogens. Citric acid (CA) is a weak organic acid which is a natural preservative and can add an acidic or sour taste. Its inclusion at 0.5% in the diet improves performance and non-specific immunity of broilers. It also enhanced specific immunity against new castle disease in vaccinated broilers.

Organic acids have mainly been used in order to sanitize feed to prevent issues such as salmonella infections in animals (**Thompson and Hinton, 1997**). Their effect in animal diets may also suppress pathogenic growth and improve digestion, absorption, mucosal immunity and topical effects on the intestinal brush border (**Mroz, 2005**). Some acids increase pepsin proteolysis secretion and the release of hormones, including gastrin and cholecystokinin, which regulate the digestion and absorption of protein (**Vargas, 2002; Afsharmanesh and Pourreza, 2005**).

By reducing the pH of the feed, organic acids can decrease bacterial contamination prior to consumption by the animal, making them useful as feed preservatives (**Mroz *et al.,* 1997**). The acid decreases pH of caecaldigesta (**Jozefiak and Rutkowski, 2005**), crop, gizzard (**Andrys *et al.,* 2003**) and intestine (**Denil *et al.;* Rahmani *et al.,* 2005**) in broiler chicks. They exhibit specific antibacterial effects at low pH, which may help to reduce overall bacterial numbers or modify distribution of bacterial species in the gut and thus improved the birds health status.

**2.7. Action of citric acid against micro-organisms**

Non-ionized organic acids can penetrate the bacteria cell wall and disrupt the normal functioning of pH sensitive bacteria, so that they cannot tolerate a wide internal and external pH gradient **(Mroz, 2005).** The overall modes of action of organic acids on bacteria are:

1. Undissociated forms of acid diffuse across cell membrane of pathogens, destroying their cytoplasm or inhibiting growth
2. Intestinal dissociation liberates H+ ions serving as a pH barrier inhibiting pathogen colonization on the brush border

3. Bacterial membrane disruption

4. Inhibition of essential metabolic reactions

5. Stress on intracellular pH homeostasis

6. Accumulation of toxic anions

7. Energy stress response to restore homeostasis

**Akyurek et al. (2011)** found that addition of organic acid in ileal digesta caused lactic acid bacteria counts to be significantly increased, whereas Escherichia coli were significantly decreased. **Tolba (2010)** observed a reduction in pathogenic burden due to addition of CA in broiler chicks. Reducing the bacterial burden due to addition of organic acid in feed can caused gut health parameters to improve significantly, which may be related to an increase in the availability of nutrients.

**2.8. Applications of molasses**

Molasses is a sticky dark by-product of processing sugar cane or sugar beets into sugar. The term molasses specifically refers to the final effluent obtained in the separation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane or sugar beets **(Curtin, 1983).** In general any liquid feed ingredient that contains sugar in excess of 43% is termed molasses. Molasses can be a source of quick energy and an excellent source of minerals for farm animals. The calcium content of sugar cane molasses is high (up to one percent), whereas the phosphorus content is low. Cane molasses is also high in sodium, potassium, magnesium and sulphur. Beet molasses is higher in potassium and sodium but lower in calcium. Molasses also contains significant quantities of trace minerals such as copper, zinc, iron and manganese.

**2.9. Types of Molasses**

There are many types of molasses which have been described and specified by the Association of American Feed Control Officials. These types include:

**A. Cane Molasses**

Is a by- product of manufacture or refining of sucrose from sugar cane. It is specified by **AAFCO (1982)** to contain not less than 46% total sugars expressed as invert, its moisture content exceeds 27%.The early literature on production and processing of cane molasses has been presented by **Anonymous (1970)**, and **Meade and Chem (1977).** There are many types of sugar cane molasses. The molasses usually available for animal feeding is known as Black strap or final molasses.

**I. Black strap (Final molasses)**

This is a by- product of cane sugar industry, from which the maximum crystalline sugar has been extracted by the normal methods. It is most commonly used in animal feeding. In addition to sucrose, it contains glucose and fructose which are fermentable. Black strap molasses also contain substances which are not fermentable by yeast. The non fermentable reducing content of molasses may be present as high as 17% in black strap molasses.

**II. Integral molasses**

It is unclarified molasses, made by partially inverting sugar cane juice to avoid crystallization of sucrose.

**III. High test molasses**

High test molasses result from the conversion of clarified whole sugar cane juice into molasses. The process involves application of 5 invertase enzyme and sulphuric acid to the cane juice resulting in syrup. Because the sugar was not excreted the high –test molasses has a greater concentration of sugars and lower concentration of minerals compared to other types of molasses.

**IV. Condensed molasses**

This is the by-product developed by condensing the residue from yeast fermentation to commercial alcohol.

**B. Beet Molasses**: Is a by-product of the manufacture of sucrose from sugar beet. It carries the same specifications as cane molasses.

**C. Citrus Molasses:** It is the dehydrated juices obtained from the manufacture of dried citrus pulp.

**D. Hemicellulose Extract:** Is a by-product of the manufacture of pressed wood, obtained by the treatment of wood at elevated temperature and pressure

**E. Starch Molasses:** Is a by- product of dextrose manufacture from starch derived from corn or grain sorghum, where the starch is hydrolyzed by enzymes and / or acid.

**2.10. Composition of molasses**

The chemical composition of molasses shows a wide variation as its composition is influenced by many cultural factors such as the type of soil used for cultivation of the crop, ambient temperature, moisture, season of crop production and variety; in addition to production practices, plant processing, and the storage conditions. The variation in composition exists in nutrients content, flavor, color, viscosity and total sugar content (**Anonymous, 1970; Hendrickson and Kesterson, 1971; Presten and Willis, 1974**). All types of molasses contain relatively large amounts of total sugars and other carbohydrates; and these compounds are responsible for the feeding value of molasses. Sugar content of molasses varies according to the production technology employed, (**Baker, 1981**). In addition to sucrose, molasses contains glucose, fructose, raffinose, and numerous non- sugar organic materials.

According to **Presten (1987) and Ly (1987)**, molasses and sugar cane juice are characterized by their extremely high NFE value, and no fiber, and negligible amount of ether extract and protein (**Agrana, 2016**). All types of molasses contain a small quantity of crude protein (3% in mineral soil 6 reaching 10% in organic soil) (**Champman *et al*., 1965**). Also the nitrogenous material in molasses consists mainly of non- protein nitrogen compounds (amides, albuminoids, amino acids and other simple nitrogenous compounds).These two factors limit its nutritional value for non-ruminants.

The mineral content of molasses shows wide variation within molasses types, and variability of trace minerals can be quite high (**Curtin, 1973**). In comparison to commonly used sources of dietary energy, mainly cereal grains,

the calcium, potassium, magnesium, sodium, chlorine, and the sulfur content of cane molasses are high; whereas the phosphorus content is low. The trace minerals (Copper, Ironand Manganese) content are high in cane molasses. The vitamin content of molasses is subject to wide variations (**Olbinch, 1963; Curtin, 1973**). It is deficient in thiamine, riboflavin, vit. A and vit. D but it is rich in niacin and pantothenic acid. Soluble ash content also varies among molasses types (**Preston, 1986**). Generally molasses is low in phosphorus but cane molasses is an excellent source of trace minerals (Table 4).

**Table 2.4** Chemical composition of molasses

|  |  |  |
| --- | --- | --- |
| **Components** | **Fresh weight** | **Dry matter** |
| Dry matter | 75% | 100% |
| Total sugar | 42% | 56% |
| Crude protein | 10% | 13.30% |
| Crude ash | 12% | 16% |
| Nitrogen-free extract | 58% | 77% |
| Calcium (Ca) | <0.5 g/kg | 0.67 g/kg |
| Potassium (K) | 40 g/kg | 53 g/kg |
| Phosphorus (P) | <0.5 g/kg | 0.67 g/kg |
| Magnesium (Mg) | <0.2 g/kg | 0.27 g/kg |
| Sodium (Na) | 11 g/kg | 14.7 g/kg |

Source: Agrana (2016)

**2.11. Molasses in feeding broilers**

Sugar cane final molasses proved to be suitable when included in quite high levels in diets for both broilers and layers with no detrimental effect on health or performance (**Ricci *et al*., 1980; Lyj, 1990**). However, certain limitations were shown with total replacement of cereal grains by molasses, due to difficulties in mixing diets containing high levels of molasses, in addition to its laxative effects (**Rossenberg, 1955; Rossenberg and Palafox, 1956; Kondo and Ross,1962;Perez, 1968; Connor *et al.,* 1972**).

Inclusion of high levels of molasses in broiler diets increased body weight and feed consumption at 0-4 weeks of age, but the increase of feed intake was not statistically significant; whereas at 4-8 weeks of age molasses inclusion has no effect on either feed intake, body weight gain, feed efficiency or live weight (**Connor *et al.*, 1972; Rhman, 1984; Kabuage *et al.*, 2000)**.

Similarly **Satava *et al*. (1981**) indicated that up to 20% molasses could be used in broiler diets with no reduction in body weight. The same workers concluded that sugar molasses can be included safely at 15 and 20% in finishing diets of broiler. Storage of diets with high levels of molasses causes loss in the nutritive value of mixed feeds (**Ross, 1960**) which would reduce the growth rate of birds and feed efficiency.

**2.12. Energy requirement for broilers**

The energy requirement of birds is met by the chemical energy contained in the feed. Energy is required for maintenance function, growth and other forms of production, and any excess is stored in the form of fats. The optimum energy needs must be provided in the diet. Most energy requirement is derived from soluble carbohydrate represented by starch and sugars **(Say, 1987)**. The efficiency of feed utilization depends upon metabolic energy in nutrients containing adequate amounts of all others required nutrients **(Scott *et al*, 1982).**

**2.13. Digestion of sugar cane molasses by poultry**

Poultry shows a fast rate of passage of digesta through the gastrointestinal tract when sugar cane final molasses are included in large proportion in diet. The laxative effect of sugar cane final molasses, defined as a rapid rate of passage of digesta through the entire gastrointestinal tract, brings about a sharp decrease in the digestibility of diets, thus causing deterioration in the daily body weight gain and feed utilization efficiency. This laxative effect can be neutralized by mixing the molasses with raw sugar or high-test molasses which contains large amounts of sugar (**Perez *et al.,* 1968; Perez and Perston, 1970**). When a laxative condition appears in chicken fed cane molasses, there is no change in the ratio of water excretion between faeces and urine (**Rodriguez *et al.*, 1980**).

**2.14. Sources of Energy**

Energy is derived from the metabolism of carbohydrates, lipids, and protein. Most energy in the animals feed is derived from carbohydrates such as cereal grains, in form of starch. Maize is the most important cereal grain for poultry feed in the Bangladesh, which contains 14-46Mj/kg metabolisable energy. Molasses is the alternative source of CHO which contains high metabotisable energy of about 10-15Mj/ kg **(Smith, 1990).**

**2.15 Summary**

The above discussions have clearly identified the feasibility of using molasses in broiler diets. However, in none of the cases, lemon juice and molasses were supplemented together in drinking water in variable concentration. Although, molasses is a potential feed supplement, it is very sticky in nature which plugs mixing devices of feed mill while added with mash feed resulting decreased feed mixing efficiency. As a result, feed millers are not interested to use molasses in poultry feed. From this perspective, it appears that, there is a clear research gap for incorporation of lemon juice and molasses together in the drinking water of commercial broiler birds. Taking these views in mind, a 28-day trial was conducted to study the effects of supplementing lemon juice and molasses on productive performance, carcass characteristics and hemato-biochemical parameters of commercial broiler.

**Chapter III: Materials and Methods**

**3.1. Study area**

The study was conducted during May to June 2018 in Khagracchari Hill district, the south-eastern part of Bangladesh and research laboratories of the Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh. May-June is considered as summer season in Bangladesh. In May, average temperature was 31.5oC, average humidity was 82.0% and average precipitation was 184.8 mm. In June average temperature was 32.8o C, humidity was 88.0% and average precipitation was 67.5 mm **(BMD, 2015).**

**3.2. Design of the experiment**

The experiment was carried out in a 2×4 factorial arrangement with 2 levels of lemon juice (0 and 1 mL/L of drinking water) and 4 levels of molasses (0, 0.5, 1 and 1.5% of drinking water from 1-2 wks and 0, 2.0, 2.5 and 3.0% of drinking water from 3-4 wks). Birds were randomly distributed into 16 floor pens and 10 birds were allocated per pen.

**Table 3.1** Design of the experiment

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment  Group | Dietary combination  (1st and 2nd week) | Treatment  Group | Dietary combination  (3rd and 4th week) |
| T00 | Lemon 0 ml/L + Molasses 0% | T00 | Lemon 0 ml/L + Molasses 0% |
| T10 | Lemon 1 ml/L + Molasses 0% | T10 | Lemon 1 ml/L + Molasses 0% |
| T01 | Lemon 0 ml/L + Molasses 0.5% | T01 | Lemon 0 ml/L + Molasses 2.0% |
| T11 | Lemon 1 ml/L + Molasses 0.5% | T11 | Lemon 1 ml/L + Molasses 2.0% |
| T02 | Lemon 0 ml/L + Molasses 1.0% | T02 | Lemon 0 ml/L + Molasses 2.5% |
| T12 | Lemon 1 ml/L + Molasses 1.0% | T12 | Lemon 1 ml/L + Molasses 2.5% |
| T03 | Lemon 0 ml/L + Molasses 1.5% | T03 | Lemon 0 ml/L + Molasses 3.0% |
| T13 | Lemon 1 ml/L + Molasses 1.5% | T13 | Lemon 1 ml/L + Molasses 3.0% |

**3.3. Experimental birds and housing**

Two hundred forty Cobb 500 day old unsexed broiler chicks were purchased from CP Bangladesh Co., Ltd. All chicks were examined for abnormalities and uniform size. Average body weight of the chicks was 46.74±0.26 g. The experimental shed has brick cemented floor with no wall but metal net wiring round. Floor space for each bird was 0.25 square feet in brooding box and 1.45 square feet in the floor. The cage was further divided into 24 pens. The pens were selected in an unbiased way for uniform distribution of chicks. The chicks were brooded in the separate brooder house. After 10 days, birds were transferred to the respective pens. Each pen was allocated for 10 birds. Dry and clean newspaper was placed over the litter material (saw dust) in the brooding floor and changed for every 8 hours. Room temperature and humidity was maintained using 200 watt incandescent lamps. The birds were exposed to continuous lighting. Chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th weeks respectively by adjusting the no. of incandescent bulbs. Room temperature was recorded by using thermometers.

**3.4. Cleaning and sanitation**

The shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding house, and rearing pens were cleaned in the same manner. After cleaning and disinfection, the house was left one week for proper drying. After drying, all doors and windows were closed wrapping by curtain. The room was fumigated (Adding 35 ml of formalin to 10 g potassium permanganate per cubic meter) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders were cleaned and washed with Timsen® solution (0.5% w/v) weekly before being used further. Drinkers were washed with potassium permanganate (1% w/v) and dried up daily in the morning.

**3.5. Experimental diets**

Readymade feeds of CP Bangladesh Co., Ltd was purchased from local dealer. There are three types of feed I purchased, Starter-510 (0-13 days), Grower-510S (14-22 days) and Finisher-511 (23-28 days). The proximate composition of starter, grower and finisher feeds are given in Table 6.

**Table 3.2** Proximate analysis of different types of feed of CP Bangladesh

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Feed code** | **Type** | **ME\*** | **CP (%)** | **Ca (%)** | **P (%)** | **EE (%)** | **CF (%)** | **Moisture (%)** |
| 510 | Starter | 3050 | 21.5 | 0.8 | 0.5 | 3.5 | 5.0 | 12.0 |
| 510S | Grower | 3100 | 20 | 0.7 | 0.4 | 4.0 | 5.0 | 12.0 |
| 511 | Finisher | 3250 | 19 | 0.7 | 0.4 | 4.5 | 5.0 | 12.0 |

\***ME**=Metabolizable Energy (kcal/kg); **CP**=Crude protein; **Ca**=Calcium; **P**=Phosphorus; **EE**=Ether Extract

**3.6. Feeding of birds**

Readymade feed was purchased from local dealer and supplied ad-libitum to the birds on round small feeder and waterer for (0-10) days. After 10th day, small round feeders and waterers were replaced by medium round feeders and round waterers. At 22nd day, large round feeder and large round waterers (10 liter capacity) were provided for feeding and drinking of the birds.

**3.7. Vaccination and Medications**

All birds were vaccinated against Newcastle disease (BCRDV live) on the 4th day followed by a booster dose on 18th day and Infectious Bursal Disease (IBD) on 14th day. After each vaccination, multivitamin (Rena-WS, Renata; 1g/ 5liter of drinking water) was supplied to overcome the effect of stress due to vaccination and cold shock.

**3.8. Carcass measurement**

On 4th week of the study period, five birds were randomly selected from each replicate and killed by severing the jugular vein and carotid artery. Once a bird was adequately bled out, it was scalded and feather was removed. After defeathering, the birds were eviscerated and the head and feet were removed as per technique described by **Jones (1984)**. During evisceration process, abdominal fat, lung, liver, spleen, gizzard and proventriculus were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.

**3.9. Serum analysis**

For serological tests blood was collected without anticoagulant from four birds of each group at 21st and 28th days of age. Clotted blood in the vacutainer tube was kept overnight at normal room temperature (25oC) and serum was collected into the Eppendorf tube by micropipette. Sera samples were marked and stored in -20°C until being analyzed for glucose, total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) by Humalyzer 3000 (Semiautomatic, microprocessor-controlled photometer with large graphic LCD screen, Wisbaden, Germany).

Randox® veterinary reagent kits were used for determination of the serum parameter of interest. Serum sample was mixed with the respective reagents in an ependroff tube. The serum with reagent was aspired by spectrophotometric method which measured the target parameter and immediately the printed result was recorded.

**3.10. Data collection**

At weekly interval, weight gain, feed intake and FCR were recorded. Carcass characteristics, serological and biochemical parameters were recorded at 3rd and 4th weeks. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total amounts of feed supplied to the birds. FCR was calculated dividing feed intake by the weight gain.

**3.11. Statistical analysis**

Data were compiled in MS Excel. Raw data related to weight gain, feed intake, FCR, carcass characteristics, hematological and biochemical parameters were tested for outlier and influential factors by using graph matrix. Multicollinearity of the independent variables was tested by using collinearity diagnostic test. Normality of all the explanatory variables were tested by using Shapiro-Wilk W and Shapiro-Francia test. Equality of variance was tested by using Bartlett's test. The corrected data were tested and analyzed for 2-WAY ANOVA by using Stata (Stata/SE 14.1, Stata Statistical Software, Stata Corporation, College Station, TX, USA). Means showing significant differences were compared by Duncan’s New Multiple Range Test **(Duncan, 1955).** Statistical significance was accepted at p<0.05 for F-tests.

**Chapter IV: Results**

**4.1 Productive performance**

Live weight significantly (p<0.01) increased in 3rd and 4th weeks, weight gain increased (p<0.01) in 1st and 3rd weeks, feed intake improved (p<0.001) in 1st and 3rd weeks and FCR improved (p<0.05) only in the 1st week as the main effects of supplementing lemon juice in the drinking water of the commercial broiler birds. On average, during 0-3 weeks, lemon juice exerted positive effects (p<0.01) on feed intake and weight gain. However, only feed intake improved (p<0.01) during 0-4 weeks (Table 4.1).

Similar to lemon juice, molasses improved (p<0.01) live weight in 2nd, 3rd and 4th weeks, weight gain increased (p<0.001) in 1st and 3rd weeks and feed intake improved (p<0.05) in 1st, 2nd and 3rd weeks and FCR improved (p<0.05) at 2nd, 3rd and 4th weeks as the main effect of molasses supplementation. Cumulative feed intake (p<0.001), weight gain (p<0.001) and FCR (p<0.05) were improved during 0-3 weeks and only feed intake improved (p<0.001) during 0-4 weeks.

Unlike main effects, combined effects of lemon and molasses were significant only for weight gain in 3rd week (p<0.05) and FCR in 1st and 4th weeks (p<0.01). On average, FCR was improved (p<0.05) during 0-4 weeks due to combined effects of lemon juice and molasses supplementation.

Marginal means of the combined effects of live weight, weight gain, feed intake and FCR fitted on profile plots substantially differed while compared for the birds provided drinking water without lemon juice and with lemon juice in addition to the gradually increasing concentrations of molasses (Figure 4.1-4.6). Non-parallel trends of the mean profile scores of lemon supplemented versus non-supplemented groups indicated that, both groups were sharply contrasting at various levels of molasses supplementation.

**Table 4.1** Live weight, weight gain, feed intake and FCR of the experimental broiler birds fed diets supplemented with different levels of lemon juice and molasses in drinking water from 1st to 4th weeks.

| **Variable** | Age | **Lemon** | **Molasses** | | | | **SEpooled** | **ηp²** | **Sig.** | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 0 | 1.0 | 2.0 | 3.0 | L | M | L×M |
| Live weight (g/bird/wk) | 1st wk | 0 | 193.6 | 195.3 | 203.1 | 202.8 | 1.04 | 0.93 | NS | NS | NS |
| 1 | 197.6 | 200.2 | 204.9 | 203.4 |
| 2nd wk | 0 | 527.0 | 532.5 | 544.2 | 540.5 | 1.66 | 0.84 | NS | \*\* | NS |
| 1 | 530.2 | 538.7 | 541.1 | 542.6 |
| 3rd wk | 0 | 1076.0 | 1101.2 | 1153.4 | 1113.2 | 10.03 | 0.93 | \*\* | \*\*\* | NS |
| 1 | 1096.2 | 1108.8 | 1181.6 | 1180.2 |
| 4th wk | 0 | 1687.5 | 1712.5 | 1734.5 | 1752.5 | 6.88 | 0.92 | \*\* | \*\*\* | NS |
| 1 | 1706.0 | 1746.0 | 1760.0 | 1775.0 |
| Weight gain (g/bird/d) | 1st wk | 0 | 21.5 | 21.7 | 22.8 | 22.8 | 0.14 | 0.95 | \*\* | \*\*\* | NS |
| 1 | 22.0 | 22.3 | 23.0 | 22.8 |
| 2nd wk | 0 | 41.5 | 42.0 | 42.5 | 42.0 | 0.14 | 0.47 | NS | NS | NS |
| 1 | 41.3 | 42.1 | 41.7 | 42.2 |
| 3rd wk | 0 | 72.3 | 75.1 | 80.8 | 75.6 | 1.24 | 0.93 | \*\* | \*\*\* | \* |
| 1 | 74.7 | 75.1 | 85.2 | 84.8 |
| 4th wk | 0 | 77.2 | 79.1 | 82.8 | 83.7 | 0.83 | 0.84 | NS | NS | NS |
| 1 | 80.9 | 83.7 | 86.3 | 91.3 |
| Feed Intake (g/bird/d) | 1st wk | 0 | 23.8 | 24.9 | 25.4 | 25.6 | 0.23 | 0.88 | \*\*\* | \* | NS |
| 1 | 26.1 | 25.4 | 26.4 | 26.6 |
| 2nd wk | 0 | 58.5 | 60.8 | 62.6 | 62.5 | 4.41 | 0.86 | NS | \*\*\* | NS |
| 1 | 58.9 | 60.3 | 62.5 | 62.3 |
| 3rd wk | 0 | 112.2 | 113.6 | 116.0 | 115.7 | 1.08 | 0.87 | \*\*\* | NS | NS |
| 1 | 114.9 | 118.9 | 121.4 | 124.6 |
| 4th wk | 0 | 136.4 | 140.6 | 139.8 | 142.7 | 0.78 | 0.76 | NS | \* | NS |
| 1 | 138.9 | 142.1 | 143.9 | 148.4 |
| FCR | 1st wk | 0 | 1.1 | 1.1 | 1.1 | 1.1 | 0.01 | 0.82 | \*\* | NS | \*\* |
| 1 | 1.2 | 1.1 | 1.2 | 1.2 |
| 2nd wk | 0 | 1.5 | 1.5 | 1.5 | 1.4 | 0.01 | 0.77 | NS | \* | NS |
| 1 | 1.4 | 1.5 | 1.5 | 1.4 |
| 3rd wk | 0 | 1.6 | 1.5 | 1.4 | 1.5 | 0.02 | 0.81 | NS | \*\* | NS |
| 1 | 1.5 | 1.6 | 1.4 | 1.5 |
| 4th wk | 0 | 1.8 | 1.7 | 1.7 | 1.7 | 0.01 | 0.85 | NS | \* | \*\* |
| 1 | 1.8 | 1.7 | 1.7 | 1.6 |
| Weight gain | 0-3 wks | 0 | 45.1 | 46.3 | 48.7 | 46.8 | 0.47 | 0.94 | \*\* | \*\*\* | NS |
| 1 | 46.0 | 46.5 | 50.0 | 49.9 |
| Feed intake (g/bird/d) | 0 | 65.2 | 66.8 | 68.5 | 68.3 | 0.49 | 0.96 | \*\*\* | \*\*\* | NS |
| 1 | 66.3 | 68.3 | 70.2 | 71.2 |
| FCR | 0 | 1.5 | 1.4 | 1.4 | 1.4 | 0.00 | 0.76 | NS | \* | NS |
| 1 | 1.5 | 1.4 | 1.4 | 1.4 |
| Weight gain (g/bird/d) | 0-4 wks | 0 | 57.2 | 61.2 | 61.9 | 62.2 | 0.55 | 0.70 | NS | NS | NS |
| 1 | 60.9 | 62.4 | 62.9 | 63.0 |
| Feed intake (g/bird/d) | 0 | 84.1 | 86.3 | 85.5 | 86.4 | 0.31 | 0.94 | \*\* | \*\*\* | NS |
| 1 | 84.5 | 86.8 | 87.1 | 87.5 |
| FCR | 0 | 1.6 | 1.5 | 1.5 | 1.5 | 0.00 | 0.75 | NS | NS | \* |
| 1 | 1.6 | 1.5 | 1.5 | 1.5 |

L=Lemon; M=Molasses; L×M=Lemon×Molases; SEpooled=Pooled standard error; ηp²=Partial eta squared of the model; NS=Non-significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001)

|  |  |
| --- | --- |
|  |  |
| **Figure 4.1** Profile plot of the marginal means for live weight at 4th week. | **Figure 4.2** Profile plot of the marginal means for weight gain at 3rd week. |
|  |  |
| **Figure 4.3** Profile plot of the marginal means for feed intake during 0-4 weeks. | **Figure 4.4** Profile plot of the marginal means for FCR at 4th week. |
|  |  |
| **Figure 4.5** Profile plot of the marginal means for FCR during 0-3 weeks. | **Figure 4.6** Profile plot of the marginal means for FCR during 0-4 weeks. |

**4.2. Carcass characteristics**

Main effect of supplementing lemon was positive for wing (p<0.05), drumstick (p<0.001), neck (p<0.001), skin (p<0.05), intestine (p<0.001), breast muscle (p<0.001), liver (p<0.05), head (p<0.001), abdominal fat (p<0.001) and feather weight (p<0.001) (Table 4.2)

**Table 4.2** Carcass characteristics of the experimental broiler birds fed diets supplemented with different levels of lemon juice and molasses at 4th week.

| **Carcass characteristics** | **Lemon** | **Molasses** | | | | **SEpooled** | **ηp²** | **Sig.** | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **0** | **1.0** | **2.0** | **3.0** | **L** | **M** | **LXM** |
| Dressing percent | 0 | 68.9 | 68.7 | 68.1 | 68.4 | 0.09 | 0.74 | NS | NS | \* |
| 1 | 68.4 | 68.2 | 68.9 | 68.6 |
| Wing weight (%) | 0 | 3.3 | 3.3 | 3.4 | 3.2 | 0.03 | 0.77 | \* | NS | \* |
| 1 | 3.3 | 3.4 | 3.3 | 3.5 |
| Drumstick weight (%) | 0 | 4.4 | 4.4 | 4.5 | 4.7 | 0.03 | 0.96 | \*\*\* | \*\*\* | \* |
| 1 | 4.7 | 4.7 | 4.6 | 4.7 |
| Neck weight (%) | 0 | 2.9 | 2.9 | 2.9 | 3.1 | 0.02 | 0.91 | \*\*\* | \*\*\* | NS |
| 1 | 3.0 | 3.0 | 3.1 | 3.1 |
| Heart weight (%) | 0 | 0.7 | 0.8 | 0.7 | 0.7 | 0.02 | 0.70 | NS | NS | NS |
| 1 | 0.7 | 0.7 | 0.8 | 0.9 |
| Proventriculus weight (%) | 0 | 0.8 | 0.8 | 0.8 | 0.8 | 0.02 | 0.57 | NS | NS | NS |
| 1 | 0.9 | 0.7 | 0.8 | 0.9 |
| Skin weight (%) | 0 | 7.0 | 7.2 | 7.2 | 7.2 | 0.03 | 0.67 | \* | NS | NS |
| 1 | 7.2 | 7.0 | 7.0 | 7.0 |
| Blood weight (%) | 0 | 3.7 | 3.8 | 3.9 | 3.7 | 0.03 | 0.29 | NS | NS | NS |
| 1 | 3.9 | 3.8 | 3.8 | 3.8 |
| Intestine and viscera (%) | 0 | 5.3 | 5.2 | 5.3 | 5.2 | 0.03 | 0.91 | \*\*\* | \* | NS |
| 1 | 5.2 | 5.1 | 5.1 | 5.0 |
| Breast muscle weight (%) | 0 | 17.0 | 16.9 | 16.9 | 16.6 | 0.12 | 0.99 | \*\*\* | \*\*\* | \*\*\* |
| 1 | 16.6 | 16.1 | 16.8 | 16.7 |
| Thigh muscle weight (%) | 0 | 4.9 | 4.9 | 4.9 | 4.8 | 0.03 | 0.73 | NS | NS | \* |
| 1 | 4.9 | 4.9 | 4.9 | 5.1 |
| Shank weight (%) | 0 | 1.5 | 1.5 | 1.5 | 1.6 | 0.01 | 0.64 | NS | \* | NS |
| 1 | 1.5 | 1.5 | 1.5 | 1.6 |
| Liver weight (%) | 0 | 2.5 | 2.6 | 2.6 | 2.6 | 0.02 | 0.78 | \* | \* | NS |
| 1 | 2.6 | 2.6 | 2.7 | 2.7 |
| Gizzard weight (%) | 0 | 2.2 | 2.4 | 2.4 | 2.4 | 0.02 | 0.61 | NS | NS | \* |
| 1 | 2.4 | 2.3 | 2.3 | 2.3 |
| Head weight (%) | 0 | 1.9 | 2.0 | 2.0 | 2.1 | 0.03 | 0.87 | \*\*\* | \* | NS |
| 1 | 2.1 | 2.2 | 2.1 | 2.3 |
| Abdominal fat weight (%) | 0 | 1.5 | 1.5 | 1.6 | 1.5 | 0.03 | 0.89 | \*\*\* | \*\* | \* |
| 1 | 1.5 | 1.6 | 1.8 | 1.8 |
| Feather weight (%) | 0 | 4.0 | 3.8 | 3.9 | 3.7 | 0.05 | 0.80 | \*\*\* | NS | NS |
| 1 | 3.6 | 3.5 | 3.4 | 3.5 |

L=Lemon; M=Molasses; L×M=Lemon×Molases; SEpooled=Pooled standard error; ηp²=Partial eta squared of the model; NS=Non-significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01)

Similarly, main effect of supplementing molasses was positive for drumstick (p<0.001), neck (p<0.001), intestine (p<0.05), breast muscle (p<0.001), shank (p<0.05), liver (p<0.05), head (p<0.05) and abdominal fat weight (p<0.01).

Combined effects were positive for dressing percent (p<0.05), wing weight (p<0.05), drumstick weight (p<0.05), breast muscle weight (p<0.001), thigh muscle weight (p<0.05), gizzard weight (p<0.05) and abdominal fat weight (p<0.05).

There was a positive relationship between abdominal fat weight and liver weight (r=0.50; p<0.05) and abdominal fat weight and heart weight (r=0.54; p<0.05). However, the relationship between skin weight and dressing percent (r=-0.71; p<0.01) as well as abdominal fat weight and breast muscle weight was negative (r=-0.80; p<0.001) (Table 4.3).

**Table 4.3** Canonical correlation coefficient matrix of carcass characteristics of the experimental broiler birds fed diets supplemented with different levels of lemon juice and molasses at 4th week.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | DP | BMW | TMW | DW | LW | HW | SW | AFW |
| DP | 1.00 |  |  |  |  |  |  |  |
| BMW | -0.27 | 1.00 |  |  |  |  |  |  |
| TMW | -0.16 | -0.36 | 1.00 |  |  |  |  |  |
| DW | -0.01 | -0.65\*\* | 0.06 | 1.00 |  |  |  |  |
| LW | -0.37 | -0.61\* | -0.02 | 0**.56\*** | 1.00 |  |  |  |
| HW | -0.06 | -0.51\* | 0.70\*\* | 0.03 | 0.29 | 1.00 |  |  |
| SW | **-0.71\*\*** | **0.53\*** | -0.39 | -0.17 | 0.23 | -0.23 | 1.00 |  |
| AFW | 0.13 | -**0.80\*\*\*** | 0.40 | 0.27 | **0.50\*** | **0.54\*** | -0.46 | 0.01 |

DP=Dressing percent; BMW=Breast muscle weight; TMW=Thigh muscle weight; DW=Drumstick weight; LW=Liver weight; HW=Heart weight; SW=Skin weight; AFW=Abdominal fat weight; \*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001)

Marginal means of the dressing percent, abdominal fat weight, breast muscle weight and thigh muscle weight fitted against the profile plot differed significantly exhibiting non-parallel trends between lemon juice supplemented and non-supplemented groups at varying levels of molasses concentration (Figure 4.7-4.10).

|  |  |
| --- | --- |
|  |  |
| **Figure 4.7** Profile plot of the marginal means for dressing percent at 4th week. | **Figure 4.8** Profile plot of the marginal means for abdominal fat weight at 4th week. |
|  |  |
| **Figure 4.9** Profile plot of the marginal means for breast muscle weight during 0-4 weeks. | **Figure 4.10** Profile plot of the marginal means for thigh muscle weight at 4th week. |

**Figure 12.** Graph matrix of abdominal fat with dressing percent, breast muscle weight, wing weight, thigh muscle weight and drumstick weight

**4.3. Hemato-biochemical parameters**

Main effect of supplementing both lemon juice and molasses were significant (p<0.01) for glucose, albumin, total protein, LDL, HDL and cholesterol. However, combined effects were significant (p<0.01) only for glucose, albumin, LDL and HDL (Table 4.4).

**Table 4.4** Hemato-biochemical parameters of the experimental broiler birds fed diets supplemented with different levels of lemon and molasses at 4th week.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Carcass characteristics** | **Lemon** | **Molasses** | | | | **SEpooled** | **ηp²** | **Sig.** | | |
| **0** | **1.0** | **2.0** | **3.0** | **L** | **M** | **L×M** |
| Glucose (g/dl) | 0 | 23.3 | 18.6 | 9.7 | 7.6 | 23.2 | 0.971 | \*\* | \*\* | \*\* |
| 1 | 35.6 | 16.4 | 7.9 | 7.0 | 19.912 |
| Albumin (g/dl) | 0 | 45.3 | 60.1 | 66.5 | 59.9 | 61.325 | 0.963 | \*\* | \*\* | \*\* |
| 1 | 77.2 | 52.7 | 78.7 | 54.5 | 77.575 |
| Total protein (g/dl) | 0 | 43.1 | 32.4 | 35.7 | 37.7 | 39.225 | 0.888 | \*\* | \*\* | NS |
| 1 | 38.1 | 44.2 | 42.7 | 35.0 | 43.625 |
| SGOT (g/dl) | 0 | 250.5 | 197.4 | 277.0 | 276.8 | 303.462 | 0.419 | NS | NS | NS |
| 1 | 288.0 | 221.2 | 277.7 | 258.4 | 295.537 |
| SGPT (g/dl) | 0 | 13.5 | 18.6 | 19.0 | 19.3 | 21.687 | 0.459 | NS | NS | NS |
| 1 | 20.5 | 22.2 | 15.7 | 14.1 | 21.137 |
| LDL (mg/dl) | 0 | 44.2 | 13.1 | 13.4 | 18.2 | 33.512 | 0.988 | \*\* | \*\* | \*\* |
| 1 | 50.4 | 17.5 | 27.4 | 17.7 | 29.312 |
| HDL (mg/dl) | 0 | 49.5 | 67.2 | 77.1 | 59.4 | 56.487 | 0.978 | \*\* | \*\* | \*\* |
| 1 | 58.0 | 64.2 | 73.2 | 60.2 | 64.137 |
| Cholesterol (g/dl) | 0 | 129.2 | 118.8 | 125.1 | 103.7 | 129.537 | 0.819 | \*\* | \* | NS |
| 1 | 125.7 | 114.6 | 125.7 | 109.4 | 119.825 |

L=Lemon; M=Molasses; L×M=Lemon×Molases; SEpooled=Pooled standard error; ηp²=Partial eta squared of the model; NS=Non-significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01)

There was a strong positive relationship between cholesterol and glucose (r=0.70; p<0.01), total protein and albumin (r=0.88; p<0.001) and SGPT and SGOT (r=0.83; p<0.001). However, albumin and glucose (r=-0.77; p<0.001) and total protein and glucose (r=-0.80; p<0.001) were negatively correlated (Table 4.5).

**Table 4.5** Canonical correlation coefficient matrix of the hemato-biochemical parameters of the experimental broiler birds fed diets supplemented with different levels lemon juice and molasses at 4th week.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Glucose | Albumin | TP | SGOT | SGPT | LDL | HDL | Cholesterol |
| Glucose | 1.00 |  |  |  |  |  |  |  |
| Albumin | **-0.77\*\*\*** | 1.00 |  |  |  |  |  |  |
| TP | **-0.80\*\*\*** | **0.88\*\*\*** | 1.00 |  |  |  |  |  |
| SGOT | -0.46 | 0.09 | 0.22 | 1.00 |  |  |  |  |
| SGPT | -0.49 | 0.20 | 0.13 | **0.83\*\*\*** | 1.00 |  |  |  |
| LDL | 0.53\* | -0.30 | -0.42 | -0.31 | 0.01 | 1.00 |  |  |
| HDL | -0.37 | 0.36 | 0.46 | 0.21 | 0.12 | -0.40 | 1.00 |  |
| Cholesterol | **0.70\*\*** | -0.47 | -0.57\* | 0.10 | 0.08 | 0.45 | -0.28 | 1.00 |

\*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001)

Marginal means of glucose, albumin, LDL and HDL fitted against the profile plot differed significantly exhibiting non-parallel trends between lemon juice supplemented and non-supplemented groups at varying levels of molasses concentration (Figure 4.11-4.14).

|  |  |
| --- | --- |
|  |  |
| **Figure 4.11** Profile plot of the marginal means for glucose at 4th week. | **Figure 4.12** Profile plot of marginal means for albumin at 4th week. |
|  |  |
| **Figure 4.13** Profile plot of marginal means for LDL at 4th week. | **Figure 4.14** Profile plot of marginal means for HDL at 4th week. |

**Chapter V: Discussion**

The aim of the study was to investigate the effects of lemon juice and molasses supplementation into the drinking water below and above recommended levels in addition to basal diet and to measure its effects in terms of productive performance, carcass characteristics and hemato-biochemical parameters in commercial broiler for a typical period of 28 days. Results obtained from previous chapter have been explained, interpreted and compared with relevant studies in this chapter.

**5.1. Feed intake**

Increasing levels of lemon juice and molasses had remarkable positive effects on feed intake in commercial broiler. It was evident that, inclusion of 3% molasss increased (p<0.05) feed intake in treatment groups compared to control at 4th week. Birds consumed relatively more feed during finisher phase despite reduced total feed intake (**Liu, 2000; Karakas *et al*., 2001; Faria *et al*., 2002**). Decreased feed intake could have been due to high ambient temperature (**Ojano and Waldroup, 2002**).

Molasses is considered as a source of energy for poultry. It has been using in poultry feed since the nineteenth century. In the present study, use of molasses in the drinking water resulted in increased feed intake of the experimental birds. Lemon juice is a rich source of vitamin C (**Pisoschi *et al*., 2011**). It was reported that dietary inclusion of vitamin C increased feed intake and feed efficiency in broiler birds under heat stress condition (**McKee *et al*., 1997; Tatli *et al*., 2009; Elagib and Omer, 2012**).

In a different study, **Ndelekwute (2015)** reportedthat, 60g/litreof molasses improved feed consumption and produced higher feed intake. On the other hand, **Khalid (2007**) reported inconsistent results of cane molasses in broiler diets were above 4% level. Increased feed intake might be due to the fact that, molasses increases palatability of feed. It was reported that besides being a source of energy, the palatability of molasses makes it an excellent carrier for other unpalatable feedstuffs (**Curtin (1983)**. Similar reports were mentioned elsewhere **(Gohl, 1975; Preston and Leng, 1987).**

**5.2. Weight gain**

Supplementation of lemon juice and molasses from 1st to 4th weeks of age in commercial broiler birds substantially improved weight gain in treatment groups compared to control. The result is consistent with previous studies where, increasing levels of supplementing lemon and molasses had significant positive effects on body weight gain in broilers**.** In the present study, highest weight gain was recorded in 1 ml lemon juice/litre drinking water and 3% molasses supplemented group which is in close agreement with other studies (**Stipkovits *et al*., 1992; Denli *et al*., 2003; Shen *et al*., 2005**).

The progressive increase of feed intake from diets containing higher levels of molasses in the present study resulted marked increase in final body weight and weight gain of the experimental birds. This effect was attributed to the increased intake of ME and other nutrients which might have promoted growth and improved feed efficiency **(Sadagopan *et al.*, 1971).**

Marked increase in body weight has also been reported by **Ndelekwute (2015)** who mentioned that, after three weeks of feeding molasses, final weight was improved. In another study, **Connor *et al*. (1972)**, **Satava et al. (1981)** and **Kabuage *et al*. (2000)** reported that, incorporation of molasses up to 6% in the broiler diet may be recommended without compromise in weight gain.

Citric acid has sufficient antimicrobial activity to preserve feed against bacterial spoilage by simultaneously reducing the levels of undesirable bacteria (*Escherichia coli*) in the gastrointestinal tract and thereby improve growth rate (**Falkowski and Aherne, 1984; Eidelsburger and Kirchgessner, 1994; Deepa *et al*., 2011**). In this study, it is evident that, lemon juice supplementation enhanced weight gain at 2nd, 3rd and 4th weeks. However, in a previous study, it was evident that, vitamins C had little influence on weight gain in broilers (**Konca *et al.,* 2009**).

**5.3. Feed conversion ratio**

In present study, the efficiency of feed utilization was improved with increasing levels of molasses in the diet. This effect, however, was not consistent during the first three weeks of the age of the experimental birds. This finding is in general agreement with **Aderolu (2013)** who reported non-significant (p>0.05) improvement in the feed conversion ratio among experimental birds fed diet supplemented with molasses. The present study, however, disagrees with the previous report indicating that the inclusion of molasses in broiler diets had no significant effect on feed efficiency **(Khalid *et al*., 2007a).** However, in contrast to the present study, **Brzóska *et al*., 2013** did not find any effect of lemon juice on feed conversion in broilers. Another study demonstrated that addition of acidifier in water for broilers improved feed conversion ratio (**Král *et al*., 2011**).

**5.4. Hemato-biochemical changes**

The blood components are particularly sensitive to changes in ambient temperature, being an important indicator of physiological responses of birds to stress factors. During heat stress, increased catabolic effect and higher concentration of adrenocorticotropic hormone result more glucose, uric acid and triglycerides in blood serum. In this study, glucose level at 4th week of age was normal and lower in treatment group compared to control.

In this study cholesterol level was higher in treatment group than control group. But in another study, it was reported that in younger age, cholesterol level remained low due to higher demand of energy used for body development **(Almeida *et al*., 2006**). The increase in glucose concentration is directly responsive to an increase in glucocorticoids (**Borges et al., 2007**), which may result from various stressors including heat stress. Glucocorticoids have primary effects on metabolism, stimulating gluconeogenesis from muscle tissue proteins.

It was reported that, high environmental temperature increased plasma glucose and cholesterol levels and reduced protein level **(Kutlu and Forbes, 1993; Rashidi et al., 2010**). The increase in blood lipids under heat stress was explained by **Rashidi et al. (2010)** who reported that high temperature reduces feed intake since broilers compensate their energy need by lipolysis of body lipid which elevates blood cholesterol and triglycerides. In contrast, **Seven et al. (2009**) reported that glucose, total protein, total cholesterol, VLDL cholesterol and triglycerides in blood plasma were not significantly influenced by heat stress.

Albumin does not vary with age which is similar to the present study. In fact, life is the continuation of a series of complex biochemical reactions supported by enzymes. Therefore, changes in enzyme activities are considered as an indication of health**.** In the present study, despite various levels of supplemental molasses, all biochemical parameters remained within normal range.

Liver is the main organ for controlling metabolism in entire body. Of all the enzymes, SGPT and SGOT are the most specific types of enzymes of the liver which increase in the plasma due to destruction of cell membrane and cell necrosis in acute liver disease and also due to accumulation of toxic substances in liver **(Meyer and Harvey, 1998).** In the present study, SGOT and SGPT remained within normal range in treatment groups. Liver transaminases, SGOT and SGPT are essential in protein biosynthesis and normal range in their concentration reflects better liver function and normal health.

**5.5. Carcass characteristics**

Gradual increment of molasses and lemon juice supplementation substantially improved carcass quality in terms of dressed weight, breast weight, drumstick weight and abdominal fat weight of birds. Carcass and liver yields are usually adversely affected by high temperature because of reduced feed intake. To reduce heat stress vitamin C is usually supplemented to broiler diets. In the present study, dietary supplementation had moderate effect on carcass characteristics which is in agreement with previous studies which reported that carcass traits were hardly influenced by dietary modulations of molasses or vitamin C (**Celik and Ozturkcan, 2003; Konca *et al.,* 2009**). Liver and gizzard yields also were not affected by vitamin C (**Konca *et al.,* 2009**). However, others reported that dietary vitamin C with molasses (**Sahin *et al.,* 2002; Sahin *et al.,* 2003; Lohakare *et al.,* 2005**) supplementation significantly increased carcass weight and yield as well as the weights of internal organs.

In the present study, dietary supplementation of lemon and molasses had significant effect in case of fat yield among different treatment groups. Previous studies demonstrated that, the addition of vitamin C decreased abdominal fat pad (**Sahin *et al.,* 2002; Sahin *et al.,* 2003**) or in few cases had no obvious effect (**Celik and Ozturkcan, 2003; Konca *et al*., 2009**).

**Limitations of the study**

Due to financial constraints and technical limitations, some of the vital hemato-biochemical parameters specially, Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), White blood cell (WBC), calcium, phosphorus and other trace minerals both in feed and meat were not analyzed. These parameters could have potential impact on human health.

**Conclusion**

The study investigated the effects of lemon juice and molasses supplementation on productive performance, carcass characteristics and hemato-biochemical parameters in commercial broiler under intensive rearing system. There was a positive relationship between gradual increase of lemon juice and molasses supplementation and better performance of commercial broiler without notable unusual changes in hemato-biochemical parameters. Carcass characteristics were improved in terms of breast muscle yield.

**Recommendations**

Lemon juice and molasses are locally available and comparatively cheaper sources of feed additives having significant positive effect in terms of performance and carcass merit without adverse pathological changes in hemato-biochemical parameters. Inclusion of 1 ml/litre lemon juice and 3% molasses in drinking water in addition to regular pellet diet is recommended for better growth, optimum FCR and desirable carcass characteristics in commercial broiler.

**Future directives**

Long term consistent effect of lemon juice and molasses supplementation on productive performance and hemato-biochemical indices of broilers should be investigated in future for further validation of the study in perspective of human health. Large sample size and multi-dimensional temporal pattern is suggested to increase sensitivity and validity of the study under field conditions. This study explores new horizon for investigating additional important parameters in future applying high sensitivity and specificity diagnostic tests and advanced statistical techniques.

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