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Growth performance of novel food based on mixture of boiled-dried granulated *Tenebrio molitor* larvae and date-fruit waste in broiler chicken farming

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Abstract. *Debache K. 2021. Growth performance of novel food based on mixture of boiled-dried granulated* Tenebrio molitor *larvae and date-fruit waste in broiler chicken farming. Asian J Agric 5: 22-28.* The present study was conducted to evaluate the growth performance of a new diet based on mixture of boiled-dried granulated *Tenebrio molitor* larvae (Tm) and date-fruit waste (Dw) in broiler chicken diet. A total of 56 two-day old broilers were randomly allotted to 4 dietary groups each with 2 replicates consisting of 7 broilers (C1, C2, Diet1 and Diet2). Equal mixture of three commercial cereal-based diets (chick starter feed, chick grower feed, and chick finisher feed) was formulated. The first control (C1) was 100% the commercial mixture. Second control (C2) is obtained by mixing 50% commercial mixture with 50% Dw. While the other groups (Diet1 and Diet2) were formulated by adding three ingredients at different proportions: 50% commercial mixture: 40% Dw: 10% Tm (Diet1) and 50% commercial mixture: 10% Dw: 40% Tm (Diet2). After the evaluation of daily body weight, clinical signs, specific growth rate and other clinical tests, the chickens were slaughtered at 60 days. Hematological, biochemical, copro-parasitological, and bacterial investigations were performed based on samples taken day 60. Weight gain of broilers fed with Diet1 was almost like broilers fed with first control (C1) diet. However, those fed with Diet2 were significantly ($P<0.05$) higher than all other dietary groups (C1, C2 and Diet1). Hematological and serum biochemical traits showed no dietary adverse effect, and copro-parasitological diagnosis was negative in all different dietary groups. Moreover, similar microbial communities were detected in digestive system parts of the same animal, no matter in relation to Tm inclusion or no. In conclusion, the overall results collected in this current study propose that date-fruit waste could be used as an exclusive feed for *T. molitor* insect rearing and dietary inclusion of mixture Dw-Tm into broilers meal could become a partial substitute for commercialized cereal-based diet without affecting the health of broilers.

Keywords: Broiler, date-fruit waste, insect meal, growth performance, *Tenebrio molitor*

INTRODUCTION

Chicken meat and eggs provide high-quality protein and are a source of all essential amino acids required in the human diet. They contain both saturated/unsaturated fatty acids, minerals, and sufficiently high quantities of all essential vitamins except vitamin C. Chicken is not only healthy meat, but also is relatively inexpensive versus other livestock meats, even though it contains more protein and less fat than red meat (Kralik and Kralik, 2017). Cereal-based meals are the most used vegetable protein source in diet formulations for broiler chickens due to the high level of protein (Veldkamp et al. 2012). However, the increasing cost and the negative environmental impact of cereal cultivation have led to the need for other useful animal nutrition alternatives. Insects (larva, pupa, and adult stage) including *Tenebrio molitor* larvae, commonly known as yellow mealworm, have been suggested in poultry feeding of their natural consumption by wild birds and free-range poultry (Zuidhof et al. 2003; FAO, 2013; Debache, 2017). This is due to their protein richness, lipids and essential elements, which were evaluated (dry matter) as follows: 22 to 48% protein, 15 to 38% fat, 4% carbohydrate, 58% moisture, 11 fatty acids, 20 essential amino acids, niacin, pyridoxine, riboflavin, folate and vitamin A, C, E, B1, B6 and B12 (Jones et al. 1972; Nowak et al. 2016; Payne et al. 2016; Rumpold and Schlüter, 2013). Moreover, Dobermann et al. (2017) reported the following mineral content expressed as mg $100g^{-1}$ dry matter: 45.7 calcium, 828.2 potassium, 215.8 magnesium, 722.7 phosphorous, 133.1 sodium, 5.4 iron, 12.5 zinc, 1.1 manganese, and 1.6 copper. Furthermore, yellow mealworm larvae are characterized to turn a wide range of organic waste into valuable protein, easy for farming and fast growth without increased use of resources like space and water or carbon emission excess (Rumpold and Schlüter, 2013; Ballitoc and Sun, 2013). Therefore, in the last decades, numerous companies have started the production of *T. molitor* for feed purposes to replace cereal-based feed partially or fully. In addition, several studies have evaluated the effects of yellow mealworm meal utilization on poultry growth performance (Ballitoc and Sun, 2013; Bovera et al. 2015; Bovera et al. 2016; Biasato et al. 2018). *T. molitor* farming is mainly achieved in humid Western where there is an abundant and wide range of substrates, including valuable recycled proteins from organic waste and by-products of agriculture and the food industries (Ramos-Elorduy et al. 2002). Dry climate regions such as the Middle East and

North Africa, including southern Algeria and Biskra, that are sparsely vegetated, with lower quantities of agricultural waste, discourage any temptation to raise yellow mealworm and to use it like poultry feed substitute. Moreover, most people in these arid and/or semi-arid regions have low in-comes and are not always able to pay for chicken meat derived from cereal nutrition. In these areas, there is less vegetable cultivation and consequently, there is less green waste; however, there are date palm (*Phoenix dactylifera* L., family: Palmaceae) plantations. This tree offers date fruit at the mature stage ("tamr" Arabic language) playing an important role in the autochthones day-to-day diet and economic and social lives (Gurevich et al. 2005). However, date palm fruit cultivation is often accompanied by enormous fruit losses which mainly occur during the date picking, date storage, conditioning and processing stages including date fruit seeds (pits) and date fruits that fall from the tree before maturity. According to non-published data, date-fruit waste could be estimated to an average of 20% of total date fruit production, corresponding to thousands of tons per year. Date-fruit waste is often discarded or used for limited purposes such as animal feed. Date-fruit waste is not edible for commercial purposes but represents the same nutritional values as the following commercialized date fruit chemical composition: 62% to 75% sugar, 2.2% to 2.7% protein, 0.4% to 0.7% fat, 5% to 8% fiber, 3.5% to 4.2% ash and vitamins, especially B-complex, C, and K (Abdel-Hafez et al. 1980; Baraem et al. 2006). Besides, the fruit pulp, date pit is about 10% of the fruit's weight and on average contained 2.3 to 6.4% protein, 5.0 to 13.2% fat, 3.1 to 7.1% moisture and 0.9 to 1.8% ash (Al-faarsi et al. 2007).

This study evaluates the growth performance of a new diet based on mixture of boiled-dried granulated *T. molitor* larvae (Tm) and date-fruit waste (Dw) in broiler chicken diet. This was done by (i) using date-fruit waste as food for *T. molitor* insect breeding to compensate for the lack of local vegetable waste and (ii) using the resultant insect larvae mixed with date-fruit waste as a partial alternative feed source in broilers meat production.

MATERIALS AND METHODS

The experimental protocol was designed in accordance to the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the Algerian Association of Experimental Animal Sciences (AASEA); these guidelines are like those of the Guide for the Care and Use of Laboratory Animals.

Insects and substrate

Yellow mealworm, *Tenebrio molitor* larvae, (average weight 0.14 to 0.2 g per worm) were purchased from a fishing shop Fischereibedarf Niklaus, Berne (Switzerland). Larvae were reared in plastic containers (60 x 40 x 10 cm) with aeration slits in the side and maintained under climate-controlled chamber at 28 ± 2 °C, 45% to 65% relative humidity (RH) and a 24 hour dark photoperiod. To avoid unwanted contamination, rearing plastic containers were sanitized with active chlorine solution (3%) before use. Date-fruit waste, no matter what date varieties were procured from a local plantation in El Hadjeb, Biskra (Algeria) harvested from September 2018 to January 2019. Date-fruit waste (with date pits) was ridden of debris and milled through Corn Grinding Mill IndiaMart to pass through a 5 mm sieve, stored in airtight plastic bag at 4°C and designed in this work (Dw).

Larval growth and mass production of insects

Dw was used as diet for all yellow mealworm stages. For each container, 1 kg of Dw, a couple of vegetable strips (mainly carrot or potato), and mostly 500 *T. molitor* larvae were sampled. Larvae were allowed to feed ad libitum and based on visual observation of remaining diet and/or accumulated feces, the diet was refreshed. To provide moisture, vegetable strips were added twice a week and the old pieces were removed. Using 3% chlorine-washed sieve (3.35 mm openings), three to five months old larvae were collected directly from the feeding containers and starved for 24h before being killed by boiling in water for 3 minutes and then overnight oven-dried at 60 °C (Aguilar-Miranda et al. 2002). Dried yellow mealworm larvae were coarsely chopped using a Philips electric meat mincer and the resultant boiled-dried powdered *T. molitor* larvae (Tm) packed and stored at 4°C until further use. The Tm was examined for food poisoning pathogen contamination by assessing *Escherichia coli* and *Salmonella* spp. They were found to be safe from both cited pathogens.

Around 10% of total reared larvae per container were allowed to finish their whole development period (adult stage) in separated rearing containers. Larvae transform into pupae, and thence adult beetles emerge from the pupae stage. When reproducing, females usually lay eggs (typically produce 300 to 500 eggs at once) in meal containers. By sieving the bedding, larvae eggs mixed with substrate residues were sampled in new rearing container to maintain the future generation.

Experimented broilers feed formulation

During all studies, the broilers were fed exclusively with the followed diet formulations. Equal mixture of three commercialized cereal-based diets (Table 1). Chick starter feed, chick grower feed and chick finisher feed obtained from a local chicken food distributor in Biskra (Algeria) were formulated. The first control (C1) was 100% the commercial mixture. Second control (C2) was obtained by mixing 50% commercial mixture with 50% Dw. While the other groups (Diet1 and Diet2) were formulated by adding three ingredients at different proportions: 50% commercial mixture: 40% Dw: 10% Tm (Diet1) and 50% commercial mixture: 10% Dw: 40% Tm (Diet2) (Table 2). All experimental meal preparation of this study, including packing and storage, were carried out in ultra-clean conditions. The assessment of *Escherichia coli* and *Salmonella* spp. in all prepared meals formulation was not detectible.

Table 1. Ingredients of broiler meal used as first control (C1). This commercial mixture has resulted from equal mixture of three-broiler starter, grower, and finisher feed

Ingredients	Starter	Growth	Finisher
Corn meal	10.1	12	16.5
Wheat	50	50	50
Soybean meal 48 c.p	30	26	21.4
concentrated protein(1)	5	5	5
Sunflower oil	2.9	5.2	5.3
Calcium carbonate	0.9	0.9	0.9
Dicalcium phosphate	0.7	0.5	0.5
Sodium chloride	0.2	0.2	0.2
Mineral-vitamin-premix	0.2	0.2	0.2
Total	100	100	100
Calculated chemical analysis			
CP%	23	21.25	19.4
ME (kcal/kg)	3003	3153	3200
L-Lysine %	1.26	1.1	1.0
DL-Methionine %	0.48	0.45	0.43
Cysteine%	0.36	0.34	0.31
Methionine + Cysteine %	0.84	0.79	0.74
Arginine%	1.28	1.15	1
Ca %	0.85	0.80	0.78
Av. Phosphorus %	0.43	0.40	0.40

Note: (1) The concentrated protein type Brocon-5 special W contain the following per kg:20% crude protein, 5% fat, 2.2% fiber, 4.2% Ca, 4.68% P, 3.85% Lysine, 3.7% Methionine, 4.12% Methionine + Cysteine, 2.5% Na, 2107 ME (kcal.kg^{-1}), 2000 IU vitamin A, 4000 IU vitamin D_3, 500 mg vitamin E, 30 mg vitamin K_3, 15 mg vitamin B1, 140 mg vitamin B_2, 20 mg B_6, 10 mg Folic acid, 100 µg Biotin, 1 mg Fe, 100 mg Cu, 1.2 mg Mn, 800 mg Zn, 15 mg I, 2 mg Se, 6 mg Co, 900 mg Antioxidant.

Table 2. Composition of experimental diets

Ingredients	Diet formulation			
	C1	C2	Diet1	Diet2
Commercial mixture	100%	50%	50%	50%
Dw	-	50%	40%	10%
Tm	-	-	10%	40%

The commercial mixture resulted from equal mixture of three-broiler starter, grower, and finisher feed. Dw, Date-fruit waste; Tm, powdered *T. molitor* larvae.

Broilers feed comparison

This study was performed using Ross-308 male and female broiler chicks purchased from a local poultry corporation named CFPA Amairi Aissa located in Manbaa el-Ghozlane/Loutaya (Algeria) at one-day of age. Healthy broilers were selected for the study after being acclimatized to the lab overnight. A total of 56 two-day age broilers averaging 45.3 ± 0.9 g were randomly allotted to 4 dietary groups each with 2 replicates consisting of 7 chickens (C1, C2, Diet1, and Diet2). Each group was housed in enclosure of 1.50 m wide x 2.20 m long x 1.10 m high and was equipped with poultry drinker with capacity 3 liters, sawdust as litter, and feeder filled with the specific diet formulation for every corresponding group. Based on standard breeding practices (Aviagen, 2014), during the first 2 weeks, chicken house was heated by infrared lamps to maintain the suitable temperature of 33°C then gradually reduced according to the age of broilers (3°C every week) until reaching 21°C and then kept constant. Until day 6, chicken houses were illuminated with 22:2 light-dark cycle and then 18:6 light-dark cycle until slaughter age. Broilers were kept under similar managerial and environmental conditions with free access to water and feed. At hatching, all broiler chicks were vaccinated against Newcastle disease, Gumboro disease, infectious bronchitis, and coccidiosis and vaccine recalls were performed on day 9 for infectious bronchitis and on day 18 for Gumboro and Newcastle diseases.

Feed and water intake, individual live body weight and mortality rate were recorded daily in the morning and evening for each group, starting at day 2 until the last day of the experiment day 60. The feeding trial lasted for 8 weeks. After measurement of body weight, clinical signs rate and blood sampling, all animals were slaughtered and immediately digestive systems were recovered and processed for bacteriological and copro-parasitological analysis. All body and feed weight measurements were monitored using a precision electronic scale OHAUS Scout SE.

Hematological and biochemical parameters

At slaughter and under safe handling practices, blood of four chickens per feeding group was collected by puncturing the medial wing vein using a syringe (26-gauge x 13 mm needle). From each chicken, 1 ml blood was placed in EDTA anticoagulated tube and 1 ml in Eppendorf tube without anticoagulant. On one glass slide, unfixed blood smears were prepared, air-dried and stained in concentrated May-Grunwald stain 6 min, 1:1 May-Grunwald stain-distilled water for 90 sec and 1:9 Giemsa's stain for 15 min (Robertson and Maxwell 1990). Using Neubauer hemocytometer, the total red and white blood cell counts were determined on blood samples previously treated with a 1:200 Natt-Herrick solution. 100 leukocytes per slide, including granular (heterophils, eosinophils, and basophils) and non-granular (lymphocytes and monocytes) leukocytes, were counted and the complete blood count results are presented as the percentage of each cell occurring in each stream. The heterophils to lymphocytes ration (H/L) was calculated by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983). For biochemical analysis, the tubes without anticoagulant were left in vertical position at room temperature for approximately two hours, then centrifuged at 800g/10 min/4°C. Sera were sampled in new Eppendorf and frozen at -20°C until analysis. The serum concentrations of total protein, albumin, glucose, cholesterol, triglycerides, total bilirubin, creatinine, uric acid, iron, total calcium, phosphorus, magnesium, and activities of the enzymes aspartate-aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP) were measured by means of enzymatic methods in a clinical laboratory analysis Biskra, Algeria.

Copro-parasitological and bacteriological examination

To investigate fecal egg (nematode, cestode) and fecal coccidial oocysts, the cloacae of the same slaughtered broilers chosen for blood analysis (identified with a shank ring) were cut open and processed using supersaturated saline flotation technique as modified McMaster method (Ballweber et al. 2014). Feces were homogenized in 15 ml of water in plastic bottle, filtrated through fine-mesh sieve (aperture 150 µm) and centrifuged at 1500 rpm for 2 minutes. Resulting sediment was suspended in 10 ml of the floatation medium (400 g of NaCl in 1000 ml of distilled water). A coverslip was placed gently on the test tube and allowed to stand on a level surface for at least 15-20 min. The coverslip was carefully removed and placed on a glass slide and examined immediately for intestinal eggs under x10 and x40 objective lens. Examination was aided by the addition of Lugol's Iodine solution to the sample on the glass slide. For bacteriological investigation, inoculum from the gastrointestinal tract (esophagus, crop, gizzard, colon, and cloacae) were made on various selective and differential media for bacteria isolation. All inoculated media were incubated at 37°C for 24-48 h. For enrichment, samples collected for salmonella isolation were inoculated in Selenite-Cystine Broth and incubated at 37°C for 24 h followed by inoculation on MacConkey agar. All bacteriological culture media are commercially available (BioRad) as pre-made or as bases, which can be prepared according to the manufacturer's recommendations. After 24-48 h of incubation at 37°C, colonies were examined for cultural and morphological properties on growth media.

Thereafter, smears on slides were prepared from the colonies for gram staining to classify the isolates into Gram-positive or Gram-negative under light microscopy at x100 magnification under oil immersion. The Gram stain procedure was performed following the protocol described in a standard microbiology laboratory manual (Leboffe and Pierce, 2002). For species identification, pure colonies obtained from subculture were subjected to standard biochemical tests as described elsewhere (Holt et al. 1994).

Statistical analysis

NCSS 2019, version 19.0.2 was used for data analysis. Data collected for growth performance and serum components were tested by one-way ANOVA, using analysis of variances followed by Tukey's multiple comparison test to evaluate dietary Dw and/or Dw-Tm inclusion. *P* values <0.05 were considered statically significant. Results were expressed as mean and standard deviation (SD).

RESULTS AND DISCUSSION

Insect

Yellow mealworms were allowed to feed ad libitum Dw supplemented with vegetable strip until larvae stage started pupating, which can occur after 3 to 5 months. At this time, pupae (pupa is a free-living creature) were sexed based on their morphology. Almost 50 pupae, 35 were females and 15 were males with sex identified by structural differences in the 4th and 5th visible abdominal sternites (Bhattacharya et al. 1970). Pupae were carefully removed and placed in new containers containing 1 kg of Dw and vegetable strips to allow adults to lay eggs which happened 4 to 17 days after copulation.

This maintaining step is very important to ensure the breeding of future generations who are kept under similar managerial and environmental conditions. Once pupae were kept separated, all yellow mealworms supposed at last instar larvae were removed into collector container to be processed as was mentioned before. Insect development time and mortality rate until pupation were similar in all larvae batches whose weight at the later larval stage was about 0.17 ± 0.3 g for 25 to 35 mm long. No difference in number of days from pupation and the corresponding pupae size (12 to 18 mm long) until the pupae metamorphosis into beetles was observed throughout the study period.

Growth performance

On study feeding start day, average live body weight of all birds regardless of related diet group was 45.3 ± 0.9 g. From this day (day two of age) until the end of experiment, all group's birds showed an increasing body weight. However, group C2 broilers showed greater growth depression versus all other dietary groups. The growth weight of broilers recorded in group Diet2 was significantly higher (P<0.05) than two broilers groups fed respectively with the mixture of commercialized cereal-based diets C1 and Diet1 (Figure 1), which suggested that DW and Tm mixture (Dw-Tm) are an effective ingredient for broiler feed that increases the live body weight. Bodyweight (BW) (Table 3) of broilers in group Diet2 (2601.69±168.89) was significantly higher (P<0.05) than C1 and Diet1. The lowest BW was noted in C2 (1458.95±239.03).

C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. (*) C1 is significantly higher (P<0.05) than C2, (**) Diet2 is significantly higher (P<0.05) than Diet1 and significantly higher (P<0.05) than C1 (***), with statistical significance: (P<0.05).

Feed intake of broilers (Table 3) in group Diet2 was higher (6035.42±215.89) than birds in other groups. The lowest feed intake (5019.02±203,67) was recorded in broilers group C2. The feed intake differed significantly higher (P<0.05) when broilers in groups Diet1 and Diet2 were compared with broilers of groups C1 and C2. The results further suggested that inclusion of Dw-Tm in broilers diet could be adequately used as an ingredient for broilers feed to get higher body weight and growth weight over commonly used as commercial broiler feed. It can be noted that live body weight increased with increasing level of Tm.

The difference improvement of the FCR efficiency was not so pronounced when the broilers were fed either with commercial diet (C1) or with diet containing Dw-Tm. The FCR was statically non-significant (P>0.05). The significantly lowest (P<0.05) FCR in all other dietary groups was calculated in broilers of group C2. The results

showed essentially similar feed conversion efficiency in C1 (2.35±0.05), Diet1 (2.49±0.09) and Diet2 (2.32±0,07) which suggested that Dw could be substitute for almost half of commercial diet but only when is associated with Tm. Diet2 (C150%:0% Dw: 40% Tm) presents relatively poor FCR compared with Diet1 (C150%: 40% Dw: 10% Tm) this suggested that increasing the level of Tm to 40% in broilers feed caused improving the FCR.

Hematological and biochemical parameters

No significant (P>0.05) differences were observed among values in hematological and serum parameters when comparing all feeding groups (Table 4).

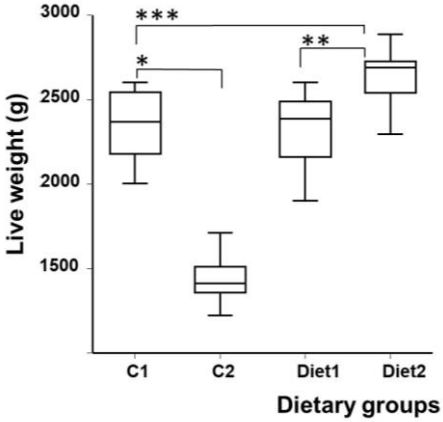

Figure 1. Live weight body of the dietary groups at slaughter day

Table 3. The growth performance of broilers as influenced by feeding various dietary regimes

Variables	Dietary groups			
	C1	C2	Diet1	Diet2
BW (g)	2358.40±140.67[a]	1458.95±239.03[b]	2332.84±202.47[a]	2601.69±168.89[c]
CFI (g)	5548.71±195.83[a]	5019.02±203.67[a]	5808.08±200.47[b]	6035.42±215.89[b]
FCR (g/g)	2.35±0.05[a]	3.44±0.08[b]	2.49±0.09[a]	2.32±0.07[a]

Note: [abc] Means within each row with no common superscript differ significantly (P<0,05) (ANOVA). C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. CFI = cumulative feeding intake; BW = Body weight; FCR = feed conversion ratio. Variables were given in mean ± standard deviation.

Table 4. The effect of partial substitution of commercial diet by Dw and Tm inclusion on the hematological and serum parameters in broilers.

Parameters	Dietary groups			
	C1	C2	Diet1	Diet2
Eryt. (10^6 cell.µL^{-1})	2.4±0.39	2.1±0.44	2.5±0.31	2.1±0..56
Leuk. (10^3 cell.µL^{-1})	7.89±0.49	8.14±0.53	7.63±0.41	7.75±0.42
H/L ratio	0.89±0.02	0.92±0.04	0.87±0.05	0.91±0.02
Lymphocyte (%)	57.18±5.23	52.18±7.02	49.18±6.15	55.35±9.03
Heterophil (%)	38.3±4.11	32.5±7.65	33.9±6.21	37.7±6.82
Monocyte (%)	3.40±1.09	3.00±1.14	3.20±1.2	3.50±1.10
Basophil (%)	2.1±0.52	1.7±0.60	2.2±0.12	1.9±0.47
Eosinophil (%)	3.38±1.49	3.04±1.05	3.40±1.01	2.08±1.14
Total protein (g.dL^{-1})	4.21±0.40	3.65±0.72	4.05±0.25	4.25±0.65
Albumin (g.dL^{-1})	2.23±0.09	1.83±0.08	1.92±0.05	2.31±0.07
Glucose (mg.dL^{-1})	271.2±30,07	261.2±60.0	252.8±28.12	241.9±39.20
Trigly. (mgdL^{-1})	68.18±20,81	74.18±40.3	78.54±36.55	89.51±41.36
Cholesterol (mg.dL^{-1})	122.25±5.14	118.25±7.4	122.26±8.14	125.12±6.03
Total bilir. (mg.dL^{-1})	0.40±0.12	0.38±0.18	0.40±0.20	0.39±0.13
Direct bilir. (mg.dL^{-1})	0.16±0.07	0.09±0.01	0.06±0.05	0.67±0.04
Uric acid (mg.dL^{-1})	6.05±0.71	5.14±0.80	8.11±0.28	8.05±0.501
Creatinine (mg.dL^{-1})	0.32±0.05	0.40±0.07	0.37±0,08	0.35±0.04
Total calcium (mg.dL^{-1})	10.15±0.76	09.56±0.46	10.15±0.76	11.04±0.49
Phosphorus (mg.dL^{-1})	6.58±0,62	6.14±0.42	6.28±0.38	6.50±0.41
Magnesium (mg.dL^{-1})	2.31±0.53	2.11±0.41	2.28±0.41	2.20±0.31
Iron (µg.dL^{-1})	100.52±8.52	79.52±10.81	95,74±4,55	91,52±8,22
AST (UI.mL^{-1})	251.25±19.30	181.25±22.14	201.25±40.26	262.25±50.11
ALT (UI.L^{-1})	9.10±2.01	9.88±5.38	10.12±5.19	11.02±3.36
GGT (UI.L^{-1})	29.17±7.80	21.17±9.62	30.17±8.12	22.17±6.41
ALP (UI.L^{-1})	12.45±9.06	15.32±8.10	17.05±8.44	18.23±10.27

Each mean represents two replicates with 3 broilers/replicate. C1 = mixture of three commercialized cereal-based diets; C2 = 50% C1 + 50% Dw; Diet1 = 50% C1 + 40% Dw + 10% Tm; Diet2 = 50% C1 + 10% Dw + 40% Tm. Eryt., erythrocyte; Leuk., leukocyte; H/L, heterophils to lymphocytes ration; bilir., bilirubinr; AST, aspartate-aminotransferase; ALT, alaninoaminotransferase; GGT, gamma glutamyl transferase; ALP, Alkaline phosphatase. Parameters were given in mean ± standard deviation

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