

Original Research Article

Evaluation and nutrient quality of toasted Flamboyant Seed (*Delonix regia*) meal in the diet of *Clarias gariepinus* fingerlings

Abstract

56 days feeding trial was conducted to evaluate the effect of toasted *Delonix regia* seed meal (TDRSM) in the diet of *Clarias gariepinus* fingerlings with respect to their growth performance, feed efficiency, nutrient utilization, and body composition. Four iso-nitrogenous and iso-lipidic experimental diets were formulated to contain 0%, 10%, 20%, and 30% of toasted *Delonix regia* meal and were designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), and D4 (30% inclusion) in a completely randomized design. Fifteen net hapa (0.5x0.5x1m) were suspended in two outdoor concrete ponds with the aid of kuralon twine tied to plastic poles, the concrete ponds were filled to 5/6 of its volume (40m³) with filtered and dechlorinated tap water. Each treatment had three replicates with 20 fish were accommodated in each hapa (mean initial body weight (1.02±0.01g) per fish. Water temperature and other water quality parameters were monitored daily. The result of the study showed that there was no significant difference ($P>0.05$) in the survival rate, However D3 gave the best value in all the growth parameters values measured and was significantly different from others while D4 gave the lowest value but was not significantly different from D1. Except for D3 which was significantly higher in all the nutrient utilization values measured, there was no significant difference between D1, D2 and D4. Proximate composition result revealed that increase in inclusion of TDRM lead to an increase in the carcass lipid content and a decrease in moisture content of the fish fed experimental diet. This result indicate the inclusion of toasted *Delonix regia* meal at 20% in the diet of *Clarias gariepinus* fingerlings improves the nutritive value for fish , hence can serve as alternative ingredient in the fish feed production.

Key words: *Clarias gariepinus*; Growth performance; *Delonix regia*; Toasted seed meal

31 With aquaculture gaining global attention in recent time, and fish being the cheapest
 32 source of animal protein however, due to the decline in the wild catches the
 33 industrialized fishing sector has become unpredictable and unsustainable. Hence
 34 aquaculture production intensification is of paramount importance especially the
 35 developing nations of the world [1]. One of the major concerns in aquaculture
 36 production is the high cost of feed and heavy reliance on fish meal and fish oil as the
 37 primary protein and energy source in aqua feed production. Fish meal is very palatable
 38 and has exceptional nutrition value including an excellent balance of essential amino
 39 acid and essential fatty acid, which closely meet the requirement of most farm fish, and
 40 also provides an excellent source of digestive energy and vitamin [1]. The high cost,
 41 scarcity and insufficient supply of some of these conventional plant protein sources has
 42 necessitate the need for a search for alternative unconventional plants protein ingredients[2] . [3]
 43 Obun, suggested that the alternative plant protein source to replace conventional once may have
 44 comparative nutritive value or preferably be cheaper than the conventional protein sources i.e.
 45 feed ingredients that are rarely consumed by human and less competed for by the livestock sector,
 46 hence locally available and cheap feedstuff are of paramount interest to aquafeed producers and
 47 fish farmers especially those from the sub-Sahara.

48 Several feed ingredients have been investigated in an attempt to find alternatives for soybeans in
 49 the diets of fishes. These include plant protein sources such as cottonseed meal, groundnut meal,
 50 sunflower, rapeseed, sesame seed, copra, macadamia and palm kernel along with aquatic plants
 51 such as *Azolla pinnata*, duckweed (Lemnaceae) and single-cell proteins[4]. These feeds are not
 52 only considerably cheaper than soybeans but also enjoy high availability and accessibility in
 53 certain regions of the world especially in Nigeria.

54 To attain a more economically sustainable and viable production, research interest has

55 been redirected towards the evaluation and use of unconventional plants protein sources
56 [5,6,7].

57 *Delonix regia* is a wild plant otherwise called flame of the forest, it is an ornamental,
58 leguminous plant, which produces tones of pods containing seed in the fruiting season
59 [8]. These seed are left unutilized since they are neither consumed by any animal nor
60 utilized for any other medicinal purpose. It produces 25-40cm long pods containing
61 seed which laboratory analysis has reported to contain crude protein of 36.92%, crude
62 lipid: 4.17%, and crude fiber: 11.39% [9]. Catfish *Clarias gariepinus* belongs to family
63 of *Claridae*, they are the most cultured freshwater fish in Nigeria. They are
64 characterized by their ability to grow on a wide range of artificial and natural feed, they
65 grow fast and have high potential hardness and tolerance to dissolved oxygen and other
66 aquaculture routine [10]. Hence emphasis has been on the increase on how to improve
67 productivity of catfish. This study was conducted to evaluate the suitability of toasted
68 *Delonix regia* seed meal (TFSM) in the diet of *Clarias gariepinus* fingerlings through
69 their growth performance, nutrient utilization and haematological indices.

70 **Materials and Methods**

71 *Ingredients and diet formulation*

72 ***Soybean meal***

73 Soybean was obtained from the Kure's new modern market and was toasted using a
74 frying pan and allow to cool before milling with the aid of a grinding machine. Toasting
75 was done to remove the anti-nutritional factor present in the seed.

76 All the ingredients were separately milled and mixed with warm water to form
77 consistent dough, which was then pelleted, sun dried, packed in polyethylene bags and
78 stored. The feed composition tables shown in table 1.

79 ***Flamboyant seed meal***

80 Matured and dried Flamboyant seedpods were collected from ornamental garden of the
81 centre for preliminary and extra moral studies (CPES) of Federal University of
82 Technology Minna. The seed were collected by manually opening the pods. The viable
83 seeds were sorted out by soaking the seed inside water. The viable seed were sun dried
84 and seeds were toasted within the temperature range of 60⁰C to 80⁰C to reduce the
85 effect of anti-nutritional factor using frying pan. After toasting, the seed were allow to
86 cool and later grounded to powder.

87 ***Fishmeal***

88 The fish meal used for these experiments was obtained from Mosegoaler farms Minna
89 Niger state. The crude protein and lipid were 65.34% and 11.36%.

91 ***Experimental diets***

92 Based on the nutritional requirements of *Clarias gariepinus* fingerlings [11], four
93 isonitrogenous and isolipid diets were formulated at 40 % protein and 9.5 % lipids,
94 containing 10-30% toasted *Delonix regia* seed meal at different levels of inclusion
95 (Table 3).

96 ***Experimental System and Fish***

97 The experimental fish, pure- bred *C. gariepinus* fingerlings, with an initial mean weight
98 of (1.01 - 1.02g) were purchased from Tagwai fish hatchery of Ministry of Livestock
99 and Fisheries development Minna, Niger state. The fish were transferred in a
100 well-oxygenated water plastic container from the hatchery to the Department of Water
101 Resources, Aquaculture and Fisheries Technology experimental fish farm, Federal
102 University of Technology, Bosso campus, Minna where the feeding trial was conducted.
103 Upon arrival they were acclimatized in a transitional tank in the farm for four days and

104 were fed commercial feed (coppense feed) at 40% crude protein once a day before the
 105 experiment commenced. The fish were subsequently fed with 40% iso-nitrogenous diet
 106 and 9.5% lipid, containing different inclusion level of toasted *Delonix regia* seed
 107 meal, designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), D4
 108 and (30% inclusion) for 56 days. Fifteen net hapa (0.5x0.5x1m) were suspended in two
 109 outdoor concrete tanks (8mx5mx1.5m) with the aid of kuralon twine tied to plastic
 110 poles. The concrete tanks were filled to 5/6 of its volume (40m³) with filtered and
 111 dechlorinated tap water. Twenty (20) fish were accommodated in each hapa and each
 112 treatment was randomly allocated to three hapas. Photoperiod depends on the natural
 113 light, and water temperature was monitored daily. The water quality parameters in the
 114 system were monitored weekly, the temperature ranged between 24°C-29°C while the
 115 concentration of dissolved oxygen ranged between 5.94-7.82 mg/L and the pH values of
 116 the treatments ranged from 7.18-7.60. No critical values were detected for nitrite and
 117 nitrate. Two replicates of each treatment using 20 fish per hapa were reared on each of
 118 the four diets. The feed was manually administered and the fish were fed three times
 119 daily at 5% of body weight at 09:00 am, 12:00pm and 16:00pm. Feeding rate was
 120 subsequently adjusted according to their growth rates per hapa. The uneaten and faecal
 121 matters were siphoned out of the hapa every morning before feeding, and 45 minutes
 122 after the fish have been fed. The fish were denied feed 24 h prior to sampling. Five fish
 123 were randomly sampled on weekly basis, and weights were measured using a digital
 124 electronic weighing balance (*CITIZEN MP-300*) model.

125 ***Biochemical Analysis***

126 About 10g initial sample and 15g of final samples from each hapa were pooled
 127 separately and then homogenized using laboratory mortar and pestle. The major

ingredient used for the diet; the formulated diet and the fish body samples were subjected to chemical analysis. The proximate composition analysis was determined according to AOAC procedures [12]. Moisture content was determined by drying samples at $105 \pm 2^\circ\text{C}$ until a constant weight was obtained. Dried samples were used for determination of crude fat, protein and Ash contents. Crude fat was measured by solvent extraction method in a soxhlet system where n-hexane was used as solvent. Crude protein content was calculated by using nitrogen content obtained by Kjeldahl method. A conversion factor of 6.25 was used for calculation of protein content according to AOAC [12]. Anti-nutritional factors of the seeds; tannins and trypsin inhibitor activity (TIA) were analyzed by modifying the procedures of [13]. Phytic acid was determined by the method of Latta and Eskin [14].

Blood collection and haematological analysis

Blood samples were collected in triplicate following the procedure of Klontz and Smith [15 and 16] and Wedemeyer and Yasutake, and subsequently taken to the Laboratory of Department of Biochemistry Federal University of Technology Minna for haematological analysis. The direct measurement of erythrocyte values (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC) and absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The white blood cell and differential count (neutrophils and lymphocytes) were analyzed as described by Dacie and Lewis [18].

Evaluation of blood parameters

$\text{MCV} = \text{PCV} / \text{Erythrocytes count} \times 10$

$\text{MCH} = \text{Haemoglobin} / \text{Erythrocytes count} \times 10$

$\text{MCHC} = \text{Haemoglobin} / \text{PCV} \times 100$

Evaluation of growth parameters

Growth performance and diet nutrient utilization were analyzed in terms of weight gain (WG), feed efficiency (FE), specific growth rate (SGR), feed intake (FI) protein efficiency ratio (PER) and apparent protein digestibility. The following formulas were

156 used:

157 $WG (\%) = (\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)} \times 100$

158 $FE (\%) = \text{weight gained (g)} / \text{feed fed (g)} \times 100$

159 $SGR (\%) = ((\ln \text{final weight (g)} - \ln \text{initial weight (g)}) / \text{feeding period (day)}) \times 100$

160 $FI (\text{mg/fish/day}) = \text{dry feed (mg) fed} / \text{number of fish} / \text{feeding period (day)}$

161 $PER = \text{wet body weight gain (g)} / \text{protein intake (g)}$

162 *Statistical analyses*

163 Data were analyzed using one-way analysis of variance (ANOVA) using Statistica
164 8.0 (Stat-Soft, Inc., Oklahoma, USA). Differences between treatments were compared
165 by Tukey's test. Level of significance was tested at $P < 0.05$.

166 **Results and Discussion**

167 This study investigates the possibility of utilizing toasted *Delonix regia* meal in the diet
168 of *Clarias gariepinus* fingerlings. Table 1 shows the proximate composition of the
169 major ingredients used in formulating the experimental diets. Fish meal has the highest
170 crude protein and lipid content (65.34% and 11.36%) followed by soybean meal
171 (43.63% and 7.00%), while the crude protein and lipid content of both the raw and
172 toasted *Delonix regia* meal was (22.45-29.58% and 5.68-9.39%) respectively. The value
173 of the *D. regia* reported in this experiment was higher than the value reported earlier by
174 Balogun *et al* [19] but lower than the value reported by Bake *et al* [20] the differences
175 might be attributed to differences in environmental conditions and the processing
176 technique used. The increase in the protein and lipid content of the TFMS may be
177 attributed to the processing technique used, this is in line with [20, 21, 22, and 23] that
178 heating and toasted of plant protein base ingredients boost their proximate composition
179 especially protein and lipid. Table 2 showed the anti-nutritional factor composition of
180 both the untreated raw *D. regia* (RFSM) and the treated toasted *D. regia* (TFMS). All
181 the anti-nutritive factors parameters measured were lower in the treated TFMS
182 ingredient as compared to RFSM. The lower values of the anti- nutritive factors may be
183 as a result of the toasting of the flamboyant seed, this agrees with the findings of [7, 21
184 and 22] that heat and toasting processing technique usually reduced the level of
185 anti-nutritive factors in plant protein based ingredients. It could be seen from table 3
186 that the diets used in the present study were isonitrogenous and all experimental diets
187 were similar in terms of their chemical and proximate composition. The composition of

the diets indicates that, increase in the inclusion level of TFSM in D2, D3, and D4 did not have any negative affect on their physical and chemical properties. This further affirms that *Delonix regia* seed as a fish feed ingredient may not be a starchy material. The food quality of a diet is directly proportional to its ability to support growth, and its dietary content is of paramount importance. One of the limiting growth factors in fish is protein, and it is well known fact that it is essential for building new tissues [24]. The good growth of *C. gariepinus* fed the tested diets in the present study indicated that the protein values of these diets were appropriate despite being lower than the 40% formulated on paper.

The growth performances of *C. gariepinus* fingerlings in terms of WG, SGR, FE, FI PER and PR are shown in Table 4. Among the formulated experimental diets, *C. gariepinus* fingerlings fed on D3 (20% inclusion of TFSM) gave the best and the highest significant ($P<0.05$) value in all the growth parameters measured while D4 (30% inclusion of TFSM) had the lowest WG, SGR, and FI, although there was no significant ($P<0.05$) differences between fish fed D4 and D1 based diets. Fish fed D2 had a higher significant ($P<0.05$) growth performance value than those fed D1 and D4. In the nutrient utilization parameters measured (FE, PER and PR) fish fed D3 had the highest value and was significantly different from other fish fed other experimental diets. However there was no significant difference between D1 and D2 and D4. Fish mortality was low and relatively uniform in all the treatments, furthermore all the experimental fish remaining in the hapas were morphologically normal at the end of the feeding trial. In this present study the water temperature which ranges between $26\pm0.5^{\circ}\text{C}$ - $29\pm 0.7^{\circ}\text{C}$ and the dissolved oxygen 6.5 to 8.4 mg/L were within the acceptable range for catfish culture. [25].

There was variations in the proximate composition analysis of whole body *Clarias gariepinus* fingerlings (wet basis) fed experimental diets for 56 days, as showed in Table 5. The results of the body composition revealed that except for moisture catfish fed toasted *Delonix regia* meal display a marginal increase in carcass protein, lipid, and ash, at the end of the experiment and were significantly higher than the initial body composition at the start of the experiment. There were no significant differences in the

218 carcass ash contents among all the treatments fed experimental diets, fish fed D3 had the
 219 highest crude protein content value and was significantly higher than the other fish fed
 220 other experimental diets ($P < 0.05$), fish fed D1 was significantly higher ($P < 0.05$) than
 221 those fish fed D2 and D4 however D2 and D4 do not show any significant different
 222 ($P > 0.05$) from each other. Fish fed D4 had the highest significant lipid carcass
 223 composition ($P < 0.05$) while D1 had the lowest value but was not significant different
 224 from fish fed D2, fish fed D1 show the highest moisture among experimental diets
 225 although no significantly different from D2 ($P < 0.05$) but were significantly different
 226 from D3 and D4.

227 In the present study, the inclusion of TFMS significantly influenced the proximate
 228 composition of fish muscle tissue. Increased crude fat deposition was observed in
 229 response to increase in the levels of toasted flamboyant seed inclusion, in this
 230 circumstance, the lipid was inversely proportional to moisture. This result then to agree
 231 with the findings of [26, 27, 28, 7, and 29]. When plant base protein source are included
 232 in fish diets there is tendency for increase in the carcass lipid.

233 Table 6, revealed the haematological parameters of the experimental fish before and
 234 after the feeding trial. Fish fed the experimental diet had a higher value in all the
 235 Haematological parameters measured as at the end of the experiment as compared to the
 236 initial before the feeding trial. The fish fed D3 had the highest Packed Cell
 237 Volume, while fish fed D4 had the lowest PCV value and was significantly ($p < 0.05$)
 238 different from those fed the other experimental diets. However there was no significant
 239 difference between fish fed D1, D2 and D3 ($P > 0.05$). WBC values showed that fish fed
 240 D4 had the highest value and was significantly different from others ($P < 0.05$), although
 241 D1 had the lowest WBC value it was not significantly different from D2 and D3 ($P >$
 242 0.05). The RBC and Hb followed the same pattern as PCV. LYMPH showed that D3 was
 243 higher and significantly different from other fish fed experimental diets, however there
 244 was no significant differences between D1, D2 and D4. While MCH followed the same
 245 pattern as WBC, there was no significant difference in MCHC and MCV among all fish
 246 fed the experimental diets ($P > 0.05$).

247 The results of the Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell
 248 (RBC), and White blood Cell (WBC) in this present study were within the acceptable
 249 range of a healthy *C. gariepinus* fingerlings[30 and 31], indicating that TFSM is not
 250 toxic to *Clarias gariepinus* fingerlings, and are safe to feed up to 30% level of inclusion.
 251 The values obtain in this study was higher than those reported by [30, 31, 32, 33, 34 and
 252 35]. The increase in all of the hematological parameters measured in all the fish fed the
 253 experimental diets is an indication that there was no blood lost in fish fed those diets
 254 when compared with what was reported by Sotulo and Faturori [33], when *Leucaena*
 255 *leucocephala* was fed to *Clarias gariepinus* fingerlings.

256 In summary the growth performance of *C. gariepinus* fed toasted flamboyant seed
 257 meal diets as presented in Table 4 shows that there was no feed rejection during the
 258 experiment, the diets were accepted by the fish and they ingested the feeds actively,
 259 although the acceptability differs among the treatment as could be seen in their total
 260 feed intake. When plant materials are used in fish diets, one common problem is usually
 261 the acceptability of the diet by the fish which has to do with the level of inclusion and
 262 palatability of the diet [21]. Proper processing of ingredients has been reported to have
 263 effect on the texture, palatability, and the level plant material incorporated, hence can
 264 affect the acceptability and level of feed intake [20, 21, 22 and 23]. In this present study
 265 the high FI obtained in this feeding trial may also be ascribed to the toasting processing
 266 technique employed in processing the flamboyant seed. This may have reduced the
 267 effect of anti-nutritive factors and other secondary compound activities in the seed
 268 which tend to have enhance the palatability and boosted the feed intake as evident in the
 269 high feed intake of all the fish fed the experimental diets. In conclusion, the results of
 270 this study demonstrate that 10–30% of TFSM can be included in the diet of *C.*
 271 *gariepinus* fingerlings without any adverse effect on their growth performance and
 272 blood system. Further research need to be carry out on the amino acid and essential fatty
 273 acid profile of the toasted flamboyant seed on a long term study.

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382

383 **Table 1: Proximate composition of the major ingredients used in the formulation of**
 384 **the experimental diet for *C. gariepinus* fingerlings.**

Ingredients	Fishmeal	Soybean meal	Maize meal	Millet meal	RFSM	TFSM
Proximate composition						
Moisture (%)	5.79	2.09	4.66	3.22	4.02	3.78
Crude protien (% d.b. ^{*1})	65.34	43.63	9.32	12.86	22.45	29.58
Crude lipid (% d.b. ^{*1})	11.36	7.00	4.20	4.36	5.68	9.39
Ash (% d.b. ^{*1})	14.34	8.15	3.22	2.33	6.35	4.14

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386 **Table 2: Effect of toasting treatment on anti-nutritional factors in *Delonix regia***
 387 **seed meal.**

Anti-nutritive factors	RFSM	TFSM	(%) decrease of anti-nutritive factors after fermentation
Phytic acid (mg/100g)	540.07	141.33	73.83
Cyanide (mg/100g)	19.45	7.15	63.24
TIA (mg/100g)	38.92	10.21	73.77
Tannin (g/kg)	22.74	8.82	59.43
Total Oxalate	45.46	10.15	77.67

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389 **Table 3: Formulation of the experimental diet and proximate composition of the**
 390 **experimental diet for *C. gariepinus* fingerlings (g/kg).**

Ingredients	D1	D2	D3	D4
FM	529.30	483.80	438.50	393.00
SBM	100.00	100.00	100.00	100.00
TDRM	0.00	100.00	200.00	300.00
MM	50.00	50.00	50.00	50.00
Millet	50.00	50.00	50.00	50.00

starch	55.00	55.00	55.00	55.00
Vitamin premix	15.00	15.00	15.00	15.00
SBO	24.30	20.10	15.90	11.70
Cellulose	161.40	111.10	60.60	10.30
Mineral premix	15.00	15.00	15.00	15.00
Total	1000.00	1000.00	1000.00	1000.00
Moisture (%)	4.18	4.29	4.15	4.31
Crude protien (% d.b. * ¹)	37.51	37.98	37.46	37.22
Crude lipid (% d.b. * ¹)	8.75	8.82	8.66	8.92
Ash (% d.b. * ¹)	12.52	12.63	12.78	12.95

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Table 4. Growth performances and nutrient utilization of *C. gariepinus* fry fed experimental diets for 56 days.

Diet code	Body weight (g)		Weight gain (%)	Survival rate (%)	Specific growth rate (%)	Total feed intake (g)	Feed efficiency	Protein efficiency ratio	Protein retention (%)
	Initial	Final							
D1	1.02±0.04	13.25±0.05 ^c	1199.02±14.32 ^c	98.80±1.14 ^a	4.58±0.01 ^c	16.95±0.31 ^b	0.72±0.01 ^b	1.92±0.05 ^b	34.50±0.24 ^b
D2	1.01±0.06	14.03±0.28 ^b	1289.11±27.51 ^b	98.96±1.07 ^a	4.70±0.04 ^b	17.78±0.17 ^a	0.73±0.01 ^b	1.95±0.02 ^b	34.59±0.38 ^b
D3	1.02±0.06	15.73±0.03 ^a	1442.16±37.25 ^a	98.97±1.02 ^a	4.88±0.02 ^a	17.58±0.12 ^a	0.84±0.04 ^a	2.20±0.08 ^a	40.18±0.75 ^a
D4	1.02±0.02	13.18±0.03 ^c	1191.83±32.22 ^c	98.82±1.05 ^a	4.57±0.06 ^c	16.75±0.12 ^b	0.73±0.03 ^b	1.95±0.03 ^b	34.42±0.25 ^b

Values in the same column with different superscript letters are significantly different ($p<0.05$) from each other.

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Table 5 Proximate composition analyses of whole body *C. gariepinus* (wet basis) fed experimental diets for 56 days

Component (%)	Initial	Final* ¹			
		D1	D2	D3	D4
Moisture	77.82	75.02±1.4 ^a	73.76±1.3 ^b	72.45±1.4 ^c	71.82±1.4 ^d
Protein	13.45	17.59±1.7 ^b	17.39±1.7 ^a	17.93±1.2 ^c	17.32±1.5 ^c
Lipid	3.87	4.47±0.6 ^c	4.72±0.6 ^c	5.98±0.2 ^b	6.97±0.7 ^a
Ash	2.96	3.21±0.4 ^a	3.22±0.4 ^a	3.25±0.4 ^a	3.28±0.6 ^a

*1 Values in the same row with different superscript letters are significantly different ($p<0.05$) from each other (n=3).

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397 **Table 6. Haematological parameters of *C. gariepinus* fingerling fed experimental diets for 56 days**

Blood parameters	Initial	Final ^{*1}			
		D1	D2	D3	D4
PCV (%)	20.62	28.34±1.27 ^a	28.42±1.34 ^a	28.68±1.36 ^a	26.55±1.47 ^b
WBC (10 ³ mm ⁻³)	7.18	7.29±0.24 ^b	7.37±0.28 ^b	7.41±0.52 ^b	7.67±0.32 ^a
RBC (10 ³ mm ⁻³)	2.56	3.28±0.35 ^a	3.24±0.26 ^a	3.18±0.42 ^a	2.95±0.38 ^b
Hb (g/100 ml)	6.19	8.61±0.67 ^a	8.87±0.24 ^a	8.96±0.43 ^a	8.02±0.52 ^b
LYMPH (100)	60.15	61.40±4.47 ^b	61.66±3.33 ^b	62.38±2.54 ^a	61.45±3.27 ^b
MCHC (%)	30.02	30.40±1.55 ^a	31.21±1.23 ^a	31.24±1.61 ^a	30.21±1.28 ^a
MCH (pg)	24.18	26.25±1.88 ^b	27.38±1.76 ^b	28.19±1.42 ^b	27.19±1.65 ^a
MCV (fl)	80.55	86.39±5.36 ^a	87.72±3.48 ^a	90.25±6.32 ^a	90.00±3.28 ^a

PCV, Packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; LYMPH, lymphocyte; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

***1 Values in the same row with different superscript letters are significantly different (p<0.05) from each other (n=3).**

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