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## ECONOMIC, GROWTH PERFORMANCE AND HEAMATOLOGICAL IMPLICATIONS OF RAISING CLARIAS GARIEPINUS ADVANCED FRY ON HIGH PROTEIN DIETS IN CONCRETE HOMESTEAD PONDS.

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### ABSTRACT

The commercial production of fish in concrete homestead ponds is fast gaining acceptance in Nigeria, but the growth rate of such fish is constrained by the stocking of good-sized fingerlings and the availability of quality fish feed. Hence, this study was designed to investigate the economic analysis, growth performance and hematological changes in advanced fry raised on high protein diets in concrete tanks. The design of the experiment is completely randomized and the economic viability of the project was determined by comparing the cost: benefit ratio of the four feeding regimes. One thousand, two hundred *Clarias gariepinus* advanced fry with initial mean weight of  $0.74 \pm 0.18\text{g}$  were randomly divided into 4 groups. Three  $1\text{m}^3$  net cages were suspended in each tank of (3m x 2m x 1.5m) dimension. The treatments were fed practical diets containing 40% (control); 48%; 56%; 64% CP levels respectively for 12 weeks. The following parameters were monitored as stated: weight gains (bimonthly); water quality (weekly); haematology (initial and at end of experiment); carcass analysis at (initial and end of experiment). Feed conversion ratio, proximate analysis and cost: benefit ratio (were calculated at end of the experiment). Data generated were statistically analyzed using the 2-way analysis of variance (ANOVA) and Duncan's multiple range tests at 95% and 99% confidence limits as appropriate. The results of the study showed that raising of fingerlings in concrete tanks can be enhanced optimally through the feeding of a 48% CP inclusion as against the convectional 40%CP. in practical diets used in earthen ponds. The incidence of cost showed that feeding advanced fry on 40% and 48% CP diet are not significantly different but both values are significantly better than fish fed on diet 56% and 64% CP. While the profit index showed that it is more profitable to feed the fish on diets containing either 40%, 48% and 56% CP. However, fish fed on the 48%CP diet in this study had optimum mean weight gain of (9.49g) and the highest PPV value (2.44), which was significantly different from fish, fed 40%( 2.41), 56% (0.75) and 64% (1.15). In addition, this research further highlighted the fact that high CP inclusion beyond the optimum does not necessarily give higher weight gains nor give the best economic reward because fish diets 3 (56%CP) and diets 4(64%CP) had lower weight gains and lower profit index. There were no appreciable derangements in hematological parameters in fish fed 40%CP and 48% CP. However fish fed 56%CP and 64% CP developed normocytic normochronic anemia and hypoproteinaemia conditions. This implies wastage of resources and confirms that inclusion of CP levels higher than 48%CP in diets of *C gariepinus* fry had negative effects on haemopoiesis and does not have growth, hematological nor economical advantage in raising advanced fry to fingerling in concrete tanks.



## **INTRODUCTION**

Aquaculture is the fastest growing food production sector in the world, with an average annual increase of about 10% since 1984 as compared to 3% increase for livestock meat and 1.6% for capture fisheries FAO (1997). To sustain this high rate of growth in aquaculture production, there is a need to increase the levels of production of fish feeds. Fish feed production is currently one of the fastest expanding agricultural industries in the world, with an annual growth rates in excess of 30% per year (Francis et. al., 2001). The latter authors reported that on the global scene, almost a third of the 122metric tons of fish harvested in 1997 was converted into fishmeal or fish oil and used in aquaculture feeds. Sargent and Tacon (1999) and FAO (1999) reported that 2 metric tons of global production of fishmeal was used in fish farming in 1996 out of which 1.3% went into catfish farming and that this represented about 40% of total fish feed production.

Fagbenro and Adeparusi (2003) reported an increasing attempt to develop standard practical diets for farmed fish in Nigeria. Two sources of fish feeds were identified, namely, farm-made and commercial feeds. These authors reported that the majority of fish feed produced and used locally for fish farming are farm –made with very few commercial sources. A few specialized animal feed millers engage in fish feed production on demand. Even then, two main types of feeds are produced by both sectors: feeds for herbivores (tilapias) with 25%-30 % crude protein (CP) and carnivores (mainly catfish) containing 40- 50% CP. In 2000, the Nigerian aquaculture industry consumed an estimated 35,570 tones of feed, (Fagbenro and Adeparusi, 2003).

The Nigerian aqua cultural history of over 50 years showed that the early fish pond owners had sad experiences. These pioneer fish farmers had to grapple with low fish production in the absence of technical know-how, lack of commercial feeds and fish seeds but more prevalent was the poor security coverage for such fish farms. However, in recent years there has been a conspicuous increase in the construction of fish farms, especially backyard ponds, (Olukunle, 2000.) The number of homestead ponds increased from 3 in 1994 to over 50 in 2000 in Oyo state, Nigeria (IFAD, 2000) but the number must have doubled since then. The upsurge in the number of fish farms and homestead ponds encourages increase in fish feed production. However, Nigeria is not a producer of fishmeal a major component in fish diet preparation. Hence the observable rise in the cost of fishmeal from 30,000 Naira/ton in 2002 to 250,000 Naira/ton in January 2004 (market prices). This trend engendered researchers in Nigeria to work on the inclusion of non- convectional protein sources in the diet of catfishes to lower the cost of fish feed production, (Faturoti et. al., 1986; Arowosoge, 1987; Balogun and Ologhobo, 1989; Omitoyin, 1995; and Olukunle, 1996). Faturoti et. al. (1986) recommended 40% CP diets for raising catfish fingerlings in earthen ponds. However, poor growth has been recorded in concrete tanks on 40% CP containing diets without supplementary fly larvae or zooplankton.



Hence, this study was designed to determine the growth response of *C. gariepinus* raised in concrete homestead ponds on practical diets containing varying high crude protein (CP) inclusion levels, their subsequent effects on the pond water quality, the economic implications of the feeding regimes and the hematological responses of the test fish investigated.

## **MATERIALS AND METHODS**

### **Experimental Diets, Rearing System, Fish and Feeding Regimes**

Four diets were formulated at 40%, 48%, 56% and 64% crude protein (CP) inclusion levels as shown in Table 1. The sources of CP in the diets were fish meal purchased from a commercial outlet in Ibadan, Nigeria, cow blood meal prepared by absorbing fresh cow blood in wheat bran in the ratio of 2.5:1 (v/v), dried and ground as described by FAO (1983 and 1989), and sesame seed meal prepared by soaking and fermentation for 5 days as described by Francis *et al.* (2001).

The experiment was carried out in open concrete homestead 3m x 2m x 1.5m fish tanks, at the University of Ibadan, Nigeria. Continuous water flow was supplied from an overhead 3,000-liter capacity tank; separate moveable 1" stand pipes served as inlets and outlets for each sub-unit and water depth was approximately 1.2m in all the sub-units for most of the time.

One thousand and two hundred *Clarias gariepinus* advanced fry, initial mean weight of 0.74 ± 0.18g, were divided into four groups in 1m<sup>3</sup> net cages suspended in triplicates of 100 fingerlings in each of the four concrete tanks. Each group of the test fish were fed diets containing 40% (diet 1) which is the control, 48%CP (diet 2), 56%CP (diet 3) and 64%CP (diet 4) for 12 weeks at the rate of 5% of their body weight.

### **Measurements of Water quality, Growth performance, Hematology and Cost: benefit analysis**

Weight gains of the test fish in each group were determined by batch weighing of fish in each triplicate net cage before commencement to give (initial weight), feeding for two weeks and weighing again till the end of 12 weeks when the final weights were taken. Feed conversion efficiency and proximate analysis of the carcasses of the test fish given the practical diets were determined over the experimental period. Water samples from the ponds were analyzed for dissolved oxygen (DO) and temperature were monitored daily throughout the experimental period as described by Boyd (1981). Blood samples were collected from 4-5 fish at initial and at the end of 12 weeks, analyzed for packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) and total white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total plasma protein (TPP) as described by Adedeji *et al.* (2000). The cost:



benefit analysis including incidence of cost, profit index and PPV for each diet in the feeding regime was calculated as in Olukunle (1996) and the prevailing market price of fish, and feed ingredients. Data generated were statistically analyzed using the 2-way analysis of variance (ANOVA) and Duncan's multiple range tests at 95% confidence interval.

## RESULTS AND DISCUSSION

The composition and proximate analysis of the experimental diets fed to *C. gariepinus* fingerlings raised in concrete homestead ponds are presented in Table 1. The proximate analysis of the different feed samples revealed that crude protein levels followed the pattern of protein composition, except for the 64% CP diet, which had slightly lower proximate CP level of 60.25% (Table 1). The levels of NFE, which consist mainly of carbohydrates, are negatively correlated with proximate CP levels and follow the graded decline in the content of *Mesolima*, the only major source of carbohydrate in the diets. This is an indication of progressive declining energy content and increasing protein content especially in Diets 3 and 4 (Table 1). This is confirmed by the high-calculated values of P: E values for diets 3 (31.63) and 4 (48.2) as compared to the low P: E value of diet 1(10.49). Diet 2 however, shows an optimum P: E value of 15.78 and similar to the value obtained for juvenile channel catfish (16), Stickney (1978)

The growth performance of the test fish in response to the treatments is shown in Table 2. All the diets were acceptable as shown by the adequate Metabolizable Energy (M.E.) the treatments contain (Table 1) and the growth shown by the test fish in all the treatments. However fish fed diets 1 (40% CP), and 2 (48%CP) had higher mean weight gains (MWG) than fish on diets 3 (56%CP) and diet 4 (64%CP). The MWG of diet 2, (9.49g) and the % weight gain of 92.32. % were the highest, while the fish on diets 3 and 4 recorded very poor MWG of 3.93g and 5.40g respectively. The lower growth performance of diets 3 and 4 on high protein content imply that the protein in the diets were probably catabolised for energy. Fish on diets 1(1.76) and 4 (1.78) had insignificantly different Food Conversion Ratios (FCR) from each other but are higher than diets 2 (1.52) and diet 3 (1.48). This implies that diet 2 and 3 were better utilized than diets 1 (the control) and 4. The low Protein to Energy (P: E) ratio content of the control diet (10.49) compared to the other diets is probably responsible for the higher FCR of diet 1. This agrees with the observation of Ringrose (1971) and Phillips (1972) who stated that there is need for a proper balance of protein and non-protein energy to supply calories and raw materials for rapid growth and efficient feed utilization. These authors concluded that this P: E ratio concept is only applicable to diets adequate in metabolizable energy (ME). These diets were adequate in M.E using values of ME for protein, carbohydrate and lipid, (Brody, 1945; Garling and Wilson, 1976).

Table 3 shows the carcass composition of the fish fed the different diets. The carcass proteins follow an increasing trend, while the NFE values a decreasing pattern. as the dietary composition within the treatments. This shows that the diets influence the composition of the



fish carcass. This is in agreement with the observation of Sena et. al. (1989) working with *O. niloticus* fry, varied levels of dietary protein level and different levels of plant ingredient (*P. aureus*) incorporated to determine performance as measured by consumption, growth performance and carcass composition concluded that protein deposition was higher with high protein diets.

The water quality showed considerable variability in Dissolved Oxygen (DO) values between the mornings and evenings. The DO values within the treatments range between 5.8 and 8.0mg/L through out the duration of the experiment which is adequate for good fish growth. Body (1982) and Adekoya et. al. (2004). DO levels are known to be high during the daytime when photosynthesis is high and low during the night when respiratory activities are higher. Samples of analyzed experimental tank water were taken during the daytime in the present study. A much wider and more frequent sampling frame would be required than was possible in this experiment, just as Madrian Sterling (1985) advised. Table 4 shows the values obtained monitoring the temperatures of the water in the treatment tanks. The water temperatures ranged from 25.5° to 26.5°C in the morning hours and 28.0° C to 29.0 °C in the evenings during the first 4 weeks, and 23.5° to 25°C in the mornings but remained the same in the evenings in the last 8 weeks of the experiment, which incidentally fell within the harmattan season (mid-October/December). There is a differential lowering of temperatures in the mornings, which became warmer in the evenings, during the harmattan season. This affected fish growth and survival. Viveen *et al.* (1985) recommended an optimum temperature of 30°C for good survival of fry-fingerlings of *Clarias sp.* during the first 5 weeks of life. They also suggested heating the indoor gutters or tanks with overhead bulbs when night temperatures fall during the harmattan. However, this may seem impracticable in the large outdoor concrete tanks used for this experiment, but the use of black polystyrene as to enhance survivals within the treatments.

The economic evaluations of the experimental diets are shown in Tables 5a and 5b. From the cost analysis, the cost of the ingredients per treatment is cheapest in diet 1 (10.19) and highest in diet 4 (14.14). Diets 2 (12.23) and 3 (13.00) are not significantly ( $P < 0.05$ ) different from each other but differ significantly ( $P < 0.05$ ) from diets 1 and 4. Protein Productive Value (PPV) is a measure of body protein stored in the flesh of animals as a result of growth. However, Table 5b shows the PPV of diets 1 and 2 as significantly ( $P < 0.05$ ), different from PPV of diets 3 and 4. The implication of these observations in this growth study is that the cheapest (Diet 1 with an incidence of cost (IOC) of 0.180) or the most expensive diet (Diet 4 with an IOC of 0.274) did not give the optimum growth performance. Diet 2 gives the optimum values for PPV with insignificantly different values for profit index and IOC to Diet 1, which is the control. If the environmental conditions were not as harsh as it was in this experiment thus affecting the survival (Table 6) of the advanced fingerlings during the harmattan months (November and December), there would have been a higher



over all monthly mean survival in all the treatments. In spite of the negative effect of the environment on this research, the higher monthly mean survival of fish fed diet 2 (21.67%) was greater than those fed diet 1 (10.0 %). This observation correlates with the observation of Viveen and Huisman (1985). Table 7 shows the hematology of the test fish fed the various diets before and at the end of the 12-week feeding trial. While the fish fed diets 1 and 2 (40% and 48% CP inclusion) did not show any appreciable derangement in haematological parameters and plasma protein levels, the fish fed diets 3 and 4 (54% and 60% CP inclusion) developed normocytic normochromic anemia and hypoproteinaemia, despite the high CP levels in their diets. This implies wastage of resources and that the high CP in their diets had negative effects on haemopoiesis. The hypoproteinaemia may be related to protein catabolism for energy production, rather than NFE (carbohydrates), which were very low in these fish (Table 3).

### **CONCLUSION**

It can be concluded from this growth studies, that fish raised in concrete tanks and fed diet with a 48% CP inclusion has optimum growth performance, higher profit index and insignificantly lower incident of cost than those grown on the usual recommended 40% CP. This research further confirms the observation that very high CP inclusion levels do not necessarily give the best weight gains or the best economic returns. Therefore, special care must be taken when growing and fattening fry in open concrete tanks. Such tendering must involve reduction of temperature differential between the hot afternoon temperatures and the extreme cold ones in the night especially during the harmattan seasons in the tropics. Special care must also be paid to the P: E ratio component of the diets utilized for feeding catfish fry.

### **Recommendation:**

It is recommended that specialists should take it upon themselves and use their expertise to raise advanced fry to fingerlings as it is done in Asian aquaculture to assist local fisher folks to improve survival of fingerlings in culture media. This will increase aqua cultural fish production. A regulating body like Fisheries Society of Nigeria should monitor and ensure that hatcheries stop the practice of selling fingerlings weighing less than 5g for stocking homestead and earthen ponds.

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**Table 1: Composition and proximate analysis of experimental feeds**

Composition	% CP inclusion levels			
	40	48	56	64
Fish meal	37.45	48.28	52.59	59.05
Sesame seed cake	9.36	12.07	13.50	14.70
Blood meal (in wheat bran)	15.00	15.00	15.00	15.00
Mesolina*	28.18	14.65	9.41	1.15
Plant oil	2.50	2.50	2.50	2.50
Animal oil	2.50	2.50	2.50	2.50
Vitamin C	2.00	2.00	2.00	2.00
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
<b>Proximate analysis (%)</b>				
Crude protein	38.87	47.20	55.85	60.25
Lipid	5.53	5.46	4.87	4.80
Ash	15.70	15.09	17.82	17.27
Crude fibre	0.16	0.15	0.23	0.17
Moisture	8.23	7.64	8.44	9.81
NFE	31.51	24.46	12.79	7.70
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

P: E                    10.49    15.78    31.63    48.2  
M.E kcal /g            4032.9   4212.0   4170.6   4326.5

Calculated from channel catfish D: E values (4.0, 9.0, 4.0) for protein, lipid, CHO. (Winfree and Stickney, 1981)

Metabolizable Energy (ME) content calculated from

M.E. of Protein        = 4.5 kcal/g  
Carbohydrate = 3.5kcal/g  
Lipid                    =8.5kcal/g (Brody, 1945; and Garling and Wilson, 1976)

\*Made from maize (source of carbohydrate along with wheat bran used to absorb blood meal)



**Table 2: Growth performance of *C. gariepinus* fingerlings raised in concrete homestead ponds with high crude protein diets**

Parameters	% CP inclusion in diet				Means ±SE
	40	48	56	64	
Initial mean weight (g)	0.88 <sup>a</sup>	0.79 <sup>a</sup>	1.00 <sup>b a</sup>	0.89 <sup>a</sup>	0.89± 0.08
Final mean weight (g)	10.25 <sup>a</sup>	10.28 <sup>a</sup>	3.93 <sup>b</sup>	5.40 <sup>c</sup>	7.47± 0.23
Mean weight gain (g)	9.37 <sup>a</sup>	9.47 <sup>a</sup>	2.93 <sup>c</sup>	4.51 <sup>b</sup>	6.58± 0.21
Mean weight of feed consumed (g)	326.5 <sup>a</sup>	326.4 <sup>a</sup>	335.82 <sup>b</sup>	273.14 <sup>c</sup>	315.47±1.48
% Weight gained	91.41 <sup>a</sup>	92.32 <sup>b</sup>	74.55 <sup>c</sup>	83.52 <sup>d</sup>	85.45± 0.77
FCR	1.76 <sup>b</sup>	1.52 <sup>a</sup>	1.48 <sup>a</sup>	1.94 <sup>c</sup>	1.78±0.11

**Table 3: Carcass analysis (%) of *C gariepinus* at the end of the experiment**

Parameters	Initial	% CP. inclusion in diet			
		40	48	56	64
Crude protein	51.43	51.90	60.94	61.60	63.30
Crude fibre	1.28	0.83	1.37	1.24	1.12
Lipid	6.56	6.83	7.04	6.18	5.69
Ash	15.72	20.81	18.76	19.54	21.51
Moisture	5.16	4.70	7.26	6.13	6.86
NFE	19.85	14.93	4.63	5.31	1.52

**Table 4: Water Quality Monitoring (Average Temperatures 0 °C**

Date	Time of day	Tanks			
		1	2	3	4
16 – 30 Oct. 2003	Mornings	26.0	26.0	26.2	25.5
	Evenings	28.0	28.0	29.0	28.5
1st Nov-Dec. 24 2003	Mornings				
	Inlet	24.0	24.5	24.8	25.5
	Outlet	23.5	24.2	25.0	25.0
	Evenings	28.0	28.0	29.0	28.5

Table 5a: Cost Analysis of Diets /100g (Naira and Kobo)

Ingredients	D	I	E	T	S	Mean
Naira/Kg	40% CP		48%CP	56%CP	64%CP	
Fish meal 200	37.45 (7.49)		48.28 (9.66)	52.59 (10.52)	59.05 (11.81)	
Beniseed cake 44	9.36 (0.42)		12.07 (0.53)	13.50(0.59)	14.76 (0.65)	
Blood meal + wheat bran 15	15.00 (0.023)		15.00 (0.23)	15.00 (0.23)	15.00 (0.23)	
Mesolina 280/10Kg	28.19 (0.85)		14.65 (0.41)	9.41 (0.26)	1.18 (0.03)	
Plant oil (150/ Liter bottle)	2.50 (0.38)		2.5 (0.38)	2.50 (0.38)	2.50 (0.38)	
Animal oil (395/litre bottle)	2.50 (0.99)		2.50 (0.99)	2.50 (0.99)	2.50 (0.99)	
Vitamin C 2200	2.00 (0.004)		2.00 (0.004)	2.00 (0.004)	2.00 (0.004)	
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> 4000/50kg bag	2.00 (0.008)		2.00 (0.008)	2.00 (0.008)	2.00 (0.008)	
Salt 30/1.5	1.00 (0.02)		1.00 (0.02)	1.00 (0.02)	1.00 (0.02)	
	<b>100.00</b> <b>(10.19)<sup>b</sup></b>		<b>100.00</b> <b>(12.23)<sup>a</sup></b>	<b>100.00 (13.00)</b> <b><sup>a</sup></b>	<b>100.00</b> <b>(14.14)<sup>d</sup></b>	<b>12.39± 0.29</b>

Source; Adom Feed mill, Oyo Road, Ibadan, Nigeria  
Prevalent Current market Prices October (2003)

Table 5b: Economic evaluation of the different diets

Parameters	% CP inclusion in diet				Mean's ±SD
	40	48	56	64	
Incidence of cost	0.180	0.185	0.192*	0.274**	0.208±0.04
Profit index	0.54	0.53	0.53	0.30**	0.48±0.06
PPV	2.41	2.44	0.75**	1.15*	1.69±0.11

\*,\*\*Significantly different from corresponding values in diets with 40 and 48% CP inclusions levels at p<0.05 and P<0.01, respectively.

Incidence of cost = Cost of feed/ kg of fish produced  
Profit Index = Weight of fish x cost of fish/ cost of feed/kg  
PPV = Increment of body protein/Protein intake / day  
Source: Olukunle (1996)



Table 6 : % Monthly Mean Survival

Treatment	1st Oct.	30 <sup>th</sup> Oct.	29 <sup>th</sup> Nov.	24 <sup>th</sup> Dec.
1	100	65.00 <sup>b</sup>	19.00 <sup>d</sup>	10.00 <sup>d</sup>
2	100	58.67 <sup>c</sup>	23.00 <sup>c</sup>	21.67 <sup>c</sup>
3	100	73.33 <sup>a</sup>	29.00 <sup>b</sup>	19.67 <sup>b</sup>
4	100	51.00 <sup>d</sup>	29.67 <sup>a</sup>	26.33 <sup>a</sup>
Mean	100	62.00	25.17	19.42
S.E±	0.83	0.66	0.42	0.36

Table 7: Hematology of *C. gariepinus* *C. gariepinus* fingerlings raised in concrete homestead ponds with high crude protein diets

Parameters	40	48	56	64
<b>Day 0</b>				
PCV (%)	30.8±0.2	31.6±0.3	31.5±0.2	30.9±0.1
RBC count (x10 <sup>6</sup> /µl)	2.6±0.1	2.7±0.1	2.6±0.1	2.2±0.0
Hb. Conc. (mg/dl)	10.1±2.1	9.8±1.1	10.0±1.1	8.9±1.3
MCV (fl)	120.4±5.6	118.3±4.4	117.2±3.9	138.1±3.4
MCHC (%)	31.2±2.1	31.9±2.3	32.3±3.1	28.9±2.3
WBC (x10 <sup>3</sup> /µl)	29.8±3.3	30.3±3.1	30.1±2.2	30.8±2.5
TPP (g/dl)	5.3±0.3	5.1±0.1	5.2±0.0	5.2±0.1
<b>Week 12</b>				
PCV (%)	34.2±0.3	32.3±0.5	26.3±0.3**	22.8±0.2**
RBC count (x10 <sup>6</sup> /µl)	2.9±0.2	2.8±0.3	2.0±0.0*	1.7±0.1*
Hb conc. (mg/dl)	11.5±1.2	10.2±0.5	7.1±0.4*	5.5±0.3**
MCV (fl)	118.5±3.7	117.4±2.2	131.3±4.6	134.4±5.3
MCHC (%)	33.5±3.2	31.7±3.1	26.7±2.2	24.0±2.6
WBC (x10 <sup>3</sup> /µl)	32.3±4.8	32.6±3.4	33.4±4.5	32.6±2.7
TPP (g/dl)	5.2±0.1	5.0±0.2	4.9±0.3	4.1±0.2*

\*,\*\*Significantly different from corresponding values at Day 0 and across CP levels at P<0.05 and P<0.01, respectively.