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# Effects of dietary potassium diformate (KDF) on growth performance and immunity of the sea bass, *Dicentrarchus labrax*, reared in hapas

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**ABSTRACT**

An experimental investigation was conducted to examine the effects of potassium diformate (KDF) with different levels of (0, 2, 3, and 4 g $kg^{-1}$ diet) in the diets for European sea bass, *Dicentrarchus labrax* juveniles on the growth performance, feed utilization, and haematobiochemical parameters for 90 days. Latterly, the highest final weight (35.1 g) and weight gain (29.6 g) were detected in the fish fed diet supplemented with potassium diformate at the level of 2 g $kg^{-1}$ (KDF2). Significant differences in the survival rate (%) were observed among the experimental groups, enhanced by increasing the level of KDF in the experimental diets and the highest value (98.89%) was observed in the fish fed diet supplemented with potassium diformate at the level of 4 g $kg^{-1}$ (KDF4). Increased values of white blood cells, red blood cells, haemoglobin, and haematocrit were observed in the fish fed KDF2 diet. Serum analyses of sea bass fingerlings fed the experimental diets with different levels of KDF had a significant ($P < 0.05$) effect on the immunophysiological parameters such as phagocytic percentage and lysozyme activity. The increasing levels of KDF in the experimental diets did affect the phagocytic percentage and the lysozyme activity positively ($P < 0.05$). It can be concluded that the dietary potassium diformate (KDF) has a positive effect on the growth performance, feed utilization and survival rate of European sea bass fingerlings. These results show the potential for KDF application in the cultivated sea bass juvenile diets to serve as a growth promoter and a basic defense module, when given at 2 or 3 g $kg^{-1}$.

## INTRODUCTION

Compared to terrestrial animals, feeding methods in aquaculture currently tend to increase the use of feed additives which have been applied successfully to other animal species and have generally been recognized in terms of feed and food safety (**Liebert *et al.*, 2010**). Potassium diformate (KDF) is unscented, low eroding, flowable and dietary **(Hebeler *et al.*, 2000**); however, dietary inclusion is successful in improving the growth performance of terrestrial animals (**Øverland *et al.*, 2000; Canibe *et al.*, 2001; Mroz *et al.*, 2002**). The European Union permitted KDF as the major material of a potential antibiotic (**Février *et al.*, 2001**). Research on

potassium diformate (KDF) supplemented fish diets is of great interest but its antimicrobial mode of action in aquatic animals is therefore unclear (**Ramli *et al.*, 2005**). Acidifiers are hopeful unconventional materials as well as gaining significant interest in improving the performance and safety of treated animals as a possible replacement in the feeds and the digestive tract (**Freitag, 2007**).

Organic acids are the example of a group of additives that will play a major role within the future in aquaculture diets. Numerous studies have demonstrated a wide range of organic acids in cold water animals (**Gislason *et al.*, 1994 and 1996**) and tropical species (**Ramli *et al.*, 2005 and Petkam *et al.*, 2008**), indicated that organic acids, their salts and/or combinations may improve the growth, feed utilization and disease resistance in fish. Organic acids were first used in piglets for animal feed to recover their restricted capability to maintain a low gastric pH, which linked to problems with digestion (**Easter, 1988**). Antibiotics prevent the development of microbes, while acidifiers are more selective in their activity (**Cromwell, 1990**). They can reduce harmful microbes and promote useful microflora colonization of the gastrointestinal tract (**Mathew *et al.*, 1991**).

*Dicentrarchus labrax*, European Sea bass (ESB) is a precious marine fish grown commercially, so it is crucial to assess specific acidifiers as useful feed additives for the species. Insufficient research on the impact of dietary acidifiers on the development and/or resistance abilities of European sea bass (**Wassef *et al.*, 2017**). It was found that the weight improvement and survival percentage increased by 2% and 5% of polybeta hydroxy butyrate in ESB diets (**De Schryver *et al.*, 2010**). While there may be a wide range of indications at the efficacy of nutritional acidifiers for other fish species, the findings are nevertheless conflicting and no facts are to be had for marine European sea bass, especially diformates. Hence, the effects of these feed supplements on ESB's capability in health and immunity remain minimal (**Wassef *et al*. 2017**). Addressing KDF effects and the proper dose in sea bass diets would be essential. So, the current research was conducted to explore the effects of KDF with different levels on growth performance, feed utilization, then resistance indices in the diets for European sea bass cultivated in the hapa system.

## MATERIALS AND METHODS

### *2.1. Experimental diets*

The experimental diets were prepared to be isonitroginous (45.1 ± 0.06%) and crude lipids (15.4 ± 0.14%) (Table1). All ingredients were first ground to small particle size (approximately 1mm) in a Wiley mill (Labx Company, Midland, ON, Canada). The formulated diets were performed as, 0 (CTRL), 2.0 (KDF2), 3.0 (KDF3), and 4.0 (KDF4) g potassium diformate per kg diet provided by "Misr Feed Additives, hy-mix" Company in Cairo The local distributor of

ADDCON Group (GmbH, Germany). The experimental diets were prepared by thoroughly mixing ingredients (Table 1). Water was added to the ingredients of each diet and the mixture was undergone the kitchen tool to have pellets. Then dried, preserved and kept in sealed shut compartments. The proximate composition of the experimental diets was determined as stated by **AOAC (2005)**. Whole examinations were set in the National Institute of Oceanography and Fisheries, Alexandria, Egypt.

**Table 1** Ingredients (g $kg^{-1}$) of the experimental diets fed to sea bass, *Dicentrarchus labrax* for 90 days.

| | Diets (g $kg^{-1}$) | | | |
|---|---|---|---|---|
| Ingredients | CTRL | KDF 2 | KDF 3 | KDF 4 |
| Fish meal (65%) | 450 | 450 | 450 | 450 |
| Soybean meal (48%) | 200 | 200 | 200 | 200 |
| Gluten (corn) | 50 | 50 | 50 | 50 |
| Wheat (14CP) | 130 | 128 | 127 | 126 |
| Fish oil | 100 | 100 | 100 | 100 |
| Corn (7.5% CP) | 50 | 50 | 50 | 50 |
| Di calcium Phosphate | 10 | 10 | 10 | 10 |
| Premix† | 10 | 10 | 10 | 10 |
| Potassium di format (KDF) | - | 2 | 3 | 4 |
| Proximate analyses (%DM) | | | | |
| Dry matter | 90.73 | 90.32 | 90.01 | 90.43 |
| Crude protein (CP) | 45.01 | 45.12 | 44.98 | 45.07 |
| Total Lipids (L) | 15.49 | 15.34 | 15.22 | 15.52 |
| Ash | 9.72 | 9.44 | 9.76 | 9.67 |
| Fiber | 1.40 | 1.40 | 1.39 | 1.40 |
| Nitrogen Free Extract (NFE) ‡ | 28.38 | 28.70 | 28.65 | 28.34 |
| Gross energy (Kcal /100g) § | 517.62 | 518.13 | 516.06 | 518.07 |
| Metabolizable energy (ME, kcal/100g DM) ¶ | 427.07 | 427.45 | 425.68 | 427.44 |

† Premix Composition: Each 1 kg contains: Vit A 4.8 million IU; Vit D3, 0.8 million IU; Vit E 4 g; Vit K 0.8 g; Vit B1 0.4 g; riboflavin 1.6 g; Vit B6 0.6 g; Vit B12 4 mg; Vit C 150 mg; Nicotinic acid 8 g; Choline chloride 200 g; Folic acid 0.4 g; Biotin 20 mg; Pantothinic acid 4 g; Magnesium sulphate 22 g; Copper sulphate 4 g; iron sulphate 12 g; Zinc sulphate 22 g; Cobalt sulphate 100 mg; Selenium 0.4 g.

‡ Nitrogen Free Extract = 100 – (%Protein + %Fat + %Fiber + %Ash).

§ GE= Gross energy based on protein (5.65 kcal/g), Fat (9.45 kcal/g), and carbohydrate (4.12 kcal/g) according to (NRC, 2011).

¶ ME (kcal/100g DM) = metabolically energy was calculated by using factors 3.49, 8.1 and 4.5 kcal/g for carbohydrates, fat and protein, respectively according to Pantha (1982).

### *2.2. The experimental design*

Fingerlings of European Sea bass, *Dicentrarchus labrax* at the mean weight of 5.46 ± 0.20 g were distributed haphazardly in twelve hapas, net enclosure (each of 1m x 1m x1m) were introduced in four concrete tanks each one of 1 m x 8 m x 3 m. All procedures and handling of animals were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, USA. All tanks were washed cautiously and full with saltwater provided with constant air circulation, and the hapas were washed week by week to remove the residuals. Ten percent of the water was replaced weekly. Each hapa was loaded with 30 fish, three replicates for every treatment. Fish from each hapa were counted at regular intervals (two weeks) to evaluate the growth. Five percent of body weight were fed to every three groups of the experimental fish with one of the experimental diets three times per day (9.00 a.m., 12 p.m. and 3.00 p.m.) for 90 days. Fish were fed the experimental diets for 6 days weekly.

### *2.3. Water quality parameters*

Water tests taken week after week for the examination of ammonia, pH, water temperature and dissolved oxygen were controlled by the advanced YSI Model A.P.H.A (1995). During the 90 days feeding trial, the water-quality parameters arrived at the average of (±SD): water temperature, 26.97± 0.32°C; dissolved oxygen, 5.44 ± 0.09 mg $^{-l}$; pH, 7.32 ± 0.04; ammonia, 0.02±0.00 mg $^{-l}$ and salinity, 32 ppt.

### *2.4. Blood analysis*

After 90 days of the feeding trial, 6 fish per treatment were individually weighed. Then nearby one milliliter of fish blood from the caudal vein was drained and exchanged into small tubes with heparin. The plasma was separated by using a centrifuge device for five minutes at the speed of 1500 × g at room temperature (24°C) then put away at -18°C till biochemical analysis. Hemoglobin (g $dL^{-1}$) was measured by hemoglobin kits spectrophotometrically and the haematocrit (Ht) was calculated by **Stoskopf (1993)** and stated as a percentage of the packed volume of cells (percentage of PCV).

### *2.5. Sample examination*

Body compositions of the experimental fish after 90 days were investigated in terms of moisture, protein, lipids, and ash using the standard methods (**AOAC, 2005**). Six fish from each treatment were sampled for analysis. Blended samples were kept at -18°C and used exclusively for biochemical examination at the research facility of the National Institute of Oceanography and Fisheries, Alexandria, Egypt. The dry matter, crude protein, and crude lipids were analyzed respectively after dryness in drying oven (105°C for 24 h), by micro kjeldahl (N x 6.25), and ether extraction (by soxhlet method). Fish growth parameters were calculated as average daily gain (ADG; [Final BW – initial BW] /days), specific growth rate (SGR; [ln final BW – ln initial

BW] × 100/days) and survival ([no. of fish at the end of the experiment/no. of fish at the start of the experiment] × 100). Feed utilization parameters such as feed conversion ratio (FCR; dry feed consumed/WG), protein efficiency ratio (PER; WG/protein intake) and protein productive value (PPV; 100 (protein gain, g)/protein fed (g) were calculated. High-performance liquid chromatography (HPLC) analysis was conducted by the amino acid analyzer manual to analyze the amino acid composition of tested diets and fish.

### *2.6. Statistical analysis*

The variances between tested groups were examined by one-way ANOVA test. Before the ANOVA analysis, the percentage of specific growth rates were arcsine converted. Differences were considered significant at $P < 0.05$, Spss (2008) program. The differences among means were determined by Duncan ´s multiple range test (**Duncan, 1955**).

## RESULTS

### *3.1. Fish growing and feed consumption*

Growth performance of the experimental fish such as final weight, weight gain, Specific growth rate (SGR%/fish/day), and survival rate (%) are presented in Table 2. The highest final weight, weight gain (g) were observed in the fish fed KDF2, and differed significantly among the examined groups ($P < 0.05$). Furthermore, increasing the levels of KDF in the experimental diets reflected on the survival rate of examined fish positively ($P < 0.05$). However, SGR data did not show significant differences ($P > 0.05$) among the examined groups. FCR, PER, and PPV are performed in Table 2. The inclusion of KDF in the experimental diets did not affect significantly ($P > 0.05$) FCR among all groups. The control group gained the worst value (1.79). The higher protein efficiency ratios and protein productive values were detected in the fingerlings fed KDF2 and KDF3 supplemented feeds in comparison with the control group.

**Table 2** Growth performance and feed utilization of sea bass, *Dicentrarchus labrax* fed the experimental diets for 90 days. Values are mean ± SD.

| Treatment* | CTRL | KDF2 | KDF3 | KDF4 |
|---|---|---|---|---|
| Final weight (FBW, g) | 30.71±0.80$^{b}$ | 35.12± 1.55$^{a}$ | 32.25±0.59$^{b}$ | 31.57± 1.02$^{b}$ |
| Weight gain (WG, g) | 25.50±0.91$^{b}$ | 29.61± 2.71$^{a}$ | 26.57±1.03$^{ab}$ | 26.16±1.42$^{b}$ |
| Average daily gain (ADG, g) | 0.28±0.01$^{b}$ | 0.33± 0.03$^{a}$ | 0.29± 0.02$^{b}$ | 0.29± 0.02$^{b}$ |
| Specific growth rate (SGR, %/d)$^{\dagger}$ | 1.97± 0.05 | 2.05± 0.09 | 1.93±0.12 | 1.96±0.12 |
| Feed conversion ratio (FCR)$^{\S}$ | 1.79±0.02a | 1.56± 0.13$^{b}$ | 1.56±0.07$^{b}$ | 1.68± 0.10$^{ab}$ |
| Protein efficiency ratio (PER %) $^{\P}$ | 1.27±0.03$^{b}$ | 1.47± 0.13$^{a}$ | 1.47±0.07$^{a}$ | 1.35±0.09$^{ab}$ |
| Protein productive value (PPV%)$^{\|}$ | 23.90±0.46$^{b}$ | 27.54± 2.91$^{a}$ | 27.54± 1.68$^{a}$ | 25.54± 0.62$^{ab}$ |
| Survival (%) | 86.67± 3.34$^{c}$ | 92.22± 5.09$^{bc}$ | 94.44±1.93$^{ab}$ | 98.89± 1.92$^{a}$ |

* Values in the same row with a different letter differed significantly ($P < 0.05$).

† SGR (%/day) = 100 (Ln final weight – Ln initial weight) / Time (days)

§FCR = Total feed consumed (g fish$^{-1}$) / weight gain (g fish$^{-1}$)
¶ Protein efficiency ratio (PER) = weight gain (g fish$^{-1}$) / protein intake (g)
‖ Protein productive value (PPV %) = protein gain (g) / protein fed (g)

### *3.2. Body composition*

Fish composition analysis is stated in Table 3. The inclusion of KDF in the experimental diets did not affect the protein and lipids content of the experimental fingerlings ($P > 0.05$). The highest value (27.20) of lipids content was found in the fish fed KDF3. The ash content of the fish fed KDF4 differed significantly from the experimental groups and gained the highest value (16.57). The carcass energy of the fish body fed KDF3 did differ significantly from the other groups and gained the highest value (580.62). Potassium diformate (KDF) supplemented diets did not affect significantly (Table 4, $P > 0.05$) the amino acid profiles of the examined fingerlings.

**Table 3**. Body composition of sea bass, *Dicentrarchus labrax* fed different levels of dietary KDF for 90 days.

| Treatment* | Initial | CTRL | KDF2 | KDF3 | KDF4 |
|---|---|---|---|---|---|
| Dry matter | 33.10±0.79 | 32.73±0.47 | 32.90±0.70 | 32.30± 0.53 | 33.00±1.21 |
| Protein | 54.40±0.46 | 56.93± 0.91 | 56.67±0.32 | 57.33 ±0.55 | 56.73±0.47 |
| Lipid | 23.70±0.56 | 25.37±0.38$^{b}$ | 26.03±0.42$^{b}$ | 27.20 ±0.79$^{a}$ | 26.23±0.31 $^{b}$ |
| Ash | 16.70±1.31 | 15.10±0.44$^{b}$ | 15.20± 0.40 $^{b}$ | 15.30 ±0.20 $^{b}$ | 16.57±0.25 $^{a}$ |
| Carcass Energy | - | 560.57±3.61$^{b}$ | 565.35±5.19$^{b}$ | 580.13±4.45$^{a}$ | 567.62±5.35$^{b}$ |

*Values are mean ± SD. Means in the same row with a different letter differed significantly ($P < 0.05$).

**Table 4** Amino acids composition (% total) of the experimental diets and European sea bass, *Dicentrarchus labrax* after 90 days feeding trial.

| Amino acid (AA) | CTRL | | KDF2 | | KDF3 | | KDF4 | |
|---|---|---|---|---|---|---|---|---|
| | Diet | Fish | Diet | Fish | Diet | Fish | Diet | Fish |
| ***Essential amino acids (EAA)*** | | | | | | | | |
| Arginine | 7.05 | 6.39 | 6.21 | 6.28 | 5.82 | 6.27 | 6.29 | 6.19 |
| Histidine | 2.16 | 2.84 | 2.41 | 2.75 | 2.45 | 2.67 | 2.15 | 2.72 |
| Isoleucine | 4.33 | 4.28 | 4.24 | 4.26 | 4.25 | 4.25 | 4.24 | 4.27 |
| Leucine | 9.17 | 7.51 | 8.18 | 7.36 | 8.46 | 7.28 | 8.23 | 7.31 |
| Lysine | 6.69 | 8.72 | 7.31 | 8.49 | 7.42 | 8.47 | 7.53 | 8.42 |
| Methionine | 1.68 | 2.63 | 1.95 | 2.54 | 1.98 | 2.62 | 2.00 | 2.69 |
| Phenylalanine | 4.63 | 4.21 | 4.49 | 4.24 | 4.49 | 4.31 | 4.50 | 4.27 |
| Threonine | 3.98 | 4.26 | 3.93 | 4.37 | 3.97 | 4.25 | 4.10 | 4.36 |
| Tryptophan | 1.02 | 0.60 | 0.84 | 0.58 | 0.76 | 0.59 | 0.89 | 0.61 |
| Valine | 4.52 | 4.91 | 4.73 | 4.85 | 4.68 | 4.79 | 4.74 | 4.89 |
| **Total EAA** | **45.23** | **46.35** | **44.29** | **45.70** | **44.28** | **45.50** | **44.67** | **45.71** |
| ***Non-essential amino acids (NEAA)*** | | | | | | | | |
| Alanine | 6.15 | 6.22 | 6.32 | 6.18 | 6.56 | 6.13 | 6.58 | 6.06 |
| Aspartic acid | 8.42 | 9.21 | 8.82 | 9.18 | 8.85 | 9.24 | 7.79 | 8.88 |
| Cysteine | 1.00 | 0.73 | 0.92 | 0.62 | 0.97 | 0.63 | 0.85 | 0.62 |
| Glycine | 6.26 | 7.38 | 6.66 | 7.27 | 6.84 | 7.28 | 6.65 | 7.39 |
| Glutamic acid | 17.24 | 14.25 | 16.90 | 14.53 | 16.45 | 14.32 | 16.64 | 14.43 |
| Proline | 4.11 | 4.12 | 5.79 | 4.23 | 5.62 | 4.31 | 6.03 | 4.30 |
| Serine | 6.07 | 4.51 | 4.26 | 4.36 | 4.26 | 4.43 | 4.52 | 4.45 |
| Tyrosine | 3.62 | 3.12 | 3.58 | 3.15 | 3.42 | 3.20 | 3.63 | 3.22 |
| **Total NEAA** | **52.87** | **49.54** | **53.24** | **49.53** | **52.98** | **49.53** | **52.69** | **49.34** |
| **Total Free AA** | **98.10** | **95.89** | **97.53** | **95.23** | **97.26** | **95.03** | **97.36** | **95.05** |

### 3.3. *Blood analysis*

Potassium diformate (KDF) supplemented feeds did affect the standard hemoglobin levels (Hb) of sea bass fingerlings ($P < 0.05$, Table 5). The highest values of hematocrit (Ht) and erythrocytes count were found in the fingerlings fed KDF2 ($P < 0.05$). There were significant

differences ($P < 0.05$) in leucocytes count among all groups and the fish fed KDF2 diet recorded the highest value. However, total protein values differed significantly among all groups ($P < 0.05$). Moreover, fingerlings fed KDF3 diet (supplemented by 3 g $kg^{-1}$) showed the highest total protein content (7.42 g $dL^{-1}$) whereas, the lowest total protein content (5.57 g $dL^{-1}$) was detected in the control group. The count of leucocytes in the blood of examined fingerlings showed significant differences among the examined groups ($P < 0.05$). The highest value was found in the fingerlings fed KDF2 diet. There were significant differences ($P < 0.05$) among all the examined groups in the mean cell volume (MCV) of erythrocytes and the highest value was detected in the fingerlings fed KDF2 diet. The inclusion of KDF in the experimental diets did affect significantly ($P < 0.05$) the mean cellular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). The highest values were detected in the fingerlings fed KDF2 diet. Furthermore, Lymphocytes percentage was differed significantly ($P < 0.05$) among all the experimental groups. The highest value was obtained in the fingerlings fed KDF2 diet. Fish fed KDF2 and KDF4 diets differed significantly ($P < 0.05$) in the eosinophil percentage comparable to the other treatments. While there is no effect of dietary KDF on the percentage of monocytes among all treatments (Table 5, $P > 0.05$).

**Table 5 Biochemical analysis of sea bass blood fed the experimental diets for 90 days.**

| Values* | CTRL | KDF2 | KDF3 | KDF4 |
|---|---|---|---|---|
| Haemoglobin (Hb,g$dL^{-1}$) | $8.26\pm1.35^{b}$ | $12.38\pm0.81^{a}$ | $11.52\pm1.24^{a}$ | $8.78\pm0.91^{b}$ |
| Haematocrit (Ht, %) | $28.89\pm1.30^{b}$ | $40.19\pm1.48^{a}$ | $30.87\pm1.71^{b}$ | $31.04\pm2.00^{b}$ |
| RBC (x $10^{6}$ $L^{-1}$) | $2.97\pm0.12^{b}$ | $4.13\pm0.16^{a}$ | $2.98\pm0.46^{b}$ | $3.22\pm0.30^{b}$ |
| WBC (x$10^{3}$ $mL^{-1}$) | $34.06\pm1.63^{d}$ | $57.42\pm1.79^{a}$ | $40.98\pm4.12^{c}$ | $46.58\pm1.09^{b}$ |
| MCV (FL) | $91.25\pm3.88^{c}$ | $103.77\pm2.64^{a}$ | $96.83\pm3.30^{bc}$ | $99.50\pm2.64^{ab}$ |
| MCH (pg) | $25.30\pm0.98^{b}$ | $31.95\pm0.94^{a}$ | $25.76\pm0.79^{b}$ | $27.14\pm1.58^{b}$ |
| MCHC(mg $dL^{-1}$) | $26.59\pm1.21^{b}$ | $31.18\pm0.82^{a}$ | $29.42\pm0.75^{a}$ | $28.77\pm2.22^{ab}$ |
| Lymphocytes (%) | $32.22\pm0.96^{d}$ | $46.54\pm1.27^{a}$ | $37.36\pm1.49^{c}$ | $40.61\pm0.70^{b}$ |
| Monocytes (%) | $4.18\pm0.20$ | $4.63\pm1.15$ | $4.55\pm0.58$ | $5.56\pm0.82$ |
| Eosinophils (%) | $0.67\pm0.04^{b}$ | $1.31\pm0.08^{a}$ | $0.69\pm0.09^{b}$ | $1.18\pm0.16^{a}$ |
| **Serum analysis** | | | | |
| Total proteins (g $dL^{-1}$) | $5.57\pm0.11^{c}$ | $5.79\pm0.30^{c}$ | $7.42\pm0.03^{a}$ | $6.41\pm0.34^{b}$ |
| Phagocytic (%) | $3.97\pm0.37^{c}$ | $5.22\pm0.25^{b}$ | $10.04\pm0.63^{a}$ | $9.15\pm0.97^{a}$ |
| Lysozyme (unit $mL^{-1}$) | $457.99\pm35.24^{c}$ | $526.54\pm24.35^{b}$ | $596.82\pm7.25^{a}$ | $565.05\pm34.12^{ab}$ |
| AST **(**U $dL^{-1}$) | $83.27\pm1.12^{b}$ | $88.60\pm1.21^{a}$ | $81.97\pm1.51^{b}$ | $83.73\pm1.58^{b}$ |
| ALT **(**U $dL^{-1}$) | $22.33\pm0.76^{b}$ | $24.80\pm0.36^{a}$ | $22.67\pm0.40^{b}$ | $22.60\pm1.01^{b}$ |
| Urea (mg $dL^{-1}$) | $4.67\pm0.25^{b}$ | $5.43\pm0.42^{a}$ | $4.50\pm0.10^{b}$ | $4.23\pm0.23^{b}$ |
| Creatinine (mg $dL^{-1}$) | $0.65\pm0.04^{b}$ | $0.75\pm0.04^{a}$ | $0.62\pm0.03^{b}$ | $0.63\pm0.03^{b}$ |

* Values are mean ± SD. Values in the same row with a different letter differed significantly ($P < 0.05$). RBC, erythrocytes, WBC, leucocytes; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Serum analyses of sea bass fingerlings fed the experimental diets with different levels of KDF had a significant ($P < 0.05$) effect on the immunophysiological parameters such as phagocytic percentage and lysozyme activity (Table 5). The increasing levels of KDF in the experimental diets did affect the phagocytic percentage positively ($P < 0.05$) and lysozyme activity. The highest values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were detected in the examined fingerlings fed KDF2 diet and differed significantly among all the experimental groups (Table 5, $P < 0.05$). The same trend was revealed in the values of Urea and Creatinine.

## DISCUSSION

The achieved results for the final weight (g) were parallel to those obtained for weight gain (g) and specific growth rate (SGR%/fish/day) (Table 2) showing that the supplementation of potassium diformate (KDF) in the experimental diets for sea bass fingerlings improved significantly the growth performance. The same manner was observed with (**Ramli *et al*., 2005**), who examined different levels of KDF (0.2, 0.3 or 0.5%) in the diets for *Oreochromis niloticus* and resulted in significant improvement of body weight and weight gain. Furthermore, another study on the organic materials, **Eid (2012)** studied the organic acid mixture (0.3% acitic acid, 0.3% formic acid and 0.3% benzoic acid), the mixture of organic acid salts (0.3% sodium benzoate and 0.3% potassium sorbate) and the significant enhancement of weight gain and specific growth rate of *O. niloticus* were observed.

**Hossain *et al*. (2007)** examined 1% each of citric acid, malic acid, and lactic acid in three experimental diets for sea bream, *Pagrus major*. They found that citric acid significantly improved weight gain compared to the control group, nevertheless, malic or lactic acid did not affect the specific growth rate. Our results are in agreement with that found by (**Wassef *et al*., 2017**) who indicated that the inclusion of NaDF (Sodium Diformate) in the diets fed to sea bass for 13 weeks at levels of (0, 0.3, 0.4, and 0.5%) lead to improve weight gain and specific growth rate. In our study, sea bass fingerlings fed KDF2 (2 g $kg^{-1}$) and KDF3 (3 g $kg^{-1}$) diets gained the best feed conversion ratio (FCR) compared to the control diet (Table 2, $P > 0.05$). It has been noticed that the 1.5% mixture of formic acid and its salts plus sorbic acid added to the diets for rainbow trout juveniles, *Oncorhynchus mykiss* improved FCR (**De Wet 2005**). Also, **Ringø** (**1992**) studied the inclusion of 1% NaDF or 1% Na-acetate in the diets for Arctic charr, *Salvelinus alpinus*, and found improved the feed conversion ratio (FCR).

Our reached results of higher protein efficiency ratios and protein productive values were observed in sea bass fingerlings fed KDF2 and KDF3 diets in comparison with the control group. Comparable results were noticed by **Ramli *et al*., 2005**. Who found that PER was significantly ($P < 0.01$) improved by the addition of 0.2 and 0.5% KDF in the diets for Nile tilapia, *O. niloticus* for 85 days. Furthermore, the higher PER and PPV were observed with sea bass juveniles fed 0.4 or 0.5% NaDF (Sodium Diformate) supplemented diets for 13 weeks at (0, 0.3, 0.4, and 0.5%) (**Wassef *et al*., 2017**). The development in fish growing and feed intake because of the acidification might be because of expanding the absorbance and accessibility of various minerals and expanding the discharge of certain enzymes such as proteases. It has been indicated that the citric acid (10 g $kg^{-1}$) added to the diets for hybrid tilapia, (*O. niloticus* × *O. aureus*) increased protease activities in the stomach by 29.6% then reduced it in the intestine by 35.1% (**Li *et al*., 2009**).

The achievements of this study directed that potassium diformate (KDF) did not affect the protein; lipids contents of sea bass fingerlings and a slight increase in ash content were observed (Table 3). Also, there was no effect of KDF on the amino acid content of the whole body of sea bass fingerlings (Table 4). Comparable results were perceived with the inclusion of NaDF (Sodium Diformate) in the diets for sea bass for 13 weeks (**Wassef *et al*., 2017**). Moreover, **Baruah *et al*., (2007)** stated that the whole-body ash content of rohu, *Labeo rohita* fingerlings was not significantly ($P > 0.05$) affected by citric acid. In the current investigation, increasing the fish growth, feed intake, energy usage, and survival; however, certain resistance parameters are observed in the fish fed KDF (2 or 3 g $kg^{-1}$) supplemented diets than in the other dietary or the control groups (0 KDF). Such discoveries demonstrate a comparative pattern of variability in the impacts of a 0.3% potassium diformate (KDF) fed to tilapia or other fish species, with increasing the fish performance and the immune indices **(Abu Elala and Ragaa, 2015; Hoseinifar *et al*., 2017a).**

In the present study, the stimulation of some immunophysiological indices was observed by increasing the level of potassium diformate (KDF) in the experimental diets for sea bass fingerlings (Table 5). These results might be due to the KDF effects on the bacteria modulation compact the pathogenic stress (**Lückstädt, 2008**), consequently stimulating the normal fish protection systems. Serum total protein performs a substantial role in the immune response and used as a basic index for fish health status (**Mulcahy, 1971**).

In our investigation, sea bass fingerlings fed potassium diformate (KDF3) (supplemented by 3 g $kg^{-1}$ diet) obtained the highest total protein content, phagocytic percentage, and lysozyme activity compared to the control group (Table 5). These observations are in agreement with **(Wassef *et al*., 2017)**, who added NaDF (Sodium Diformate) in the diets for European sea bass (ESB) with the different levels of 0.3, 0.4, and 0.5 %. Eventually, in the present research, data of haemato-immunological parameters directed that potassium diformate (KDF) can act as normal resistances of European sea bass fingerlings at the dosage of 2 g $kg^{-1}$ feed. Further studies are

needed to determine the optimum time to use this KDF-dose and its effects on the gut microflora of fish. Besides, the economic analysis of this study would be desirable in the future research.

Supplementation of dietary potassium diformate (KDF) has a positive effect on the fish growth, feed efficiency and survival rate of European sea bass fingerlings. Furthermore, the improvement of immunity by these KDF dosages suggests a powerful basal health promotional impact. Also, the results presented here may facilitate improvements in using potassium diformate in the feeds for European sea bass fingerlings with two doses 2 or 3 g $Kg^{-1}$ serving as fish growing enhancer and a protection component.

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## ARABIC SUMMARY

### تأثير إضافة ثنائي فورمات البوتاسيوم على نمو و مناعة أسماك القاروص المرباة في الهابات

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**أجريت هذه الدراسة لتقييم تأثير إضافة ثنائي فورمات البوتاسيوم في علائق أسماك القاروص الأوروبي بمستويات مختلفة (صفر - ٢ - ٣ - ٤ جرام لكل كجم عليقة) على النمو و الاستفادة الغذائية و مكونات الدم لمدة ٩٠ يوم. أظهرت النتائج أن أعلي وزن نهائي (٣٥.١ جم) و الزيادة في الوزن (٢٩.٦ جم) وجدت في الأسماك التي تم تغذيتها على عليقة مضاف لها ٢ جم ثنائي فورمات البوتاسيوم و ظهرت اختلافات معنوية في معدل الحيوية بين كل المعاملات التجريبية و التي تحسنت بزيادة مستوي إضافة ثنائي فورمات البوتاسيوم في العلائق التجريبية و كانت أعلي قيمة ٩٨.٨٩% تم ملاحظتها في الأسماك التي تم تغذيتها علي عليقة مضاف لها ٤ جم ثنائي فورمات البوتاسيوم. أيضا من النتائج المتحصل عليها من تحليل دم الأسماك التجريبية وجد أن زيادة قيم خلايا الدم البيضاء و الحمراء و الهيموجلوبين و الهيماتوكريت تم ملاحظتها في الأسماك التي تم تغذيتها علي العليقة المضاف لها ٢ جم ثنائي فورمات البوتاسيوم وكذلك كان هناك تأثير إيجابي لإضافة ثنائي فورمات البوتاسيوم في العلائق التجريبية علي قياسات المناعة الفسيولوجية لإصبعيات أسماك القاروص الأوروبي. و كذلك فإن إضافة ثنائي فورمات البوتاسيوم في العلائق كان له تأثير ايجابي علي الكفاءة الغذائية ومعدل النمو والإعاشة لإصبعيات أسماك القاروص الأوروبي، ومن ذلك يمكن التوصية بإضافة ثنائي فورمات البوتاسيوم في العلائق بمعدل ٢ أو ٣ جم لكل كجم عليقة و الذي يمكن أن يخدم كمحسن للنمو وكوقاية طبيعية لإصبعيات أسماك القاروص الأوروبي المستزرعة.**