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# Improved Growth Rate and Feed Utilization in Farmed African Catfish Clarias gariepinus (Burchell 1822) Through a Growth Promoter Biogen® Supplementation

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Abstract: The present study was conducted to examine probiotic treatment in the diet of African catfish Clarias gariepinus. A total of 240 of African catfish (weight ranged from 81.13 to 91.35 g) were divided in the five experimental groups. The experiment was conducted for 120 days. Experimental diets were identical in all except for the variation in probiotic levels. A probiotic (Biogen®) was used at 0% (control), 0.5% (diet 2), 1.0% (diet 3), 1.5% (diet 4) and 2.0% (diet 5) inclusion rates in the experimental diets. The growth performance and nutrient utilization of African catfish including Weight Gain (WG), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Protein Productive Value (PPV) and Energy Retention (ER) were significantly (p≤0.05) higher of fish fed diets containing 0.5% Biogen® than for fish fed control diets. In the same trend fish fed 0.5% Biogen® recorded the best-Feed Conversion Ratio (FCR) (p≤0.05) compare to the control diet. However, among the probiotic fed groups no significant differences were recorded (p≥ 0.05). Concerning the influence of different dietary probiotic levels on chemical proximate analysis of whole carcass, protein content was significantly higher (p≤0.05) at 0.5% inclusion, while, fish fed diet containing 1.0% Biogen® recorded the highest ash content without significant (p $\ge$ 0.05) difference with the other treatments. Differences were observed in carcass lipid and gross energy content with significantly lowest (p≤0.05) values recorded in fish fed 2.0% Biogen®. The production performance and subsequent cost-benefit analyses clearly indicated that the diets containing 0.5% probiotic Biogen® recorded the highest net return compared to the control diet. Based on the result of this study, it is concluded that Biogen® positively influenced growth performance and feed utilization of African catfish as well as the economic efficiency.

**Key words:** Probiotics, African catfish, growth, feed utilization, economic evaluation

# INTRODUCTION

The African catfish, *Clarias gariepinus* (Burchell, 1822), is one of the most highly valued species in Africa (Egypt, Ethiopia, Ghana, Mali and Nigeria) and Asia (China, Indonesia, Malaysia, Philippines and Thailand). Recently, *Clarias gariepinus* has been introduced in some European and Latin American countries and its culture has increased in scale (FAO, 1997; Ali, 2001). African catfish is considered one of the best suitable alternatives to tilapia for subsistence fish farming in Africa. Yields of catfish from ponds could be higher than those of tilapia while using low grade feed composed of local agricultural by-products (Hogendoorn, 1983; Goda *et al.*, 2007).

Catfish is cultured for its high growth rate, disease resistance and it's tolerant of a wide range of temperature, as well as oxygen and high salinity levels (Huisman and Richter, 1987; Haylor, 1993; Bovendeur *et al.*, 1987). The superior performance of *Clarias gariepimus* compared to other *Clarias* species in terms of growth rate has probably contributed to the fact that *C. gariepimus* has been widely introduced to areas outside its natural range (Verreth *et al.*, 1993).

Antibiotics had been frequently used to enhance growth and/or resistance to diseases in aquaculture systems. However, the excess use of antibiotics to prevent bacterial diseases in aquaculture, have led to an increase in antibiotic-resistant bacteria (Teuber, 2001). In recent years, there have been increasing the beneficial effects of the probiotic in the aquaculture of many economically important marine and freshwater fish species as an alternative to the use of antibiotics in feeds. Evidence of the beneficial effects of probiotics increased the demand of probiotic in aquaculture, which are defined by Gatesoupe (1999) as "microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health".

Recently, many kinds of probiotics have been commercially produced and used as feed additives in farmed fish such as turbot, Nile tilapia, salmon, trout, eel, sole and gilthead seabream (Robertson *et al.*, 2000; Lara-Flores *et al.*, 2003; Nikoskelainen *et al.*, 2003; Hjelm *et al.*, 2004; Lategan *et al.*, 2004; Chabrillon *et al.*, 2005, 2006; Picchietti *et al.*, 2007).

Probiotic Biogen® consists of *Bacillus* species bacteria. The advantage of these bacteria is that they are able to survive the pelletization process. After transit through the stomach, they germinate in the intestine and use a large number of sugars (carbohydrates) for their growth and produce a range of relevant digestive enzymes (amylase, protease and lipase).

The present study was conducted to determine the effect of feeding commercial probiotic Biogen® on growth performance and nutrient utilization in African catfish.

### MATERIALS AND METHODS

#### **Experimental Fish and Culture Technique**

This study was performed at the Middle East Land Reclamation Company (MELARC) Farm, Tamoo, Giza, Egypt. African Catfish *Clarias gariepinus* was obtained from a commercial supplier (Elobor market) Cairo, Egypt. The average initial body weight for African catfish ranged from 81.13 to 91.35 g. In March-15-2003, 240 African catfish were equally divided into 15 groups and carefully stocked into 15 ponds 8 m³ each and 1.25 m depth 16 fish in each pond. The fish were adapted to experimental conditions for 2 weeks prior to the start of the experiment and were fed a commercial diet twice a day. The actual experimental period extended for 17 weeks. The ponds were supplied with freshwater. The turnover rate of water was 0.4 m³ day⁻¹ pond⁻¹ and fish were held under natural light. Photoperiod was maintained at a 12:12 h light: dark schedule. During the trial, water temperature and Dissolved Oxygen (DO) were measured using an YSI model 56 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA) and pH by using a pH meter (Orion pH meter, USA). Ammonia and alkalinity were measured at weekly intervals according to APHA, AWWA, WPCF (1985).

All dietary treatments were tested in triplicate groups where each pond was considered as an experimental unit. During the experiment, all fish were fed their respective diets at a level of 3% of body weight for 6 days a week. The daily ration was divided into three equal amounts and offered three times a day (0900, 1200 and 1500). A random sample of fish from each treatment was weighed biweekly and the amount of daily diet was adjusted accordingly.

#### **Experimental Diets**

Five isonitrogenous and isocaloric diets were formulated for the experiment (Table 1). The control diet had no Biogen® added and four other test diets included Biogen® added at 0.5, 1.0, 1.5 and 2.0% levels, respectively. The fish meal, soybean meal, wheat bran and yellow corn were purchased from the Islamic Company (APICO), Dokki-El-Giza Egypt. Biogen® was obtained from the El-Zahra Veterinary Trading Company, an exclusive agent of the manufacturer, China Way Corporation Taiwan, Roc). The Biogen® is reported to contain: *Allicin* (not less than 0.247 M mole g<sup>-1</sup>), *Bacillus subtilis* not less than 6×10<sup>7</sup> g<sup>-1</sup>) and *High Unit Hydrolytic Enzyme* (not less than 3690 Unit g<sup>-1</sup>). The diets were processed by blending the dry ingredients into a homogenous mixture and then passing the mixed

Table 1: Formulation and proximate analysis of experimental diets (% dry matter)

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Ingredients	Control	0.5% Biogen®	1.0% Biogen®	1.5% Biogen®	2.0% Biogen®
Fish meal, herring	15.00	15.00	15.00	15.00	15.00
Soybean meal	32.00	32.00	32.00	32.00	32.00
Yellow com	20.00	20.00	20.00	20.00	20.00
Wheat bran	28.00	27.50	27.00	26.50	26.00
Vitamin and mineral premix <sup>1</sup>	2.00	2.00	2.00	2.00	2.00
Corn oil	3.00	3.00	3.00	3.00	3.00
Biogen®	-	0.50	1.00	1.50	2.00
Analyzed composition (dry m	atter basis)				
Dry matter (%)	85.32	84.88	84.43	83.99	83.54
Crude protein (%)	29.60	29.52	29.46	29.38	29.30
Lipid (%)	6.90	6.88	6.86	6.84	6.82
Ash (%)	12.72	13.17	13.59	14.04	14.49
Gross energy (MJ kg diet-1)	18.65	18.55	18.47	18.38	18.36
Calculated composition					
Metabolizable energy <sup>2</sup>					
(MJ kg diet <sup>-1</sup> )	13.99	13.92	13.85	13.79	13.77
Protein: energy ratio (mg kcal-	1) 88.54	88.76	88.94	89.12	89.01

<sup>1</sup>Vitamin and mineral mixture each 1 kg of mixture contains: retinyl acetate (vit. A), 3000 IU; cholecalciferol (vit. D), 2400 IU; all-rac-α-tocopheryl acetate (vit. E), 60 IU; menadione sodium bisulfite (vit. K), 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid, vit. C), 120 mg; cyanocobalamine (vit. B12), 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate (FeSO<sub>4</sub>.7H<sub>2</sub>O, 20% Fe), 65 mg; manganese sulfate (MnSO<sub>4</sub>, 36% Mn), 89 mg; zinc sulfate (ZnSO<sub>4</sub>.7H<sub>2</sub>O, 40% Zn), 150 mg; copper sulfate (CuSO<sub>4</sub>.5H<sub>2</sub>O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000 mg. <sup>2</sup>ME was calculated as 75% of GE according to Hepher *et al.* (1983)

feed through a laboratory pellet mill (California Pellet Mill Co., San Francisco, CA). The chemical composition of experimental ingredients and diets are presented in Table 1.

### **Sampling and Chemical Analysis**

At the beginning of the experiment, a sample of 10 fish was taken for determining chemical body composition (initial carcass sample). At the end of the experiment, 5 fish from each tank were sampled. Fish were killed with an excess concentration of anesthetic (t-amyl alcohol) and then weighed. The fish were then pooled per pond (final carcass sample) for chemical analyses. The pooled fish samples were cooked in an autoclave, ground into homogeneous slurry in a food processor and oven dried, finely ground in a coffee grinder and stored at -20°C until analyzed. Diet, ingredients and carcass samples were analyzed for Dry Matter (DM) and ash according to AOAC (1995), crude protein (%N×6.25) by Kjeldahl method using a Kjeltech autoanalyzer (Model 1030, Tecator, Höganäs, Sweden) and total lipid according to the method of Bligh and Dyer (1959). Gross energy content of carcass samples was measured using an automated bomb calorimeter (Model 1272, Parr Instruments Inc., Moline, IL USA).

# **Calculations Parameters**

Mean fish weight of each treatment pond was determined by dividing total fish pond weight by number of fish in each pond. Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Protein Productive Value (PPV) and Energy Retention (ER) was calculated using the following equations:

WG = Final body weight (g) - initial body weight (g);

 $SGR = [(\ln FBW - \ln IBW) \times 100] \text{ period day}^{-1},$ 

Where,

FBW: Final Body Weight (g); IBW: Initial Body Weight (g);

ln = Natural logarithmic.

FCR was calculated from feed intake (g)/wet weight gain (g); PER was calculated as:

Wet weight gain (g)/protein intake (g); PPV as (Retained protein/protein intake) ×100 and ER = (Retained energy/energy intake)×100. Net production (kg) pond<sup>-1</sup> = TBMH - TBMS,

where.

TBMH is the total body mass at the harvest (kg) pond<sup>-1</sup>, TBMS is the total body mass at the start (kg) pond<sup>-1</sup>;

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TBMH (kg) = (FBW/1000) *16;

TBMS (kg) = (IBW/1000) *16;

Total income (LE) pond<sup>-1</sup> = TBMH * price (6.0 LE kg<sup>-1</sup>);

Net return (LE) pond<sup>-1</sup> = Total income (LE) pond<sup>-1</sup> - total cost (LE) pond<sup>-1</sup>;

Profit (%) = (net return (LE) pond<sup>-1</sup>/total cost (LE) pond<sup>-1</sup>) * 100.
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# Statistical Analysis

Growth performance, feed utilization efficiency and body chemical composition parameters were statistically compared using a one-way ANOVA ( $p \le 0.05$ ) and differences among means were identified using the Duncan Multiple Range Test. GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA, 1988). Alpha levels of 0.05 (significant) were used in all analysis and the individual tank was considered as the experimental unit.

# RESULTS

No mortality was detected for African catfish after 120 days of feeding diets with and without probiotic supplementation; this may be due to the fulfillment of dietary requirement, all the water quality parameters were within the acceptable range for African catfish *Clarias gariepinus*. The water temperature ranged from 25 to 28°C, Dissolved Oxygen (DO) from 5.0 to 6.5 mg  $\rm L^{-1}$  and pH from 6.0 to 8.0.

Data in Table 2 shows that African catfish fed diets containing 0.5% Biogen® had a significantly higher (p≤0.05) weight gain (406.87±8.66 g), specific growth rate (1.50±0.15% day<sup>-1</sup>), protein efficiency ratio (1.58±0.09), protein productive value (30.28±1.70) and energy retention (16.48±0.83) than fish fed control diet. However, among the probiotic fed groups no significant differences were

Table 2: Growth performance and nutrient utilization of African catfish *Clarias gariepinus* after 120 days of feeding diets supplemented with Biogen<sup>®</sup>, Mean in the same row sharing the same superscript are not significantly different (p≥0.05)

		0.5%	1.0%	1.5%	2.0%	
Parameters	Control	Biogen®	Biogen®	Biogen®	Biogen®	SE 1
Survival (%)	100.00	100.00	100.00	100.00	100.00	-
Initial body weight (g fish <sup>-1</sup> )	91.35	81.13	85.33	89.42	81.80	5.05
Weigh gain (g fish <sup>-1</sup> )	288.75 <sup>b</sup>	406.87ª	$369.47^{ab}$	$371.18^{ab}$	$369.10^{ab}$	8.66
Specific growth rate (% day <sup>-1</sup> )	$1.19^{\circ}$	1.50°	$1.39^{\rm ab}$	$1.37^{\rm ab}$	$1.42^{ab}$	0.15
Feed conversion ratio	2.58°	$2.15^{b}$	$2.31^{ab}$	$2.30^{ab}$	2.24ª	0.10
Protein efficiency ratio	1.31	1.58	1.47	1.48	1.52	0.09
Protein productive value	$21.92^{b}$	30.28°	$24.60^{ab}$	$26.70^{ab}$	$27.79^{ab}$	1.70
Energy retention	13.80 <sup>b</sup>	16.48 <sup>a</sup>	14.90 <sup>ab</sup>	$16.09^{ab}$	$14.99^{ab}$	0.83

<sup>&</sup>lt;sup>1</sup>Pooled standard error; Mean in the same raw sharing the same superscripts are not significant different (p>0.05)

Table 3: Chemical composition of the whole carcass of African catfish *Clarias gariepinus* after 120 days of feeding diets supplemented with Biogen<sup>®</sup>. Mean in the same row sharing the same superscript are not significantly different (p≥0.05)

		0.5%	1.0%	1.5%	2.0%	
Parameters	Control	Biogen®	Biogen®	Biogen®	Biogen®	SE 1
Moisture (%)	75.73	74.90	74.88	74.64	75.67	0.54
Crude protein (%)	14.22 <sup>b</sup>	16.30°	$14.10^{\circ}$	15.13 <sup>ab</sup>	$14.60^{\circ}$	0.78
Lipid (%)	5.63a	$4.20^{bc}$	5.03 <sup>ab</sup>	5.23ab	3.73°	0.72
Ash (%)	$4.43^{ab}$	$4.60^{ab}$	$6.02^{a}$	5.00 <sup>ab</sup>	4.39 <sup>ab</sup>	0.21
Gross energy (kcal 100 g <sup>-1</sup> )	133.49a	131.83a	126.91ab	134.97⁴	121.18 <sup>b</sup>	6.75

<sup>&</sup>lt;sup>1</sup>Pooled standard error; Mean in the same raw sharing the same superscripts are not significant different (p>0.05)

Table 4: Fish production and the economic efficiency of the experimental diets

		0.5%	1.0%	1.5%	2.0%
Items	Control	Biogen®	Biogen®	Biogen®	Biogen®
Fish No./pond	16.00	16.00	16.00	16.00	16.00
Total body mass/pond kg-At the start (TBMS)	1.46	1.30	1.37	1.43	1.31
Total body mass/pond kg-At harvesting (TBMH)	6.08	7.81	7.28	7.37	7.21
Net production/pond (kg)	4.62	6.51	5.91	5.94	5.90
Relative per cent of control net production	100.00	141.00	128.00	129.00	128.00
Total income LE¹/pond	36.48	46.86	43.68	44.22	43.26
Total costs LE/pond	27.46	31.19	30.57	30.59	29.83
Net return, LE/pond	9.02	15.67	13.11	13.63	13.43
Profit (%)	32.85	50.24	42.89	44.56	45.02

<sup>&</sup>lt;sup>1</sup>One American dollar (\$) = 6.0 Egyptian pond (LE)

recorded (p≥0.05). Fish fed control diet without any probiotic had the numerically lowest WG, SGR, PER, PPV and ER. The best-feed conversion ratio was recorded for fish fed diet containing 0.5% Biogen® treatments (2.15±0.10); while the worst FCR was recorded for the fish fed control diet (2.58±0.10).

Concerning the influence of different dietary probiotic levels on chemical proximate analysis of whole carcass (Table 3), no statistical differences ( $p \ge 0.05$ ) were observed in the moisture and ash content. Differences were observed in carcass protein content, with the highest significantly ( $p \le 0.05$ ) value recorded in fish fed with 0.5% Biogen® supplement. Carcass lipid and gross energy content was also affected by Biogen® supplemented, the lowest significantly ( $p \le 0.05$ ) lipid and gross energy content were recorded for the fish fed diet supplemented with 2.0% Biogen®.

The higher total net production values were 6.51, 5.91, 5.94 and 5.90 kg pond<sup>-1</sup> for fish fed increment levels of Biogen® levels (0.5, 1.0, 1.5 and 2.0%, respectively), while the lowest value's recorded for fish fed the control diet (4.62 kg pond<sup>-1</sup>) (Table 4). The same trend was recorded for total pond income (LE) and net pond return (LE). Referring to economic analysis (Table 4) and according to market price in 2006, the input-output analysis indicated that the percentage of 50.24, 42.89, 44.56 and 45.02 were recorded as profit (%) for fish fed diet contents Biogen® at levels of 0.5, 1.0, 1.5 and 2.0%, respectively.

### DISCUSSION

All the diets supplemented with Biogen® resulted in growth performances and feed utilization better than that of the control basal diets (Table 2), suggesting that the addition of Biogen® enhance the growth performance and feed utilization of African catfish *Clarias gariepinus*. These results are in agreement with the findings of Wang and Zirong (2006), who found that the addition of different bacterial strain (photosynthetic bacteria (PSB) and lyophilized *Bacillus* sp. (B) and their mix) in common carp basal diets improved growth performances, feed utilization and digestive enzyme activities compare to the control diet. In the same trend, bacterial probiotics have been found to influence positively on growth rate in juvenile carp *Cyprinus carpio* L. (Noh *et al.*, 1994; Bogut *et al.*,

1998). Lara-Flores et al. (2003) observed that, yeast produced the best growth performance and feed efficiency in Nile tilapia Oreochromis niloticus. Kennedy et al. (1998) showed that the addition of a gram-positive probiotic bacterium to diets increased survival and growth rate of marine fish larvae (snook, red drum, spotted seatrout and stripped mullet). In contrast, Gildberg et al. (1995) and Efthimiou (1996) found that there was no effect of probiotics on growth performance was observed in Atlantic salmon fry and dentex, respectively. Also, Shariff et al. (2001) and McIntosh et al. (2000) found that treatment of P. monodon and Litopenaeus vannamei with a commercial Bacillus probiotic did not significantly increase (p≥0.05) either survival or growth. The mechanism by which Biogen® improved growth efficiency in catfish in the current study could be due to the synthesis of a range of relevant digestive enzymes (amylase, protease and lipase). These enzymes would enhance growth performance as a result of higher nutrient digestibility, which could explain the better growth and feed efficiency seen with the supplemented diets. Also, probiotics have been shown to affect on digestive processes by promoting the population of beneficial micro-organism, microbial enzyme activity, improve intestinal microbial balance, consequently improving digestibility and absorption of food and feed utilization (Bomba et al., 2002). Another possible reason for improving growth performance and feed efficiency may be due to the role of probiotics by inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and space and alteration of microbial metabolism. Probiotics can also improve nutrition by detoxifying the potentially harmful compounds in feeds, by denaturing the potentially indigestible components in the diet by hydrolytic enzymes including amylases and proteases, by producing vitamins, such as biotin and vitamin B12 (Sugita et al., 1992, 1996; Hoshino et al., 1997), by producing inhibitory compounds (Spanggaard et al., 2001) and by stimulating host immunity (Fuller, 1992; Gibson et al., 1997).

With respect to survival, African catfish survival rate was 100% after 120 days of feeding diets with and without probiotic supplementation; this may be due to the fulfillment of dietary requirement, a healthy fish used in this experiment and the best experimental conditions.

The best-feed efficiency and nutrient utilization including FCR values observed with probiotic-supplemented diets suggest that addition of probiotics improved feed utilization of African catfish *Clarias gariepinus*. The presence of the significant differences in FCR in present study has been observed in other studies, the other authors found that probiotic improved the growth and feed efficiency of Israeli carp (Noh *et al.*, 1994; Bogut *et al.*, 1998), Nile tilapia (Lara-Flores *et al.*, 2003) and common carp (Wang and Zirong, 2006). The administration of probiotic bacteria and their metabolites has found to be an effective way to promote body weight and feed conversion ratio in farm animals (Abe *et al.*, 1995; Denli *et al.*, 2003).

From a practical point of view, this means that probiotic treatments affect positively on fish growth and increase the feed efficiency, which causing decrease the amount of feed necessary for optimum growth, which could result in reducing production cost. Improving FCR may be due to synthesis of digestive enzymes in Biogen® treatments which led to enhanced digestion and increased absorption of food, which in turn contributed to the improved feed efficiency. In contrast, other studies have been observed there was no significant difference in feed conversion ratio (Efthimiou *et al.*, 1994; Gildberg *et al.*, 1995; Efthimiou, 1996; Gildberg and Mikkelsen, 1998; Hidalgo *et al.*, 2006).

Significant differences were observed in PER, PPV and ER results indicate that supplementing diets with probiotics improves protein and energy utilization in African catfish. Better efficiency of protein uptake for the probiotic groups may be due to better digestion, absorption and assimilation of the nutrients. This results may be supported by the postulation from Lara-Flores *et al.* (2003) who found that the PER and ANU recorded the best values with probiotic-supplemented diets. In contrast, Hidalgo *et al.* (2006) found that no significant differences were observed in PER and PPV for juvenile dentex fed two probiotics (Bacillus toyoi, T and B. cereus, E) at increasing levels (0.5, 1 and 2 g kg<sup>-1</sup> diet).

Yields of African catfish fed diets supplemented with 0.5, 1, 1.5 and 2% Biogen® resulted in 141, 128, 129 and 128% more production than the control diet, respectively (Table 4). African catfish seems to be more efficiently utilizing the nutrient when supplemented with probiotics. Meanwhile, the least net yield was recorded with the fish fed the control diet (without probiotic). Referring to the economic analysis (Table 4) and according to the market price in 2006, the total net return of fish production using probiotic at four levels from 0.5 to 2% were higher than the control. However, the cost-benefit analyses of the present study clearly indicated that the Biogen® diets are better for African catfish *Clarias gariepinus* than the control diet.

It could be concluded that the addition of probiotic Biogen® as a feed additive in African catfish *Clarias gariepinus* diets is recommended to improve growth performance and nutrient utilization as well as improve the economic efficiency.

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