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EFFECT OF USING SOME SPICES AS FEED ADDITIVES ON GROWTH PERFORMANCE, PRODUCTION TRAITS AND BODY COMPOSITION OF NILE TILAPIA, *Oreochromis niloticus* (L.)

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ABSTRACT

This study was conducted to evaluate the effect of black seed and marjoram leaves powder on growth performance, feed utilization, and body composition of Nile tilapia, *Oreochromis niloticus.* All diets are iso-nitrogenous (30% crud protein).and iso-caloric (4.72 kcal/g diet). Thirty glass aquaria were used, each one was stocked with 10 fish (8.2 g) and three aquaria were designated for each treatment. The ten treatments were: Treatment 1 was control without additives, treatments 2, 3 and 4 contained 1.0%, 2.0% and 3.0% black seeds (BS), respectively, treatments 5, 6 and 7 contained 1.0%, 2.0% and 3.0% marjoram leaves (ML), respectively and treatments 8, 9, and 10 contained BS and ML (1 : 1 w/w) at a rate of 1.0, 2.0%, and 3.0%, respectively. Fish were fed on one of the experimental diets at feeding level of 3% of body weight daily for 12 weeks. The feed was offered twice daily, five days a week. Fish growth (indicated as final weight, weight gain, daily gain, relative grouth rate (RGR),and specific growth rate (SGR)) was increased gradually and the maximum growth was obtained when fish fed T4. The lowest growth was obtained at the control group. The highest feed intake and the lowest feed conversion ratio (FCR) were obtained when fish fed 30 g BS/kg diet (T4). Apparent protein utilization (APU), and energy utilization (EU) were enhanced significantly when fish fed BS and ML (each alone or together), while the lowest values were obtained at the control group. Dry matter content in fish body was significantly incrased by BS and/or ML. Crude protein increased significantly also in all treatments compared with the control. Total lipids contents refleacted significant increases in all treatments compared with the control.Yet, ash content decreased significantly with each black seed and/or marjoram leaves level.

Keywords: Black seeds, Marjoram leaves Nile tilapia, Growth, Feed utilization, Fish body composition .

INTRODUCTION

The use of medicinal herbs as feed additives has greatly increased because they contain some chemical components that may enhance the growth and health (Salem and El-Mahdy, 2001). Nowadays, there is an increased demand for using these herbs as natural growth promoters via improving the general health (Abdelhamid *et al*., 2002, 2004 a, b, c, d &e and 2005 a & b, Abd Elmonem *et al*., 2002; Shalaby *et al*., 2003 and El-Dakar *et al*., 2004 a&b) instead of using synthetic drugs (Abdelhamid *et al* .1997 and1998 and Hussein *et al*., 2000). Several studies in animal nutrition showed that adding some spices or medicinal herbs to diets had favorable effects on live weight gain, feed efficiency and nutrient digestibility (Hnafy, 1995; Karaly, 1995; Gabr *et al*., 1996; Mir *et al*., 1998; Youssef *et al*, 1998; Aboul-Fotoh *et al*., 1999; Allam *et al*., 1999; El-Ayek *et al*., 1999 ; El-Saadany

et al.,1999; Abdelhamid *et al*.,20012004 a,b;Shehata *et al* .,2001 and Abdelhamid,2008)

Black seed (*Nigella sativa*) is a medical herb used for the treatment of variety of ailments (Boulos, 1983) and also commonly used as condiment in Middle East. It has also been reported to possess many pharmacological effects (Abd El-Aal and Attia, 1993 and Hussein *et al*., 2000). Thymoguinone , an active principle of black seed, is known to possess antioxidant properties (Nagi *et al*., 1999).

Marjoram (*Origanum majorana*) plants are widely used allover the world as a very popular spice, under the rernacular name "oregano". They are of great economic importance which is not only related to their use as a spice, in fact, as recent studies have pointed out, Oregano is used traditionally in many other ways as its essential oils have antimicrobial effects for thir antioxidant activity (Lagouri *et al*., 1993 and Sivropoulou *et al*., 1996).

The importance of Nile tilapia (*Oreochromis niloticus*) in aquaculture is evident from the fact it has now spread to all continents of the world because it exhibits most of the desirable qualities of a culture species and tolerates the different conditions (El-Sayed, A. F.M. ,2004). The present study therefore aimed to evaluate the use of black seed and marjoram leaves powders as feed additives and study their effects on growth performance, feed conversion, and body composition of Nile tilapia, *Oreochromis niloticus* (L).

MATERIALS AND METHODS

Diet preparation:

Ingredients of the experimental diets were obtained from the local market (Zoo –Control Factory).Ten experimental diets were formulated (30% crude protein and 4.72kcal/g diet) to contain different levels of black seed (BL) and/or marjoram leaves (ML) (Tables 1and2). The diets contained control (0), 1.0, 2.0 or 3.0 % BS, 1.0, 2.0 or 3.0% ML, 1.0, 2.0 or 3.0% BS + ML (1:1 w/w). The ingredients of each diet were separately blended with additional 100 ml of water to make a paste of each diet. The pastes were separately passed through a grinder, and pelleted (1 mm diameter) in a modified paste extruder to form the tested diets. The diets were approximately similar in texture and nutrition contents but containing different levels of BS and/or ML. The diets were stored in plastic bags in a refrigerator (-2^0 C) until use.

Fish rearing:

Fingerlings of Nile tilapia, *Oreochromis niloticus* (L) with an average initial body weight of 8.2 g/fish were obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt, and kept for 2 week as an acclimation period to the laboratory conditions. After that, fish were distributed into 30 glass aquaria; 100-L each, at a rate of 10 fish/aquarium and each treatment was represented by three aquaria.

Each aquarium was supplied with compressed air via air–stones using aquarium air pumps. Fish were acclimatized one week to the aquarium condition and fish feces were cleaned daily by siphoning with a three quarters

of aquariums, water and was replaced by aerated water from a storage tank. All aquaria were maintained at 26 – 28° C with 12-12 light – dark photoperiod cycle using fluorescent tubes as the light source. The daily feeding rate was 3% of live body weight which was offered at 2 meals daily; 5 days a week for 12 weeks. Fish were weighed every two weeks and the amount of feed quantity for each aquarium was adjusted accordingly.

Table 1: Ingredients of the experimental diets (on dry matter basis).

Ingredients	Co-ntrol	Black seeds			Marjarom			Black seed : Marjrom (1:1)		
		1%	2%	3%	1%	2%	3%	1%	2%	3%
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Herring fish meal	19	19	19	19	19	19	19	19	19	19
Soybean meal	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
Corn flour	30	30	30	30	30	30	30	30	30	30
Wheat bran	9	9	9	9	9	9	9	9	9	9
Starch	2.88	2.38	1.88	1.38	2.38	1.88	1.38	2.38	1.88	1.38
Cellulose	2.0	1.5	1.0	0.5	1.5	1.0	0.5	1.5	1.0	0.5
Corn oil	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.7 4
Fish oil	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88	1.88
Vitamins premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Minerals premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Spice	0	1	2	3	1	2	3	1	2	3
Total	100	100	100	100	100	100	100	100	100	100

1- Vitamins premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0mg; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

2- Minerals premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $COCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.

Table 2: The chemical composition of the experimental diets (%on dry matter basis).

Items	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Dry Matter	90.89	90.78	90.11	90.43	90.77	90.53	90.75	90.66	90.58	90.767
Crude protein	30.25	30.27	30.25	30.30	30.22	30.27	0.22	30.22	30.27	30.25
Total lipids	7.16	7.16	7.18	7.20	7.21	7.19	7.16	7.2	7.16	7.18
Crude fiber	5.86	5.85	5.86	5.93	5.88	5.87	5.9	5.77	5.85	7.88
Ash	6.77	6.78	6.81	6.78	6.78	6.77	6.75	6.78	6.84	6.80
NFE[1]	49.96	49.94	49.90	49.79	49.91	49.90	49.97	50.03	49.88	47.89
GE (Kcal/kg diet)[2]	4422.5	4422.5	4423	4422.48	4423.11	4423	4422.46	4423.21	4422.81	4423,12
P/E ratio(mg protein/kcal GE)	68.4	68.46	68.49	68.51	68.32	68.44	68.33	68.32	68.45	68.39

1- Nitrogen-Free Extract (calculated by difference)= 100 – (protein + lipid + ash + crude fiber).

2- Gross energy (GE): Calculated from (NRC, 1993) as 5.65, 9.45 and 4.1 Kcal/g for protein, lipid and NFE, respectively.

Proximate analysis of diet and fish:

At the start of the experiment, 20 fish with similar average body weight were taken and kept frozen for proximate chemical analysis. At the end of the experiment, the basal diet and fish from each treatment were chemically analyzed according to the standard methods of AOAC (1990) for determination of moisture, crude protein, total lipids, ash and dietary fiber.

Growth parameters:

Weight gain (WG)= W2-W1

Daily gain (DG) = W2 - W1 / T;

Relative growth rate (RGR) = [(W2 – W1) / W1] X 100;

Where W2 = average final body weight (g), W1 = average initial body weight (g), and T = experimental period (days).

Feed utilization parameters:

Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g);

Feed efficiency ratio (FER) = body weigh gain (g) / feed intake (g) x100;

Protein efficiency ratio (PER) = gain in weight(g) / protein intake in feed (g);

Apparent protein utilization (APU %) = 100 [protein gain in fish (g) / protein intake in feed (g)].

Energy utilization (EU %) = [energy gain / energy intake] x 100.

Statistical analysis

All numerical data obtained in the present study were subjected to one way ANOVA. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VI, USA) as described by Dytham (1999).

RESULTS AND DISCUSSION

In the present study, Nile tilapia fish fed the experimental diets were active and grew efficiently without external signs of nutritional deficiency. Results in Table (3) show that initial body weight of all experiment groups did not differ significantly. The use of different spices levels resulted in higher($P<0.05$) performance of Nile tilapia than those fed the control diet concerning weight gain, SGR and RGR. The highest final weight was obtained at group fed 3.0% BS (34.0±0.12 g), group fed 3% ML (33.0±0.23 g) and group fed 3% BS + ML (32.6±0.12 g),respectively , while the lowest one was obtained at the control group (26.37±0.20 g). The improvement in growth parameters and survival rate may be because these spices contain substances that enhanced fish growth and the general fish health (Abd Elmonem *et al*., 2002; Shalaby *et al*., 2003 and El-Dakar *et al*., 2004c). Similar results were obtained with different medical plants and herbs such as marjoram, basil, licorice roots, black seeds, peppermint leaves, fenugreek seeds and caraway seeds (Abd El-Maksoud *et al*., 2002, Abd Elmonem *et al.,* 2002; Sakr, 2003; Shalaby *et al*., 2003; El-Dakar, 2004 El-Dakar *et al*., 2004 a, b, c; and Shalaby, 2004).

Table (4) shows that feed intake increased significantly with added different spices levels in fish diets ($P<0.05$). The highest feed intake was obtained at groups fed 3% BS (32.06±0.51 g feed/fish), groups fed 3% BS and ML (31.84±0.34 g feed/fish), groups fed 1% BS and ML (31.25±0.29 g feed/fish), groups fed 2% BS (30.98±1.05 g feed/fish) and groups fed 2% BS and ML (30.79±0.48 g feed/fish), while the lowest one was obtained at the control groups (28.58±0.23 g feed/fish). Contrarily, FCR decreased significantly ($P<0.05$) at fish groups fed 3% ML, groups fed 3% BS, groups fed 2% ML (1.23, 1.25 and 1.35, respectively), while the highest FCR values were obtained at the control (1.58).

The PER values were enhanced significantly in 2% BS (3.75), while the lowest PER was obtained at the control groups (2.31). The percentage FER increased with the increase of different spices levels in the tested diets (P<0.05). The highest FER values were obtained by groups of fish fed 3% BS, 3% ML and 3% BS + ML (80.40, 80.39 and 79.49%, respectively), while the lowest one was obtained at the control groups (63.73%). Similarly, The APU% and EU% increased significantly due to the inclusion of BS and /or ML in fish diets (P<0.05). The highest APU was obtained at groups fed 3% ML, 3% BS and 3% BS + ML (61.25%, 58.62% and 57.78%, respectively), while the lowest one was obtained at the control groups (46.87%). The highest EU was obtained in all groups that contained different spices levels, where the highest value was obtained at groups fed ML (37.59%), while the lowest one was obtained at the control group (29.33%).

In the present study, the improved fish growth and feed utilization when supplemented diets with BS and /or ML may possibly due to the improve in feed intake and nutrient digestibility. Also, BS and ML contain several nutrients, especially essential oils,vitamins and minerals that may help in fish growth promotion. These results agree with those found by Abdel-Maaksoud *et al*.(1999); El–Dakar (2004) and Shalaby (2004).

Table (5) shows the proximate chemical composition of whole body of Nile tilapia fed diets containing different levels of spices. The inclusion of BS and/or ML in tilapia diets herein resulted in an increase in protein (with few exceptions) and ether extract contents with a decrease in ash content.This increase of both crude protein and ether extract may be attributed to the incrase in dry matter content .

Table 5: Proximate chemical analysis on dry matter basis (mean ± SE) of Nile tilapia fed diets containing different levels of black seeds and marjoram leaves for 12 weeks.

Treatments	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
Initial	21.19^{a} ±0.39	59.89^{a} ±0.23	11.72^{a} ±0.20	28.39^{f} ±0.41
Control (T1)	27.38^{b} ±0.64	65.46^{b} ±0.27	16.31^{cd} ±0.46	18.28^{ab} ±0.29
T2	28.20^{b} ±0.41	67.45^{de} ±0.60	17.51^{de} ±0.31	15.04^{a} ±0.37
T3	27.95^{b} ±0.82	66.46^{bcd} ±0.48	18.80^{f} ±0.51	14.74^{a} ±0.26
T4	26.59^{b} ±0.63	68.64^{e} ±0.29	14.62^{b} ±0.24	16.74^{cde} ±0.53
T5	29.00^{b} ±0.99	68.38^{e} ±0.64	17.15^{de} ±0.58	14.47^{a} ±0.78
T6	27.74^{b} ±0.52	66.37^{abc} ±0.63	16.56^{c} ±0.28	17.07^{de} ±0.55
T7	28.50^{b} ±0.93	66.65^{cd} ±0.75	15.76^{c} ±0.53	16.56^{cd} ±0.24
T8	28.19^{b} ±0.82	64.39^{b} ±0.47	17.45^{de} ±0.38	17.64^{e} ±0.24
T9	27.41^{b} ±0.76	66.00^{bcd} ±0.53	18.00^{ef} ±0.30	16.01^{bc} ±0.23
T10	28.27^{b}± 1.08	65.26^{bc} ±0.14	18.25^{ef} ±0.19	16.49^{cd} ±0.74

MeanS with different superscripts in the same column are significantly different (P<0.05).

These results are not in agreement with those obtained by Abd El – Maksoud (2002), Abd Elmonem *et al.* (2002), Shalaby (2004), and Abdel Wahab *et al*. (2007) who found that fish body composition did not differ due to the use of various spices in diets.Hwever,the analysis of fish at start of expriment revealed lower dry matter, crude protein and ether extract, but higher ash contents than at the end of the expriment. Moreaver, there was a negative relationship between water content and ether extract content of the whole fish

body ,This negative relationship was proved too by Abdelhamid(1988) and Abdelhamid *et al* .(1998,2005 b&2007).

In conclusion, under these experimental conditions, the use of BS and/or ML at 3.0% level in the diet can improve the growth performance and feed utilization of Nile tilapia.

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" تأثير استخدام بعض التوابل كإضافات غذائية على المعدلات الإنتاجية" وتركيب الجسم لأسماك البلطي النيلى.

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المعمل المركزي لبحوث الثروة السمكية- العباسة – أبو حماد - شرقية - قسم بحوث التغذية وتكنولوجيا تصنيع الأعلاف

أجريت هذه الدراسة بهدف تقدير تأثير استخدام كل من مسحوق بذور حبة البركة ومسحوق أوراق البردقوش كإضافات غذائية على الأداء الإنتاجي وتركيب الجسم لأسماك البلطي النيلى .العليقة الأساسية كانت تحتوى على ٣٠% بروتين خام (ونسبة طاقه٤٠٧٢ كيلو كالورى/جم عليقه).وكانت التجربة عبارة عن ٣٠حوضا زجاجيا وزعت فيها الأسماك بصورة عشوائية بمعدل ١٠ أسماك/ حوض(بمتوسط ٨٠٢جم/سمكة) وخصصت ثلاثة أحواض لكل معاملة(كمكررات).وقسمت إلى ١٠ معاملات كالتالي المعاملة الأولى (كنترول)غذيت على العليقه الأساسية بدون إضافات,المعاملات ٤,٣,٢ كل منها كانت تتغذى على العليقة الأساسيه مضافا إليها مسحوق بذور حبة البركة بنسبة١%,٢%,٣% على التوالي ,المعاملات ٧,٦,٥ كل منها كانت تتغذى على العليقه الأساسية مضافا إليها مسحوق أوراق البردقوش بنسبة ١%,٢%,٣% على التوالي, والمعاملات ١٠,٩,٨ كل منها كانت تتغذى على العليقه الأساسية مضافا إليها كل من مسحوق بذور حبة البركه ومسحوق أوراق البردقوش معا (١:١وزن: وزن) بنسبة ١%,٢%,٣% على التوالي. وكانت الأسماك تتغذى يوميا بمعدل ٣% من وزن الجسم تقدم على مرتين يوميا لمدة ١٢ أسبوعا (خمسة أيام /اسبوع) وكان يتم وزن الأسماك كل أسبوعين وتعدل كمية الغذاء المقدمة حسب الزيادة في وزن الأسماك. وفى نهاية التجربة تم تعيين الوزن النهائي للأسماك وعليه تم تقدير كل من معدل الزيادة في النمو ومعدل النمو النسبي ومعدل النمو النوعي، فكانت أعلى قيم في المعاملة الرابعة ،واقل قيم فى المعاملة الأولى (الكنترول)،كذلك ارتفعت كمية الغذاء المأكول وانخفضت نسبة معامل التحويل الغذائي في المعاملة الرابعة بينما نسبة الاستفادة من البروتين ونسبة الاستفادة من الطاقة قد ارتفعت معنويا عند تغذية الأسماك على العليقة المحتوية على إضافات سواء مسحوق بذور حبة البركة أو مسحوق أوراق البردقوش أو كليهما معا بينما انخفضت قيم المعاملة الأولى .وبالتحليل الكيماوي لجسم الأسماك وجد أن محتوى الأسماك من المادة الجافة قد زاد معنويا باضافة مسحوق بذور حبة البركة أو مسحوق أوراق البردقوش ,ومحتواه من البروتين زاد معنويا في كل المعاملات باستثناء الكنترول ، ومحتواه من المستخلص الإثيرى أيضا ارتفع معنويا فى جميع المعاملات عدا الكنترول ، بينما محتوى الأسماك من الرماد انخفض معنويا في كل المعاملات المغذاه على عليقه تحتوى اى من الإضافات المستخدمة.

Table 3: Growth performance (means ± SE) of Nile tilapia fed diets containing different levels of black seed and marjoram leaves for 12 weeks.

Items	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Initial weight (g)	8.27 ±o.o3	8.27 ±0.03	8.22 ± 0.03	8.27 ± 0.03	8.27 ± 0.03	8.27 ± 0.03	8.23 ± 0.03	8.23 ± 0.03	8.27 ± 0.03	8.30 ± 0.03
Final weight (g)	26.37^{F} ± 0.20	29.30^{de} ± 0.17	30.35^{c} ± 0.14	34.0^{a} ± 0.12	28.4^{e} ±0.17	30.75^{c} ±0.72	33.0^{b} +0.23	30.75^{c} ±0.43	30.2^{ed} ±0.17	32.6^{b} ± 0.12
Weight gain (g)	18.1^{e} ±0.17	21.0^{d} ±0.12	22.10^{c} ±0.12	25.75^{a} ±0.14	20.15^{d} ±0.14	22.5^{c} ±0.69	24.70^{b} ±0.23	22.5^{c} ±0.43	21.95^{c} ±0.20	24.35^{b} ±0.14
Gain rate (g/day)	0.21^{e} ±0.002	0.25^{d} ±0.002	0.26^{c} ±0.001	0.31^{a} ±0.002	0.24^{d} ±0.002	0.27^{c} ±0.008	0.29^{b} ±0.002	0.27^{c} ±0.005	2.26^{c} ±0.003	0.29^{b} ±0.002
Specific-growth rate(%/d)	1.39^{f} ±0.003	1.49^{e} ±0.008	1.55^{d} ±0.013	1.69^{a} ±0.011	1.47^{e} ±0.003	1.57^{cd} ±0.02	1.64^{b} ±0.008	1.58^{c} ±0.017	1.55^{cd} ±0.011	1.64^{b} ±0.016
Relative-growth rate(%)	219.38^{f} ±1.33	257.5^{d} ±4.68	267.44^{cd} ±0.63	312.15^{a} ±2.84	244.23^{e} ±0.89	272.65^{c} ±7.44	297.59^{b} ±2.78	275.0^{c} ±5.78	266.09^{cd} ±3.38	295.0^{b} ±2.88
Survival rate (%)	$94.0±1.9^{a}$	$97.0±0.8^{a}$	$98.0±0.5^{a}$	$97.0±0.5^{a}$	$98.0±0.3^{a}$	$98.0±0.5^{a}$	$97±0.5^{a}$	$98.0±0.6^{a}$	$98.0±0.3^{a}$	$99.0±0.0^{a}$

Means with different superscripts in the same row are significantly different (p<0.05).

Table 4: Feed intake, feed conversion ratio (FCR),feed efficiency ratio (FER) protein efficiency ratio (PER), apparent protein utilization (APU) and energy utilization (EU) of Nile tilapia fed diets containing different levels of black seeds and marjoram leaves for 12 weeks.

Items	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Feed intake(g)	28.58^{d} ±0.23	30.06bcd ±0.56	30.98^{ab} ±1.05	32.06^{a} ±0.51	29.01^{cd} ±0.02	30.43^{abc} ±0.0.82	30.73^{ab} ±0.08	31.25^{ab} ±0.29	30.79^{ab} ±0.48	31.84^{ab} ±0.34
FCR	1.58^{a} ±0.02	1.44^{b} ±0.03	1.40^{bc} ±0.04	1.25^{ef} ±0.02	1.44^{b} ±60.01	1.35^{cd} ±0.005	1.23^{f} ±0.005	1.38^{bc} ±0.01	1.41^{bc} ±0.008	1.37^{de} ±0.05
FER(%)	63.73^{e} ±1.14	69.91^{d} ±1.88	71.55^{cd} ±2.05	80.40^{a} ±1.73	69.46^{d} ±0.44	73.93^{bc} ±0.26	80.39^{a} ±0.73	72.14^{cd} ±0.72	71.3^{cd} ±0.47	79.49^{b} ±0.36
PER	2.31^{a} ±0.04	2.55^{cd} ±0.06	3.75^{bc} ±0.01	2.35^{a} ±0.28	2.59^{cd} ±0.01	2.69^{bc} ±0.01	2.93^{d} ±0.03	2.66^{bc} ±0.07	2.71^{bc} ±0.12	2.79^{bc} ±0.01
APU (%)	46.87^{f} ±0.76	54.89^{cde} ±1.44	54.03^{de} ±1.56	58.62^{b} ±1.28	56.62^{bcd} ±0.39	55.17^{cde} ±0.05	61.25^{a} ±0.49	54.46^{de} ±1.07	52.20^{e} ±0.41	57.78^{bc} ±0.35
EU (%)	29.33^{c} ±0.48	36.45^{ab} ±1.89	35.33^{ab} ±1.01	35.0^{ab} ±0.94	35.93^{ab} ±0.23	35.74^{ab} ±0.78	37.59^{a} ±0.19	34.33^{b} ±0.55	33.69^{b} ±0.28	35.28^{ab} ±0.22

Means with different superscripts in the same row are significantly different (P<0.05).