

## EFFECT OF STOCKING DENSITY ON PERFORMANCE, PHYSIOLOGICAL STRESS INDICATORS, AND IMMUNOLOGICAL STATUS OF BROILERS

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### ABSTRACT

The objective of this work was to investigate some physiological stress indicators and immunological status of commercial broilers grown to moderate and large weights with different housing densities. Three hundred and eight one-day-old hybrid broiler chicks were obtained from a commercial hatchery. Chicks were allotted to four groups according to stocking densities of 20, 30, 40, and 50 Kg/m<sup>2</sup> (9.1, 13.6, 18.2, and 22.7 birds/m<sup>2</sup>, respectively). Stocking density was calculated based on an expected final body weight of 2.2 Kg live body weight per bird. Some of live performance traits, physiological stress indicators, and the weight of lymphoid organs were used as indicator of immunity status. The significant effect of 30 kg/m<sup>2</sup> stocking density compared with 20 kg/m<sup>2</sup> on live performance appeared only at 42 d of age. But increasing stocking density to 40 or 50 kg/m<sup>2</sup> significantly decreased Body weight (BW), growth rate (GR), and feed intake (FI) at 28, 35, and 42 d of age. The physiological stress indicators showed that corticosterone (CS), Total Nitrites (TN), glucose (GL), and cholesterol (CHO) were not significantly affected by increasing stocking density (to 50kg/m<sup>2</sup>) up to 35 d of age. But at 42 d, CS, TN, and CHO were significantly increased, especially with 40 and 50 kg/m<sup>2</sup>. Only at 42 d of age in the present investigation, heterophil to lymphocyte (H:L) ratio was significantly increased by increasing stocking density. Absolute and relative weights of spleen, bursa, and thymus at 42 d of age were significantly decreased as stocking density increased. Thus it could be concluded that, broiler rearing up to 35 d of age (about 1750 g BW) with 20 or 30 kg/m<sup>2</sup> stocking densities can protect the birds from the reduction in live performance, reduction immunological status, and increase in physiological stress indicators, especially that this weight (1750 g) is suitable for the marketing process.

**Keywords:** stocking density-physiological stress-immunological status- performance

### INTRODUCTION

For many years, the term stocking density indicated the numbers of birds being reared in a given housing area (Rice and Botsford, 1925). Now, many in the poultry industry express stocking density as body mass (kg) per housing space unit (m<sup>2</sup>). Moderate size of bird represents the majority of broiler production. The majority of the small bird market is in the fast food service sector. Numerous studies have been conducted to characterize the effects of stocking density on broiler performance (Bolton 1972, Proudfoot 1979, Cravener 1992 and Estevez 1997). Generally broiler chickens are reared at a considerably high stocking density. Such rearing conditions may act on the birds as a stress that causes various functional disorders. On mature hen, Feldkamp and Adams, 1973 reported that the reduction of floor space per bird resulted in a significant decrease in laying rate, perhaps because of increased stress. Craig *et al* (1986) demonstrated that plasma

CS levels increased when population density was increased. According to Puvadol Pirod and Thaxton, 2000a,b, plasma concentrations of corticosterone, glucose, and cholesterol were found to be increased during the adaptive phase of stress in broilers. Additionally, total plasma nitrite concentration increased during oxidative stress. Thus, increasing stocking density would result in increased levels of stress and consequently decreased immunocompetence in the birds. There have been very few studies to determine the impact of stocking density on the immune status of birds. Also, little studies have been published concerning the influence of stocking density on physiological stress.

The objective of this work was to investigate some physiological stress indicators and immunological status of commercial broilers grown to moderate and large weights under increasing housing densities.

## **MATERIALS AND METHODS**

### **Management and Experimental procedure:**

Three hundred and eight one-day-old hybrid broiler chicks were obtained from a commercial hatchery. Chicks were wing banded, individually weighed and allotted to four groups according to stocking densities of 20, 30, 40, and 50 Kg/m<sup>2</sup> (9.1, 13.6, 18.2, and 22.7 birds/m<sup>2</sup>, respectively). Stocking density was calculated based on an expected final body weight of 2.2 Kg live body weight per bird. Each pen floor area was 2.42 m<sup>2</sup>. The treatments contained of 22, 33, 44, and 55 chicks per pen for calculated stocking densities of 20, 30, 40, and 50 kg of Bw/m<sup>2</sup>, respectively. All treatments were randomly housed in eight floor pens (two replicates for each treatment). All chicks were fed *ad libitum* on a starter (1-18 days), grower (19-28 days), and finisher diet (29–42) (Table 1). Birds exposed to continuous light (24 h/day) for the first 2 days and thenceforth 22 h/day to the end of the experiment (42 days).

### **Measurements:**

Chicks were weighed at hatch, 28, 35, and 42 d of age, whereas feed intake and feed conversion ratio were calculated at 28, 35, and 42 d of age. Mortality was recorded daily in each replicate. Blood samples (5 samples from each replicate) were collected from wing vein at 28, 35, and 42 d of age in heparinized tubes. The tubes were plunged into an ice bath immediately after each collection. One drop of whole blood from each tube was used to make a thin smear on a clean microscope slide. Slides were then air-dried, stained (Cook, 1959), and 100 leukocytes from each smear were identified for cell type and counted, then the heterophil: lymphocyte ratio (H:L ratio) was calculated. Remaining plasma samples in the tubes were stored at -20C<sup>0</sup> until later analysis. Plasma corticosterone (CS) was determined using an enzyme immunoassay procedure described previously by Thaxton *et al* (2006). Plasma Glucose (GL) and Cholesterol (CHO) were determined by colorimetric detection procedures described by Elliot (1984). Total plasma nitrate (TN) concentration, an accepted indication of nitric oxide, was determined spectrophotometrically using Griess reagent ( Green

*et al.*,1982 ). At 42 days of age, 5 birds from each replicate were weighed, slaughtered and spleen, bursa, and thymus were excised and weighed.

**Table (1): Composition and calculated analysis of the experimental diet**

<b>Ingredients</b>	<b>Starter</b>	<b>Grower</b>	<b>Finisher</b>
Yellow corn %	60	61.0	64.8
Soybean meal 44%	24	22.70	19.00
Broiler concentration 52%	10	10.00	--
Boon meal %	--	--	2.10
Gluten %	2.9	2.40	2.50
Plant oil %	1.9	3.2	3.90
Fish meal 70 %	--	--	4.00
Wheat %	--	--	3.00
Calcium Di phosphate %	0.5	0.35	0.20
Salt %	0.25	0.1	0.25
Vitamins and trace mineral mixture %	0.45	0.25	0.25
Total	100	100	100
<b>*Calculated analysis :</b>			
Crude protein %	22.9	22.04	18.90
Me Kcal / kg	3052.71	3162.3	3222.4
Crude fat %	5.172	6.499	6.6044
Crude fiber %	3.422	3.35	3.18
Calcium %	1.518	0.86	0.89
Phosphorus %	0.6015	0.57	0.45
Lysine %	1.147	1.11	1.14
Methionine %	0.576	0.57	0.50

**\*According to NRC (2000).**

**Statistical Analysis:**

The general linear models procedure of the SAS system was utilized (SAS institute, 1988). Significant differences between means were determined by Duncan's multiple- range tests (Duncan, 1955).

**RESULTS**

**Live Performance:**

Results of body weight (BW), growth rate (GR), feed consumption (FI), feed conversion (FC), and mortality percentage (MO) of the different stocking density groups (T1, T2, T3, and T4) at 21, 28, 35, and 42 d of age are summarized in Table (2). Statistical analysis revealed that no differences could be noticed between groups up to 21 d of age. However, at 28, 35, d of age, it was observed that increasing stocking density to 40 or 50 kg of Bw/m<sup>2</sup> adversely affected growth rate and feed consumption, but feed conversion and mortality percentage were not significantly affected. Also no significant differences were observed between T1 (20kg Bw/m<sup>2</sup>) and T2 (30kg Bw/m<sup>2</sup>) for all traits. Significant differences were observed for all live performance traits at 42 d of age where birds in the T4 (50kg Bw/m<sup>2</sup>) recorded the lowest body weight which reaching about 14.73% of the control group (T1), followed by T3 and T2 (5.94% and 2.78%, respectively).

**Table (2): Effect of stocking density on live performance of broilers at 21, 28, 35, and 42 day of age**

Treatments	T1 20kg/m <sup>2</sup>	T2 30 g/m <sup>2</sup>	T3 40 g/m <sup>2</sup>	T4 50kg/m <sup>2</sup>	S.E	significant
<b>At 21 day</b>						
<b>BW</b>	752.73	756.13	739.97	748.55	9.60	N.S
<b>GR</b>	704.88	708.28	692.21	700.70	8.33	N.S
<b>FI</b>	1000.13	1003.06	991.86	1002.60	7.24	N.S
<b>FC</b>	1.42	1.42	1.43	1.43	0.01	N.S
<b>MO</b>	0.9	1.0	1.0	0.9	0.01	N.S
<b>At 28 day</b>						
<b>BW</b>	1264.37 <sup>a</sup>	1270.16 <sup>a</sup>	1233.46 <sup>b</sup>	1150.11 <sup>c</sup>	11.73	*
<b>GR</b>	1216.52 <sup>a</sup>	1221.82 <sup>a</sup>	1185.41 <sup>b</sup>	1102.12 <sup>c</sup>	7.82	*
<b>FI</b>	1910.53 <sup>a</sup>	1928.87 <sup>a</sup>	1861.99 <sup>b</sup>	1718.35 <sup>c</sup>	8.00	*
<b>FC</b>	1.57	1.58	1.57	1.56	0.02	N.S
<b>MO</b>	1.7	1.6	1.7	1.7	0.02	N.S
<b>At 35 day</b>						
<b>BW</b>	1775.77 <sup>a</sup>	1767.83 <sup>a</sup>	1730.05 <sup>b</sup>	1567.19 <sup>c</sup>	13.01	**
<b>GR</b>	1727.92 <sup>a</sup>	1719.49 <sup>a</sup>	1682.00 <sup>b</sup>	1519.20 <sup>c</sup>	11.99	**
<b>FI</b>	2922.18 <sup>a</sup>	2922.13 <sup>a</sup>	2877.22 <sup>b</sup>	2596.73 <sup>c</sup>	9.01	**
<b>FC</b>	1.69	1.70	1.71	1.71	0.02	N.S
<b>MO</b>	2.00	1.90	1.90	2.10	0.10	N.S
<b>At 42 day</b>						
<b>BW</b>	2235.43 <sup>a</sup>	2173.18 <sup>b</sup>	2102.65 <sup>c</sup>	1906.21 <sup>d</sup>	22.63	***
<b>GR</b>	2187.58 <sup>a</sup>	2124.84 <sup>b</sup>	2054.60 <sup>c</sup>	1858.22 <sup>d</sup>	15.77	***
<b>FI</b>	3959.52 <sup>a</sup>	3844.96 <sup>b</sup>	3841.72 <sup>b</sup>	3567.78 <sup>c</sup>	16.81	***
<b>FC</b>	1.81 <sup>a</sup>	1.81 <sup>a</sup>	1.89 <sup>b</sup>	1.92 <sup>b</sup>	0.02	*
<b>MO</b>	2.1 <sup>a</sup>	2.8 <sup>b</sup>	3.2 <sup>b</sup>	3.6 <sup>c</sup>	0.02	*

Means within row for each item having different superscript differ significantly \* (p≤0.05)  
 \*\* (p≤0.01) \*\*\* (p≤0.001)

**Physiological stress indicators:**

The effect of stocking density on some physiological stress indicators are illustrated in Table (3). CS, TN, GL, and CHO were not significantly affected with increasing stocking density up to 35 d age. At 42 d of age, CS concentration was increased by increasing stocking density. CS concentration in T2, T3 and T4 birds was increased by 2.83%, 17.27%, and 27.54%, respectively of T1. The same trend was noticed with TN at 42 d of age where TN value in T2, T3 and T4 birds was increased by 7.35%, 38.85% and 56.14%, respectively, of T1. Also CHO was increased as stocking density increased where birds in T4 recorded the highest value followed by T3 and T2. There were no significant differences among treated groups in GL value.

**Immunological status:**

The results of H:L ratio as affected by stocking density is presented in Table (4). H:L ratio was not significantly affected with increasing stocking density up to 35 d of age. At 42 d of age, H:L ratio was increased by increasing stocking density. H:L ratio in T2, T3, and T4 birds was increased by 10.39%, 50.65%, and 66.23%, respectively of T1. Table (5) summaries

the effect of stocking density on absolute and relative lymphoid organ weights at 42 d of age. Increasing stocking density significantly decreased spleen absolute weight by 42.41%, 28.41%, and 4.67% with 50, 40, and 30 kg/m<sup>2</sup>, respectively, compared to 20 kg/m<sup>2</sup>. The same trend was observed with absolute weight of bursa by 35.60% (50 kg/m<sup>2</sup>), 32.46% (40 kg/m<sup>2</sup>), and 5.50% (30 kg/m<sup>2</sup>) compared to 20 kg/m<sup>2</sup> while the thymus absolute weight was decreased to 37.57%, 27.38%, and 5.03% for 50 kg/m<sup>2</sup>, 50 kg/m<sup>2</sup>, 50 kg/m<sup>2</sup>, respectively. Also, the relative lymphoid organs were decreased as stocking density increased where 50 kg/m<sup>2</sup> recorded the lowest relative spleen weight by 32.17% followed by 40 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup> (23.48% and 1.74%, respectively). T3 birds had the lowest bursa weight where it decreased by 24.65% followed by T4 birds (28.07%) then T2 birds (2.92%) compared to T1 birds. Also thymus relative weight was decreased by 26.47%, 23.53%, and 2.94% of T4, T3, and T2 compared to T1.

**Table (3):Effect of stocking density on physiological stress indicators of broilers at 28, 35, and 42 day of age.**

Treatments	T1	T2	T3	T4	S.E	significant
Traits	20kg/m <sup>2</sup>	30kg/m <sup>2</sup>	40kg/m <sup>2</sup>	50kg/m <sup>2</sup>		
<b>At 28 day</b>						
CS	476.15	468.12	471.03	473.17	5.83	N.S
TN	91.15	90.28	90.89	92.00	4.12	N.S
GL	198.14	199.5	200.16	198.00	2.99	N.S
CHO	110.80	111.21	111.00	112.86	3.00	N.S
<b>At 35 day</b>						
CS	462.11	458.26	462.00	0.80	0.07	N.S
TN	88.25	88.99	87.14	88.28	3.12	N.S
GL	205.44	203.21	204.00	203.99	2.68	N.S
CHO	118.84	117.81	116.08	116.99	3.68	N.S
<b>At 42day</b>						
CS	512.57 <sup>a</sup>	520.00 <sup>a</sup>	601.09 <sup>b</sup>	653.72 <sup>c</sup>	0.09	**
TN	89.13 <sup>a</sup>	95.68 <sup>a</sup>	123.76 <sup>b</sup>	139.17 <sup>c</sup>	3.62	**
GL	223.77	218.53	215.35	220.43	5.22	N.S
CHO	107.01 <sup>a</sup>	111.42 <sup>a</sup>	149.88 <sup>b</sup>	155.69 <sup>b</sup>	4.00	*

Means within column for each item having different superscript differ significantly \* (p≤0.05) \*\* (p≤0.01)

**Table (4):Effect of stocking density on H:L ratio of broilers at 21, 28, 35, and 42 day of age**

Treatments	T1	T2	T3	T4	S.E	significant
Traits	20kg/m <sup>2</sup>	30kg/m <sup>2</sup>	40kg/m <sup>2</sup>	50kg/m <sup>2</sup>		
At 28 day	0.76	0.76	0.78	0.77	0.09	N.S
At 35 day	0.81	0.79	0.79	0.80	0.07	N.S
At 42day	0.77 <sup>a</sup>	0.88 <sup>a</sup>	1.16 <sup>b</sup>	1.28 <sup>c</sup>	0.09	**

Means within row for each item having different superscript differ significantly \*\* (p≤0.01)

Table (5): Effect of stocking density on lymphoid organs weight of broilers at 42 day of age

Treatments	T1 20 kg/m <sup>2</sup>	T2 30 kg/m <sup>2</sup>	T3 40 kg/m <sup>2</sup>	T4 50 kg/m <sup>2</sup>	S.E	significant
<b>Absolute Weight</b>						
<b>BW</b>	2235.43 <sup>a</sup>	2173.18 <sup>b</sup>	2102.65 <sup>c</sup>	1906.21 <sup>d</sup>	22.63	***
<b>Spleen</b>	2.57 <sup>a</sup>	2.45 <sup>a</sup>	1.84 <sup>b</sup>	1.48 <sup>b</sup>	0.02	*
<b>bursa</b>	3.82 <sup>a</sup>	3.61 <sup>a</sup>	2.58 <sup>b</sup>	2.46 <sup>b</sup>	0.02	*
<b>thymus</b>	7.56 <sup>a</sup>	7.18 <sup>a</sup>	5.49 <sup>b</sup>	4.72 <sup>b</sup>	0.02	*
<b>Relative weight</b>						
<b>Spleen</b>	0.115 <sup>a</sup>	0.113 <sup>a</sup>	0.088 <sup>b</sup>	0.078 <sup>b</sup>	0.001	*
<b>bursa</b>	0.171 <sup>a</sup>	0.166 <sup>a</sup>	0.123 <sup>b</sup>	0.129 <sup>b</sup>	0.001	*
<b>thymus</b>	0.338 <sup>a</sup>	0.330 <sup>a</sup>	0.261 <sup>b</sup>	0.248 <sup>b</sup>	0.001	*

Means within row for each item having different superscript differ significantly \* (p<0.05)  
\*\*\* (p<0.001)

## DISCUSSION

As previously mentioned, the aim of the current study was to investigate some physiological stress indicators and immunological status of commercial broilers grown to moderate and large weights with different housing densities. In the present investigation, the significant effect of stocking density 30 kg/m<sup>2</sup> compared with 20 kg/m<sup>2</sup> on live performance appeared only at 42 d of age. But increasing stocking density to 40 or 50 kg/m<sup>2</sup> significantly decreased BW, GR, and FI at 28, 35, and 42 d of age. These results are in line with those observed by Dozier (2006) who stated that increasing stocking density above 30kg/m<sup>2</sup> adversely affected GR, FI, and FC at 28 d of age. They added that final BW gain and FI were also negatively impacted by high stocking densities at 35 d of age. Also, Shanawany (1988) reported that stocking density at or exceeding 30 birds / m<sup>2</sup> led to a greater decrease in final BW compared with stocking densities of 10 and 20 birds/m<sup>2</sup>. He showed that the reduction in growth rate due to stocking density was highly related to a decrease in feed consumption. This result is in agreement with our results where feed conversion was not affected with all stocking densities. On the other hand, Imaeda (2000) reported that there was no significant difference in body weight gain and feed intake between the stocking densities (12, 15, and 18 birds/m<sup>2</sup>). This may be due to differences in housing system where his experiment was done in closed system house.

In the current study, the physiological stress indicators showed that corticosterone (CS) , Total Nitrites (TN), glucose (GL), and cholesterol (CHO) were not significantly affected by increasing stocking density (to 50kg/m<sup>2</sup>) up to 35 d of age. But at 42 d, CS, TN, and CHO were significantly increased, specially with 40 and 50 kg/m<sup>2</sup>. These results are in agreement with Dozier (2006) who pointed out that stocking density (30 to 45 kg of BW/M<sup>2</sup>) did not

influence plasma CS, GL, CHO, and TN of broiler at 31 d of age. Also Thaxton (2006) found that plasma concentration of CS, CHO, and GL in broiler were not affected by increasing stocking density to 55 kg/m<sup>2</sup> in closed house system. On the other hand, Siegel (1960) showed that increased population density caused the adrenal gland of chicks to hypertrophy. Also, Craig *et al* (1986) demonstrated that plasma CS levels increased when population density was increased. It has also been found that plasma concentrations of CS, GL, and CHO increased during the adaptive phase of stress in broilers (Puvadol Pirod and Thaxton, 2000a). Additionally, total plasma nitrite concentration increased during oxidative stress.

Only at 42 d of age in the present investigation, heterophil to lymphocyte (H:L) ratio was significantly increased by increasing stocking density. These results are in harmony with those reported on broiler by Heckert (2002) who found that H:L ratio tended to increase across the treatment groups. Also Dozier (2006) stated that H:L ratio was increased as stocking density increased. Also Thaxton (2006) noticed that as stocking density increased from 30 to 45 kg of BW/m<sup>2</sup>, the H:L ratio increased linearly. However in caged leghorns, increased cage density had no effect on the H:L ratio (Patterson and Siegel, 1998).

Absolute and relative weights of spleen, bursa, and thymus at 42 d of age were significantly decreased as stocking density increased. These observations are compatible with the results noticed by Heckert 2002 who found that there was tendency to smaller spleen weights with increasing stocking density. They added that bursa weight was significantly lighter with increasing stocking density mainly due to a large decrease in bursa weight at the highest stocking density. They reported that increasing housing density would result in increased levels of stress and consequently decreased immunocompetence in the birds.

Conclusively, rearing broiler up to 35 d of age (about 1750 g BW) with 20 or 30 kg/m<sup>2</sup> stocking densities can protect the birds from the reduction in live performance, reduction immunological status, and increase in physiological stress indicators, and that this weight (1750 g) is suitable for the marketing process.

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تأثير كثافة الطيور على الكفاءة الانتاجية وبعض مدلولات الضغوط الفسيولوجية  
والحالة المناعية لدجاج التسمين  
سعد زغلول الدمراوى<sup>1</sup> ، فاطمة مصطفى عبد النبى<sup>2</sup> ، نعمت عبدالغنى بدوى<sup>2</sup>  
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يهتم هذا العمل بدراسة بعض مدلولات الضغوط الفسيولوجية والحالة المناعية لدجاج التسمين النامى حتى أوزان متوسطة أو كبيرة خلال كثافات اسكانية متزايدة. استخدم فى هذه الدراسة عدد 308 كتكوت تسمين عمر يوم، حيث قسمت الطيور الى أربعة مجاميع طبقا للكثافات 20، 30، 40، 50 كجم/م<sup>2</sup> وذلك يعادل 9.1، 13.6، 18.2، 22.7 طائر/م<sup>2</sup> على الترتيب. ولقد تم حساب كثافة الطيور على أساس أن وزن الجسم النهائى المتوقع للطائر هو 2.2كجم. تم دراسة بعض صفات الكفاءة الانتاجية وبض مؤشرات الضغوط الفسيولوجية وكذلك أوزان بعض الأعضاء الليمفاوية كمدلول للحالة المناعية. ولقد أظهرت النتائج أن التأثير المعنوى للكثافة 30 كجم/م<sup>2</sup> بالمقارنة بـ 20 كجم/م<sup>2</sup> على الكفاءة الانتاجية قد ظهر فقط عند عمر 42 يوم، فى حين أن زيادة الكثافة الى 40 أو 50 كجم/م<sup>2</sup> قد قلل بصورة معنوية كلا من وزن الجسم و معدل النمو و الغذاء المستهلك عند الأعمار 28، 35، 42 يوم. أوضحت النتائج أيضا أن مستوى كلا من الكورتيكوستيرون و النترات الكلية و الجلوكوز والكوليسترول لم يتأثر معنويا بزيادة كثافة الطيور (حتى 50 كجم/م<sup>2</sup>) حتى عمر 35 يوم، أما عند 42 يوم من العمر فقد لوحظ زيادة معنوية فى مستوى الكورتيكوستيرون و النترات الكلية والكوليسترول، خاصة مع الكثافات 40 و 50كجم/م<sup>2</sup>. كما لوحظ زيادة نسبة الليمفوسايت الى الهتيروفيل بزيادة كثافة الطيور عند 42 يوم من العمر، وعلى العكس فقد حدث انخفاض معنوى فى الأوزان المطلقة والنسبية لكلا من الطحال والبرسا والثيرمس عند عمر 42 يوم بزيادة كثافة الطيور. ولذلك يمكن أن نخلص الى أن تربية دجاج التسمين حتى عمر 35 يوم من العمر ( متوسط وزن 1.750 كجم) عند كثافات 20 أو 30 كجم/م<sup>2</sup> يمكن أن يقى الطيور من انخفاض الكفاءة الانتاجية أو من انخفاض الحالة المناعية أو من زيادة الضغوط الفسيولوجية التى قد تنشأ عند التربية على كثافات أعلى أو الى أعمار أطول، خاصة وأن هذا الوزن (1.75 كجم/م<sup>2</sup>) يعتبر مناسب لعملية التسويق.