SWINE MARKER SW967 AS A PROMISING GENETIC MARKER FOR RABBIT MEAT PRODUCTION-RELATED TRAITS

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SUMMARY

Reciprocal crossing between three rabbit breeds viz., Flemish Giant, New Zealand White and Papillon was made and the F_1 crossbreds were intermated within each genotype and produced the F_2 crossbreds. A minimum of 7 individuals per genotype were slaughtered and DNA was isolated. DNA amplification was performed using primers for two identified genetic markers from swine to investigate their association with meat production-related genes in rabbits. Specific-PCR profile analyses revealed two alleles for swine marker SW967 with ML ranged from 63 to 110 bp. The swine marker SW967 affected significantly dressing percentage, dissected side muscle percentage, dissected side bone percentage, dissected side muscle weight to dissected side bone weight ratio and dissected side bone weight occurring in foreleg cut. This work showed the possibility of using genetic markers from swine to improve muscling traits in rabbits through marker assisted-selection.

Keywords: *Rabbits, meat production-related genes, genetic markers*

INTRODUCTION

To achieve real progress in production traits, many generations of selective breeding are required which could be costly and time-consuming and sometimes as in the case of carcass traits are not very effective (Visscher and Haley, 1998). However, genetic progress in quantitative traits may be further enhanced by molecular genetics either by direct selection on genes that affect traits of interest or through selection on genetic markers linked to quantitative trait loci and used in marker-assisted selection (Geldermann, 1976; Soller and Bekmann, 1983; Beever *et al*., 1990). Fortunately, a large proportion of markers isolated in one species could be used in other closely related species (Moore *et al*., 1991). Until now, rabbits suffer from a scarcity of literature on the association of genetic markers with meat production-related traits.

The purpose of this paper was to investigate the detection of genetic markers associated with rabbit meat production-related traits, which could be used as a corner stone for future marker-assisted selection in this species.

MATERIAL AND METHODS

Breed groups:

At the private El-Qanater Rabbit Farm (20km north of Cairo), three rabbit breeds viz., Flemish Giant (F), New Zealand White (N) and Papillon (P) were considered in this study.

Experimental animals:

Each of the three breed groups was composed of one buck and three does taken randomly from the stock.

F_1 production scheme:

In March-April 2001, reciprocal crossing system between breed groups was made. F_1 were symbolized as: (FN), (FP), (NF), (NP), (PF) and (PN).

F_2 production scheme:

In October-November 2001, the F_1 crossbreds were intermated within each genotype group for one generation. F_2 genotypes were symbolized as (FN-FN), (FP-FP), (NF-NF), (NP-NP), (PF-PF) and (PN-PN). However, there were too few animals from the (FN-FN) genotype and it was excluded from the study.

Management:

After kidding, litters were kept with their dams in breeding batteries till weaning at 28 days of age, by which time they were ear tagged, sexed, weighed and transferred to wired cages. Dams and weaners were fed *ad. libitum* a commercial pelleted diet containing 18.5% crude protein, 14% crude fiber and 3.2% crude fat, providing 2800 Kcal digestible energy/kg diet.

Meat production-related traits:

At marketing age (90 days), a minimum number of 7 individuals per genotype were fasted for twelve hours and transferred to the Meat Laboratory where animals were weighed (fasted slaughter weight). After slaughtering, animals were dressed out then the hot carcass, skin, head, ears, feet, tail, heart, lungs plus trachea, liver, spleen, kidneys and empty digestive tract were weighed separately. Dressed carcasses were packed in polyethylene bags and deep frozen at -18˚C for one week. Offals were grouped into external (head, ear, feet, tail, and skin) and internal organs (heart, lungs plus trachea, liver, spleen, kidneys and empty digestive tract). The dressing out-related traits were calculated as a percentage of fasted slaughter weight. After being thawed, carcasses were split into right and left sides. The right sides were jointed into four cuts (Blasco *et al*., 1992), viz., hind leg , fore leg , loin and thoracic cage cut and weighed separately. The sum of the weights of side cuts gave the jointed side weight. Side weight distribution between cuts was expressed as percentage carcass side weight occurring in each cut**.** Each cut was dissected into muscle, fat and bone and their weights were recorded. The sum of a tissue weight over all the cuts gave the dissected side muscle weight (DSMW), dissected side fat weight (DSFW) and dissected side bone weight (DSBW). The sum of DSMW, DSFW and DSBW gave the dissected side weight (DSW). Side composition traits were expressed as a percentage of each tissue of DSW. Side meatiness traits were expressed as muscle to bone and muscle to fat ratios at the side level. Side muscle, fat and bone weight distribution between cuts were expressed by calculating percentage dissected side muscle, fat and bone weight occurring in each cut, respectively.

Genetic markers:

After isolating genomic DNA (Sambrook *et al.*, 1989), two identified genetic markers, from swine, were used to investigate their association with loci in connection with meat production-related traits. Table 1 summarizes information of the 2 markers considered in the present experiment.

Table 1. Information on the Swine markers used in this experiment

Species	Marker	
	SWC9	**SW967**
Chromosome no.	2	5
No. of alleles	17	10
Primers*	GGCTCAGGGATCCCACAG AAGCACCTGTACCCACACG	AGCAGACTGTTCATCTGTTCAG GGGGCAGCTGAAAAGTCC
Size (bp)	224-246	95-115
Meat production-related traits in connection with the marker-associated loci	Muscle mass	Average Daily gain
Reference	Nezer *et al*., 1999	Casas-Carrillo *et al*., 1997

*: for each marker the upper primer is the forward and the lower one is the reverse.

Polymerase chain reaction (PCR) conditions:

DNA amplification (Table 2) was performed (Williams *et al*., 1990) using specific primers (synthesized by Metabion, Germany) for the considered markers. The components of PCR reaction are given in Table (3) together with their amounts totaling to 25 µl.

Table 2. PCR conditions used for each of the four primers of the two markers used in the present experiment

Species	Marker	
	SWC9	**SW967**
Initial separation	94°C-5 min	94°C-5 min
Temperature time duration for cycle steps		
Denaturation	94°C-40 sec	94°C-40 sec
Annealing	58°C-40 sec	58°C-40 sec
Extension	72°C-40 sec	72°C-40 sec
Final extension	72°C-40 min	72°C-40 min
No. of cycles	35	35
Reference	http://www.marc.usda.gov.swine	http://www.marc.usda.gov.swine

Table 3. Components used in PCR reaction (Williams *et al*., 1990)

Component	Concentration	Amount
Genomic DNA	25 ng	2.0µl
Forward primer	10 Pico-mol	1.0µl
Reverse primer	10 Pico-mol	1.0µl
Bioron's DNTPs	-	2.5µl
Bioron's buffer (10X) mixed with $MgCl_2$	-	2.5µl
Bioron's Taq DNA polymerase (250U)	-	0.2µl
Distilled water	-	15.8µl

Gel electrophoresis and visualization of DNA bands:

The specific PCR products were resolved by electrophoresis in 2% agarose gel at 100 volts for 30-45 minutes using TBE buffer (5.5g Boric Acid, 10.8g Tris and 4ml Edta 0.5M adjusted to pH 8; the final volume was made up to 1 liter with distilled

water). The gel was stained with 0.2µg/ml ethidium bromide and examined on ultraviolet transilluminator.

Densitometric scanning and analysis:

PCR product gels were scanned using Bio-Rad Gel Doc 2000 and analysed with the Quantity One Software package supplied by the manufacturer.

Statistical analysis:

The data were analysed according to the following linear model (SAS, 1994):

$$Y_{ijklm} = \mu + G_i + GE_j + S_k + M_l + e_{ijklm}$$

where:

Y_{ijklm} = the observation on the m^{th} rabbit of the i^{th} cross type, j^{th} generation, k^{th} sex and l^{th} marker;

μ = the overall mean;

G_i = the fixed effect of the i^{th} cross type (i = 1, 2,…5);

GE_j = the fixed effect of the j^{th} generation (j = 1, 2, i.e. F1 vs. F2);

S_k = the fixed effect of the k^{th} sex (k = 1, 2);

M_l = the fixed effect of the l^{th} marker (l = 1, 2);

e_{ijklm} = the random error assumed to be N.I.D $(0,\sigma^2 e.)$.

The results were examined to identify those genetic markers which are associated with meat production-related traits.

RESULTS AND DISCUSSION

Detection of genetic markers:

The swine marker SW967 was able to produce in rabbits informative polymorphic products resolvable by agarose electrophoresis. Swine and rabbits are known to have common taxonomical grounds in being multi-parous and non-gastric species.

Swine marker SW967:

The results of specific-PCR profile analyses using swine marker SW967 are illustrated in Figure 1. This marker revealed a total of two alleles with molecular length (ML) ranged from 63 to 110 bp in the five rabbit genotypes.

Association of swine marker SW967 with rabbit meat production-related traits:

For each of the 32 traits, the effect of the marker has been tested and comparisons (C_2-C_1/C_1) between carriers of one (C_1) and two alleles (C_2) were also tested wenever the main effect was statistically significant at $P < 0.05$.

Body traits:

In Table (4) the marker SW967 did not affect significantly any body trait.

Carcass attributes:

Table 5 shows significant effect ($P < 0.05$) of the SW967 marker on dressing percentage, dissected side muscle percentage, dissected side bone percentage, dissected side muscle weight to dissected side bone weight ratio and dissected side bone weight occurring in foreleg cut. The marker explains 13.23%, 15.31%, 11.32%, 13.79 and 8.85 of the phenotypic variation of such trait, respectively. It is noteworthy that the number of alleles had significant ($P<0.05$) positive effect on dressing percentage, dissected side muscle percentage, dissected side muscle weight to

dissected side bone weight ratio and dissected side bone weight occurring in foreleg cut [(C_2 – C_1)/ C_1: +6.61%, +1.36, +8.79, +3.57 and +3.57, respectively] and significant (P<0.05) negative effect on dissected side bone percentage [(C_2 – C_1)/ C_1: -7.32]. Previous reports (Gouda, 1998; Lukefahr *et al*., 1989; Lukefahr *et al*., 1983; Lukefahr *et al*., 1982) demonstrated that dressing percentage, muscle percentage, bone percentage and muscle to bone ratio are genetically inter-related.

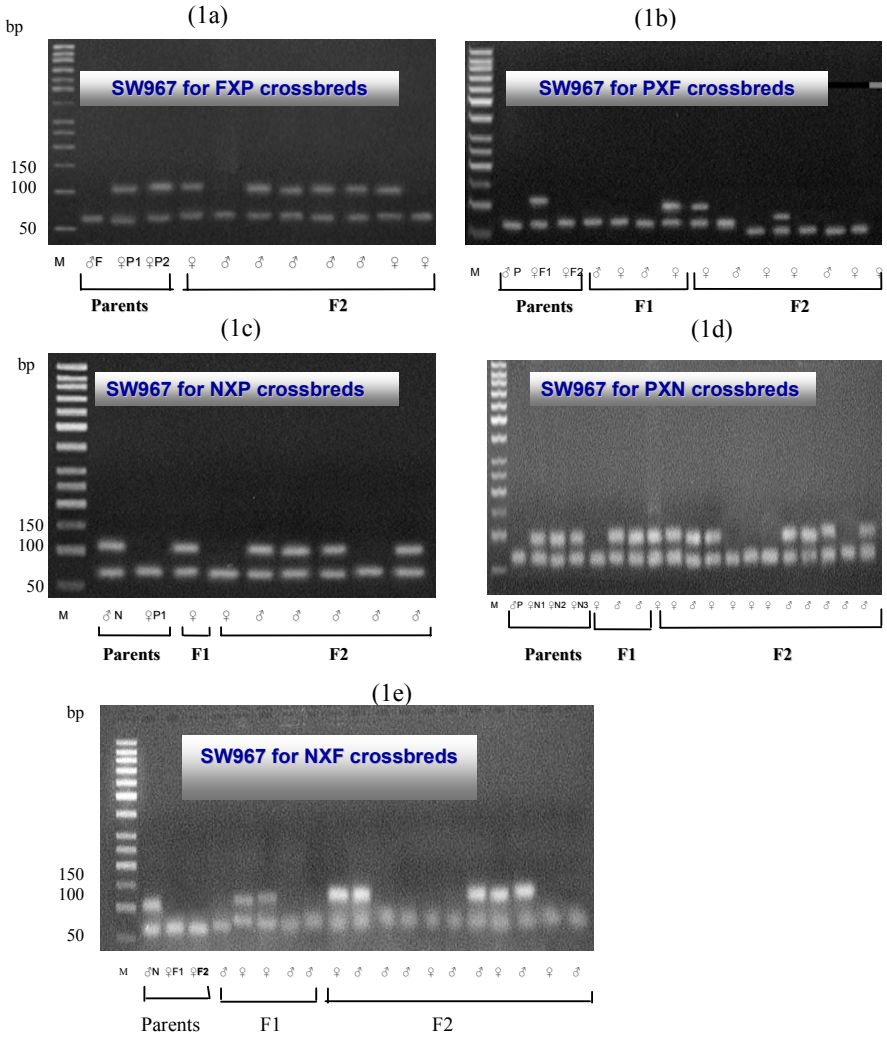

Figure 1. DNA polymorphism based on specific-PCR analysis of marker SW967
(1a) : FxP crossbreds , (1b) : PxF crossbreds
(1c) : NxP crossbreds, (1d) : PxN crossbreds (1e) : NxF crossbreds

CONCLUSION

Considering all the data obtained in the present study it can be concluded that swine marker SW967 proved to be a promising genetic marker for muscling traits in rabbits to achieve a higher genetic gain through marker-assisted selection.

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كشاف الخنازير SW967 ككشاف وراثى واعد للصفات المرتبطة بإنتاج اللحم فى الأرانب

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أجرى خلط تبادلى بين ثلاث سلالات من الأرانب هى الفليمش جاينت و النيوزيلندى الأبيض و البابيون للحصول على الجيل الأول ثم تم عمل خلط داخلي بين أفراد الجيل الأول داخل كل تركيب وراثى و الحصول على أفراد الجيل الثانى. ذبح عدد 7 أفراد على الأقل لكل تركيب وراثى و تم عزل الحامض النووى DNA . تم إكثار الحامض النووى باستخدام بادئات لكشافين وراثيين موصفين من الخنازير لبحث مدى ارتباطيهما مع الجينات المتعلقة بإنتاج اللحم فى الأرانب. أظهر تفاعل البلمرة المتسلسل (specific Primer PCR) وجود اثنين من الأليلات لكشاف الخنازير SW967 بطول جزيئى يتراوح من 63 الى 110 زوج من القواعد. أظهر كشاف الخنازير SW967 تأثيراً معنوياً على نسبة التصافي ونسبة العضلات بنصف الذبيحة ونسبة العظام بنصف الذبيحة ونسبة وزن العضلات إلى وزن العظام بنصف الذبيحة ووزن العظام فى قطعية الرجل الأمامية. تبين من هذا العمل إمكانية استخدام الكشافات الوراثية من الخنازير لتحسين صفات اللحم فى الأرانب من خلال الانتخاب بمساعدة الكشافات (MAS).

Table 4. Effect of SW967 marker on body traits and comparisons between carries of one allele (C_1) and two alleles (C_2)

Body Traits	Overall mean	Marker effect (P<0.05)	Least square means for the groups		Relative deviation [100 (C_2– C_1)/C_1)]	Percentage total variation in the trait explained by the marker
			exhibited two alleles (C_2)	exhibited one allele (C_1)		
i. Body weight (g) at age (days) of weaning (WW) and slaughtering (SW)						
WW28	376.0±14.5	ns	422.73 ±20.55	412.28 ±22.99	0.20	+ 2.55
SW90	1628.0±36.2	ns	1686.05 ±53.03	1697.79 ±59.32	0.04	- 0.69
ii. Body linear measurements (cm) at slaughter age (90 days): length of body (BL); width of loin (LW); girth of chest (CG) and round (RG)						
BL	32.0±0.34	ns	32.05 ±0.50	31.15 ±0.56	+ 2.89	0.23
LW	4.9±0.06	ns	5.00 ±0.10	4.89 ±0.11	+ 2.25	2.71
CG	23.3±0.21	ns	23.40 ±0.33	23.72 ±0.37	- 1.35	0.88
RG	19.6±0.24	ns	19.92 ±0.33	19.96 ±0.37	- 0.20	0.01
iii. Daily gain (DG, g/d) in body weight between weaning and slaughtering						
DG28-90	20.9±0.47	ns	21.05 ±0.65	21.42 ±0.73	- 1.73	1.18

ns: non significant

Table 5. Effect of SW967 marker on carcass attributes and comparisons between carries of one allele (C_1) and two alleles (C_2)

Carcass attributes	Overall mean	Marker effect (P<0.05)	Least square means for the groups		Relative deviation [100 (C_2–C_1)/C_1)]	Percentage total variation in the trait explained by the marker
			exhibited two alleles (C_2)	exhibited one allele (C_1)		
i. Dressing-out traits			51.30±0.67	48.12±0.75	+6.61	13.23
a. Dressing percentage	50.97±0.54	*	4.69±0.19	5.12±0.21	-8.40	3.01
b. Giblets percentage	4.59±0.15	ns	22.32±0.29	21.73±0.32	+2.72	3.70
c. External organs percentage	22.35±0.19	ns	12.07±0.38	12.55±0.43	-3.82	1.05
d. Internal organs percentage	11.70±0.29	ns	51.30±0.67	48.12±0.75	+6.61	13.23
ii. side cutting-out traits						
Side weight distribution between cuts percentage jointed side weight occurring in:						
e. hind leg cut	39.12±0.18	ns	38.94±0.24	39.23±0.27	- 0.74	1.07
f. fore leg cut	19.46±0.09	ns	19.42±0.12	19.19±0.13	+1.20	1.99
g. loin cut	32.58±0.17	ns	32.91±0.25	32.40±0.28	+1.57	3.34
h. thoracic cage cut	8.84±0.12	ns	8.73±0.16	9.16±0.18	-4.69	4.79
iii. Side tissue dissecting traits						
Side composition						
i. dissected side muscle percentage	83.21±0.18	*	83.58±0.23	82.46±0.26	+1.36	15.31
j. dissected side fat percentage	3.83±0.61	ns	4.01±0.26	4.15±0.29	-3.37	0.27
k. dissected side bone percentage	12.96±0.18	*	12.40±0.26	13.38±0.29	-7.32	11.32
Side meatiness						
l. dissected side muscle weight to dissected side bone weight ratio	6.49±0.09	*	6.81±0.13	6.26±0.14	+8.79	13.79
m. dissected side muscle weight to dissected side fat weight ratio	24.41±1.30	ns	23.87±2.10	22.79±2.35	+4.74	0.26

Table 5. Cont.

Carcass attributes	Overall mean	Marker effect (P<0.05)	Least square means for the groups		Relative deviation [100 (C_2–C_1)/C_1)]	Percentage total variation in the trait explained by the marker
			exhibited two alleles (C_2)	exhibited one allele (C_1)		
Side muscle weight distribution between cuts expressed as percentage dissected side muscle weight occurring in:						
n. hind leg cut	39.76±0.17	ns	39.55±0.24	39.89±0.27	- 0.85	1.46
o. fore leg cut	18.47±0.12	ns	18.29±0.16	18.19±0.18	+ 0.55	0.07
p. loin cut	34.50±0.19	ns	34.87±0.28	34.29±0.31	+1.69	3.47
q. thoracic cage cut	7.27±0.01	ns	7.28±0.15	7.62±0.17	-4.46	3.43
Side fat weight distribution between cuts expressed as percentage dissected side fat weight occurring in:						
r. hind leg cut	24.26±0.83	ns	22.89±1.37	25.58±1.53	-10.52	3.46
s. fore leg cut	55.06±0.93	ns	54.54±1.26	52.27±1.53	+4.34	2.30
t. loin cut	16.60±0.56	ns	17.79±0.89	17.45±1.00	+1.95	0.14
u. thoracic cage cut	4.07±0.26	ns	4.78±0.33	4.67±0.36	+2.36	0.05
Side bone weight distribution between cuts expressed as percentage dissected side bone weight occurring in:						
v. hind leg cut	42.22±0.33	ns	43.05±0.50	42.30±0.56	+1.77	2.00
w. fore leg cut	15.87±0.12	*	16.25±0.18	15.69±0.20	+3.57	8.85
x. loin cut	23.71±0.37	ns	23.36±0.60	23.81±0.67	-1.89	0.55
y. thoracic cage cut	18.20±0.31	ns	17.34±0.47	18.20±0.53	-4.73	2.95

* : significant ; ns: non significant

a, b, c and d: calculated as percentage of slaughter weight; e, f, g and h: calculated as percentage of jointed side weight; i, j and k: calculated as percentage of dissected side weight. n, o, p and q: calculated as percentage of the muscle in cut to the muscle in side; r, s, t and u: calculated as percentage of the fat in cut to the fat in side; v, w, x and y: calculated as percentage of the bone in cut to the bone in side.