Investigation and Modeling of Electrical Stunning Parameters of Turkey Using Response Surface Methodology

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Abstract: The aim of this work is to evaluate the effects of electrical stunning parameters of turkey on the quality of steak and stew using the response surface methodology. 1584 turkeys were used for the study. The parameters tested were the voltage and pulse. The responses were the sensory quality of steak and stew. The rejection rate varies between 0.23% (steak female) and 56.06% (female stew). Results show that it is possible to obtain a low rejection rates (< 1% for the steak of male turkey) at a voltage of 50 Volts and pulse of 7. In comparison of pulse, voltage remains the most important factor. The optimal conditions selected for the total output of the process obtained from the analysis of the response surfaces are: voltage, from 45 to 47 Volts; pulse from 6.5 to 7 and voltage from 50 to 52 Volts; pulse from 6.8 to 7, respectively for female and male turkeys. Under these experimental conditions, the rejection rates range from 0.23% to 0.80% and from 11.93% to 13.27% respectively for steak and stew. The results show that the voltage and pulse are both the parameters meaning on the global quality of steak and stew.

Keywords: Electrical stunning, turkey, steak, stew, sensory quality.

1. INTRODUCTION

The current European regulation regarding animal protection at the moment of slaughter makes stunning previous to slaughter mandatory in order to ensure that animals are unconscious and do not suffer unnecessarily [1]. Permitted methods for stunning are: (1) captive bolt pistol, (2) concussion, (3) electronarcosis and (4) exposure to carbon dioxide [2].

Stunning of animals is in the first place applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover consciousness before death intervenes *via* exsanguinations. Secondly stunning should produce sufficient immobility to facilitate the initiation of exsanguinations [2]. Stunning methods affected several meat quality parameters [3-6]. In addition, electrical stimulation of carcass was effective in improving eating quality attributes of pork [7].

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Electrical stunning induces unconsciousness by generating an epileptiform seizure, characterized by two distinct phases, a tonic phase and a clonic phase, rendering the animal insensible to pain [8]. The production of an epileptiform seizure is dependent upon the amount of current passing through the brain and possibly the area of the brain through which current flows [8]. Electrical stunning has some shortcomings such as causing blood splash [9].

Study of stunning equipment has become a higher priority, and there is greater willingness to invest in reliable and if necessary sophisticated equipment, especially if there are carcass or meat quality advantages. The electric discharge is passed through a bath electrolyte is precisely controlled so that only the subject is anesthetized without causing death (or stopping of the heart pump) to ensure complete bleeding. The electronarcosis is recognized in the Muslim ritual slaughter. To check the proper functioning of post electronarcosis a wake-up test is performed whenever a change is performed batch of poultry. This test consists of taking three subjects after electro-anesthesia check the waking time which should not exceed the limit of 2 minutes.

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In Tunisia, turkey must be rendered unconsciousness and insensible to pain prior to exsanguinations to be humanly slaughtered. Electrical stunning was used in Tunisia to stun turkey for slaughter [10]. This work is a study of electrical stunning parameters that the aims were: (1) determine the effect of electrical stunning on the meat quality of turkeys, (2) improving the quality of meat through optimization of electrical stunning parameters and (3) reducing of loss and rejection rate of steak and stew.

2. MATERIALS AND METHODS

2.1. Animal Material

These trials included 792 turkeys (*Meleagris gallopavo*) for each sex, for a total of 1584 individuals of the same strain (hybrid), and standard type but different producers for each sex. The average weight of animals was 15.175 kg for males and 6.5 kg for females.

2.2. Electrical Stunning

The principle of Electrical stunning involves passing a sufficient current, delivered by the electrode in the bath, through the brain to stop its normal activity. Equipment used for stunning poultry must be designed, constructed and maintained so consistently stunning should be efficient so that the animal immediately unconscious and insensible to pain and remains so until that he dies.

Ohm's law expresses the relationship between current, voltage and resistance. These elements are responsible for the operation of a bain-marie. The current is measured in amperes, the voltage in volts and the resistance in ohms. The current and voltage used by electric bains-marie are displayed by the voltmeter and ammeter, respectively.

$$I = \frac{V}{R}$$

Where V represents the voltage in Volt, R is the resistance in Ohm and I is the Intensity in milli-ampere.

Electrical stunning in poultry is conventionally performed by passing birds' heads through a water bath containing a powered electrode (Figure **1**).

2.3. Experimental Design

Experimental design used consists of varying two factors: voltage (U) and pulse (P). Levels of each factor depend on the sex of the animal. For each factor there are three levels and two repetitions. Each sample consisted of 22 individuals. So, we obtain: 3 × 3 ×2 × 22 = 396 individuals for each parameter (escalope and stew), or 396 x 2 = 792 individuals for each sex. The sensory quality was evaluated meaning blood splash.

2.4. Statistical Model

The study was conducted by performing analysis of variance and using statistical tools (NEMROD-W, Version 9901, LPRAI COMPANY, and SAS 9.1 Microsoft Product, France). The statistical model is a model with 2 factors. The following equation was used to express the rejection rates as a function of the independent variables:

$$R_{ijk} = \mu + \alpha\, U_{ijk} + \beta\, P_{ijk} + \gamma\, (U.P)_{ijk} + \varepsilon_{ijk}$$

Where R_{ijk} represents the response variable, μ is a constant; U_{ijk} and P_{ijk} are the effect of voltage and pulse, respectively. $(U.P)_{ijk}$ are the interaction effect of different factors. ε_{ijk} is the experimental errors.

3. RESULTS AND DISCUSSION

This study aims to determine the effect of each factor on the rejection rates as well as possible

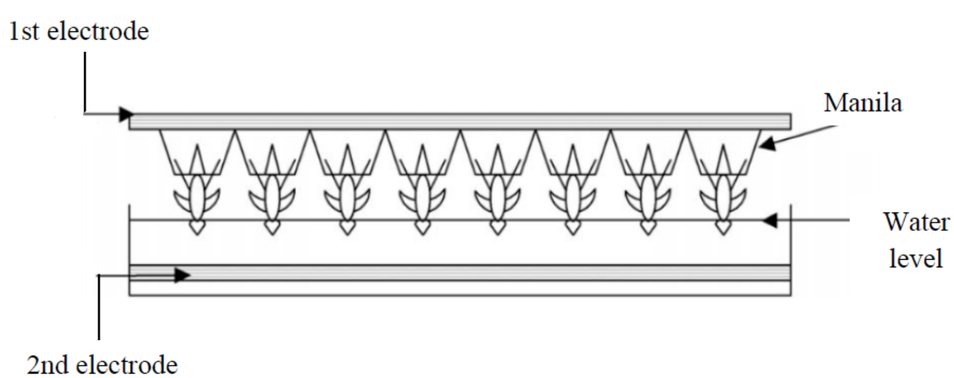

Figure 1: Diagram of electrical anesthetize.

Table 1: Independent Variables of Voltage (V) and Pulse (P) in RSM Design

Sex	Symbols	Independent variables	Coded levels		
			1	2	3
Male	U	Voltage (volts)	50	60	70
	P	Pulse	5	6	7
Female	U	Voltage (volts)	45	55	65
	P	Pulse	5	6	7

Table 2: Experimental Data for Rejection Rates

	Anatomical part	Trials	U	P	Repetition	R
	Stew	1	1	1	1	19.92
		2	1	1	2	20.68
		3	1	2	1	23.27
		4	1	2	2	22.82
		5	1	3	1	12.28
		6	1	3	2	11.93
		7	2	1	1	25.21
		8	2	1	2	26.05
		9	2	2	1	26.34
		10	2	2	2	26.46
		11	2	3	1	53.72
		12	2	3	2	53.48
		13	3	1	1	47.38
		14	3	1	2	47.84
		15	3	2	1	28.96
		16	3	2	2	30.44
		17	3	3	1	37.82
		18	3	3	2	38.18
	Steak	1	1	1	1	2.9
		2	1	1	2	3.1
		3	1	2	1	6.8
		4	1	2	2	5.2
		5	1	3	1	0.32
		6	1	3	2	0.80
		7	2	1	1	6.63
		8	2	1	2	6.21
		9	2	2	1	6.32
		10	2	2	2	6.64
		11	2	3	1	8.22
		12	2	3	2	7.78
		13	3	1	1	7.92
		14	3	1	2	7.78
		15	3	2	1	3.52
		16	3	2	2	4.04
		17	3	3	1	5.63
		18	3	3	2	5.85

(Table 2). Continued.

	Anatomical part	Trials	U	P	Repetition	R
Male Female	Stew	1	1	1	1	24.8
		2	1	1	2	25.46
		3	1	2	1	23.01
		4	1	2	2	22.61
		5	1	3	1	13.07
		6	1	3	2	13.27
		7	2	1	1	50.81
		8	2	1	2	51.61
		9	2	2	1	53.9
		10	2	2	2	56.06
		11	2	3	1	54.03
		12	2	3	2	51.99
		13	3	1	1	46.18
		14	3	1	2	46.5
		15	3	2	1	47.68
		16	3	2	2	47.32
		17	3	3	1	43.1
		18	3	3	2	44.7
	Steak	1	1	1	1	1.12
		2	1	1	2	1.22
		3	1	2	1	0.52
		4	1	2	2	0.94
		5	1	3	1	0.27
		6	1	3	2	0.23
		7	2	1	1	2.5
		8	2	1	2	2.86
		9	2	2	1	2.9
		10	2	2	2	3.08
		11	2	3	1	2.43
		12	2	3	2	3.27
		13	3	1	1	2.38
		14	3	1	2	1.9
		15	3	2	1	2.1
		16	3	2	2	1.56
		17	3	3	1	2.07
		18	3	3	2	2.85

interactions between different factors. This is a factorial trial carried out with a Random Complete Trial. Table **1** lists all the test data.

The model is therefore to propose a model having taken into account the 2 factors mentioned above, each with 3 levels and 2 repetitions. It was completed 72 trials. There are 2 factors at 3 levels each with 2 repetitions, 2 sex and 2 anatomical parts. Hence: 3 × 3 × 2 × 2 × 2=72 trials.

3.1. Models Study

The analysis of variance by the SAS software (Microsoft product) on the results is shown in Table **2** is given in Tables **3** and **4**.

3.1.1. Male Turkey

3.1.1.1. Stew

The coefficient of determination R^2, coefficient of variation CV, standard deviation σ, and average R, were 0.998276; 2.127502; 0.846391; 39.78333 respectively. It is noted that the model explains 99% (R^2=0.998276) the variability of rejection rates in the Pr<0.0001. It is therefore a relevant model.

At the end of this model we will study the effect of each factor on rejection rates and the possible interactions between different factors.

The standard deviation is not small (σ=0.846391), therefore it is not a good accuracy. Indeed σ shows that the number of trials (18) is not sufficient to achieve accurate results.

The analysis of variance (the model) involving interactions between different factors is given in Table **3**.

According to Table **3**, we find that the model depends primarily on U, P and interaction UP. Indeed, the voltage, the pulse and interaction between voltage and pulse have a highly significant (Pr<0.0001) at α=5%. So the model is:

$$R = \mu + \alpha U + \beta P + \gamma (U*P) + \varepsilon$$

Thereafter each factor has undergone the test of Waller-Duncan to see if there are significant differences between groups or classes of the same factor (Table **4**). We note that the average rejection rates yield for 6 observations according to the voltage can be classified into 3 groups (A, B and C). Thus the differences between groups are significant. For the pulse, we note

Table 3: Analysis of Variance (ANOVA) on the Model, the Factors and their Interactions

Sex	Anatomical part	ANOVA	Source of variation	DF	SSD	MS	F	Pr > F
Male	Stew	Model	Model	8	3734.039	466.754	651.55	< 0.0001
			Error	9	6.447	0.716	-	-
			Total	17	3740.486	-	-	-
		Factors and their interactions	P	2	88.203	44.101	61.56	< 0.0001
			U	2	3545.408	1772.704	2474.54	< 0.0001
			P*U	4	100.427	25.106	35.05	< 0.0001
	Steak	Model	Model	8	94.378	11.797	58.32	< 0.0001
			Error	9	1.820	0.202	-	-
			Total	17	96.198	-	-	-
		Factors and their interactions	P	2	3.040	1.520	7.52	< 0.0120
			U	2	44.900	22.450	110.98	< 0.0001
			P*U	4	46.436	11.609	57.39	< 0.0001
Female	Stew	Model	Model	8	3734.039	466.754	651.55	< 0.0001
			Error	9	6.447	0.716	-	-
			Total	17	3740.486	-	-	-
		Factors and their interactions	P	2	88.203	44.101	61.56	< 0.0001
			U	2	3545.408	1772.704	2474.54	< 0.0001
			P*U	4	100.427	25.106	35.05	< 0.0001
	Steak	Model	Model	8	15.398	1.924	15.85	< 0.0002
			Error	9	1.093	0.121	-	-
			Total	17	16.491	-	-	-
		Factors and their interactions	P	2	0.084	0.042	0.35	< 0.7163
			U	2	14.058	7.029	57.88	< 0,0001
			P*U	4	1.256	0.314	2.59	< 0.1089

DF: Degrees of Freedom, SSD: Sum of Square Deviation, MS: Mean Square, Pr: probability of error type 1.

Table 4: Test for Waller-Duncun of U and P

Sex	Anatomical part	Symbol	Level	Waller group	Average	N
Male	Stew	U	2	A	53.0667	6
			3	B	45.9133	6
			1	C	20.3700	6
		P	2	A	41.7633	6
			1	A	40.8933	6
			3	B	36.6933	6
	steak	U	2	A	6.9667	6
			3	B	5.7900	6
			1	C	3.1867	6
		P	1	A	5.7567	6
			2	A	5.4200	6
			3	B	4.7667	6
Female	Stew	U	2	A	53.0667	6
			3	B	45.9133	6
			1	C	20.3700	6
		P	2	A	41.7633	6
			1	A	40.8933	6
			3	B	36.6933	6
	Steak	U	2	A	53.0667	6
			3	B	2.1433	6
			1	C	0.7167	6
		P	1	A	1.9967	6
			3	A	1.8533	6
			2	A	1.8500	6

Averages with the same letter are not significantly different.

that the average rejection rates for 6 observations can be classified into 2 groups (A and B). The same classification was obtained for the steak.

The rejection rate rose from 12% to values above 50% for stew from a voltage of 50 to 70 volts. The effects of slaughter on the quality of the carcass are of prime importance. Bruising, redskins, blood spots, blood splash, red wingtips, red pygostyles, and ruptured blood vessels in the breast muscles are all defects that can appear in carcasses and cause downgrading [11]. Gregory [9] outlined four theories to explain the cause of the blood capillary rupture that leads to blood splash: (1) it could be due to counteracting muscle contractions during stunning causing localized tearing of the capillary bed, (2) arteriolar dilatation can be one of contributing factors, (3) the blood vessels may be unduly fragile and (4) during intense generalized muscle body contractions, such as those during electrical stunning, the venous and arterial systems experience severe external pressure.

3.1.1.2. Steak

The coefficient of determination R^2, coefficient of variation CV, standard deviation σ, and average R, were 0.981075; 8.463074; 0.449765; 5.374444 respectively. It is noted that the model explains 99% (R^2=0.981075) the variability of rejection rates in the Pr<0.0001. It is therefore a relevant model. At the end of this model we will study the effect of each factor on rejection rates and the possible interactions between different factors. The standard deviation is small (σ=0.449765), therefore it is a good accuracy. Indeed σ shows that the number of trials (18) is sufficient to achieve accurate results.

The analysis of variance (the model) involving interactions between different factors is given in Table **3**. According to Table **3**, we find that the model depends primarily on U and interaction U*P. Indeed, the voltage and interaction between voltage and pulse have a highly significant (Pr<0.0001) at α=5%. So the model:

$$R = \mu + \alpha U + \beta P + \gamma (U*P) + \varepsilon$$

Can be written in the simpler form: $R = \mu + \alpha U + \gamma (U*P)$

The incidence of ventricular fibrillation at 75, 150 or 250 mA per bird was 26, 97 and 100% respectively for turkeys stunned electrically. Haemorrhaging in the breast muscle was the only variable affected by stunning current and was greatest when 250 mA was used. So, it is recommended that turkeys should be stunned with 150 mA per bird [12]. However, electrical waterbath stunning of ducks using 150 mA of 600 Hz alternating current (AC) is ineffective [13].

3.1.2. Female Turkey

<u>3.1.2.1. Stew</u>

The coefficient of determination R^2, coefficient of variation CV, standard deviation σ, and average R, were 0.998276; 2.127502; 0.846391; 39.78333 respectively. It is noted that the model explains 99% (R^2=0.998276) the variability of rejection rates in the Pr<0.0001. It is therefore a relevant model. At the end of this model we will study the effect of each factor on rejection rates and the possible interactions between different factors. The standard deviation is not small (σ=0.846391), therefore it is not a good accuracy. Indeed σ shows that the number of trials (18) is not sufficient to achieve accurate results.

The analysis of variance (the model) involving interactions between different factors is given in Table **3**. According to Table **3**, we find that the model depends primarily on U, P and interaction UP. Indeed, the voltage, the pulse and interaction between voltage and pulse have a highly significant (Pr<0.0001) at α=5%. So the model is:

$$R = \mu + \alpha U + \beta P + \gamma (U*P) + \varepsilon$$

The test of Waller-Duncan gave 3 groups (A, B and C) according to the voltage and 2 groups (A and B) for the pulse.

Based on the results obtained and in all cases an increase in voltage causes a significant increase in blood spots and hemorrhages in the stew and in the steak. The rupture of the blood vessels following the increase in venous pressure can be a significant cause. This is confirmed by previous studies [9]. Indeed, the rejection rate rose from 12% to values above 50% for stew passing from a voltage of 45-65 Volts for females. However, in electrical water-bath systems, birds may miss the stunner completely and some birds may still be alive. For these reasons, electrical water-bath systems are increasingly under scrutiny on nonhuman-animal welfare grounds. Controlled Atmosphere Killing (CAK), a promising alternative technology, uses gas mixtures to render birds unconscious [14].

Lines and colleagues [11] have demonstrated that priorities for selecting the parameters to be used for trout slaughter were that the system should meet the requirements for humane slaughter, and that it should not increase the incidence of hemorrhages. To achieve this, a low-voltage field, with high frequency and long exposure time was selected. A suitable set of parameters was found to be an electric field of 250 V/m r.m.s. using 1000 Hz sinusoidal voltage for a duration of 60 s.

<u>3.1.2.2. Steak</u>

The coefficient of determination R^2, coefficient of variation CV, standard deviation σ, and average R, were 0.933725; 18.34152; 0.348489; 1.900000 respectively. It is noted that the model explains 93% (R^2=0.933725) the variability of rejection rates in the Pr<0.0001. It is therefore a relevant model. At the end of this model we will study the effect of each factor on rejection rates and the possible interactions between different factors. The standard deviation is small (σ=0.348489), therefore it is a good accuracy. Indeed σ shows that the number of trials (18) is sufficient to achieve accurate results.

The analysis of variance (the model) involving interactions between different factors is given in Table **3**. According to Table **3**, we find that the model depends primarily on U only. Indeed, the voltage have a highly significant (Pr<0.0001) at α=5%. So the model:

$$R = \mu + \alpha U + \beta P + \gamma (U*P) + \varepsilon$$

Can be written in the simpler form: $R = \mu + \alpha U$

In this case, the test of Waller-Duncan has established 3 groups (A, B and C) for the voltage but for the pulse the average rejection rates for 6 observations can be classified in one group (A) because the differences between groups are not significant.

Wilkins and colleagues [16] have demonstrated that increasing waveform frequency was associated with a decrease in the prevalence of ventricular fibrillation, although turkeys appeared more susceptible than broilers. Use of the higher frequency waveforms (500 and 1500 Hz) was associated with a marked improvement in carcase quality, particularly with regard to breast muscle haemorrhaging and their use may result in considerable commercial advantage. The use of a high frequency waveform (1400 Hz) resulted in a faster bleedout and an improvement in carcase quality associated with a substantial reduction in haemorrhagic downgrading conditions [17]. Blood spots continue to be a problem in the poultry processing sector. High frequency has helped to reduce their importance. The main commercial advantage with high frequencies is fewer blood spots and other hemorrhages in the carcass, and this could be linked to the reduced muscle tension during stunning [9]. However, over-application of electrical stunning increases the risk of PSE meat.

3.2. Response Surfaces and Isoresponse Curves Analysis

Figures **2** and **3** represents the performance against various parameters that are decisive. The response surfaces and isoresponse curves are easier to interpret. The colors correspond to the ranges. The

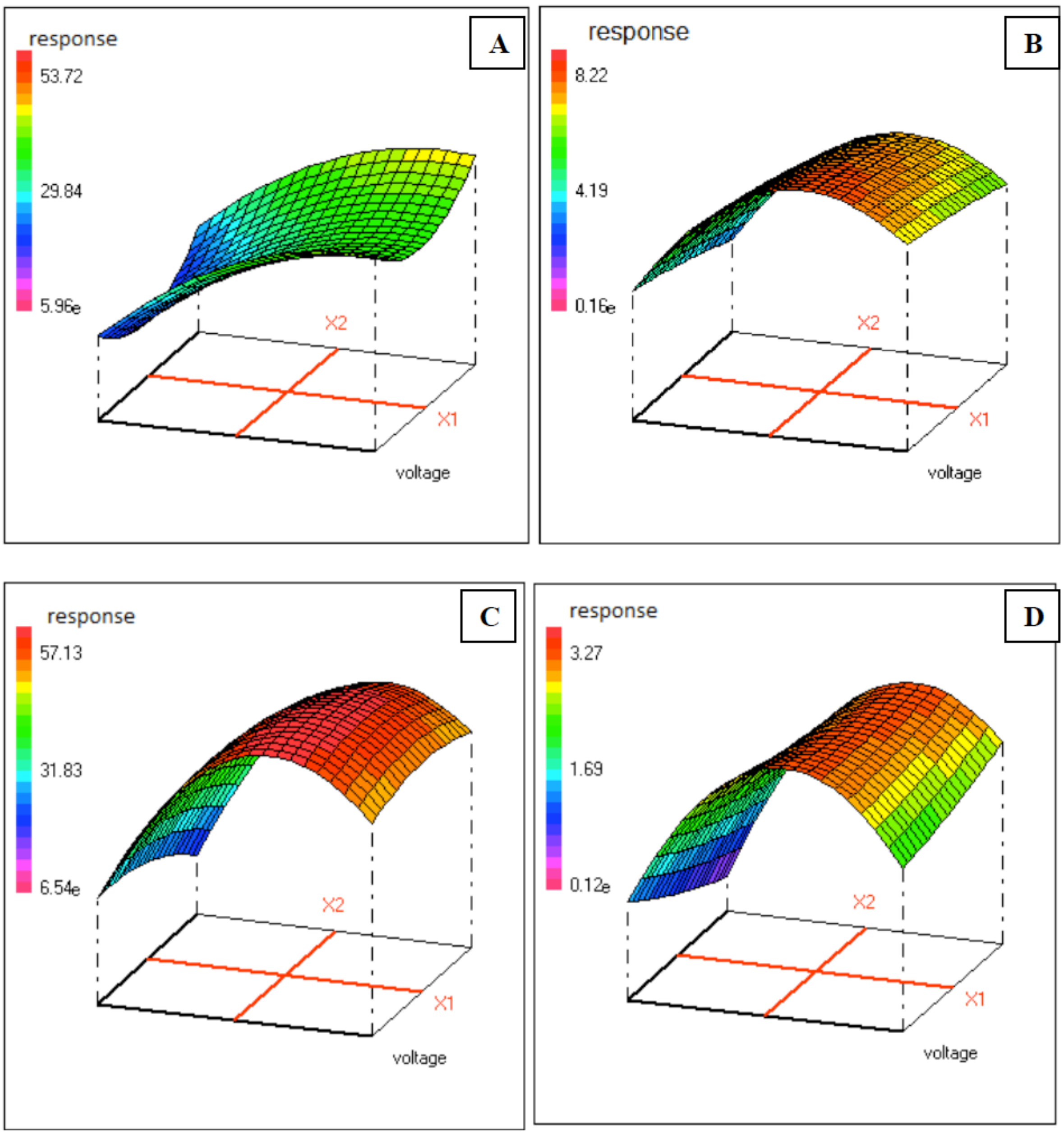

Figure 2: Response surfaces of rejection rates (**A**: stew male; **B**: steak male; **C**: stew female and **D**: steak female).

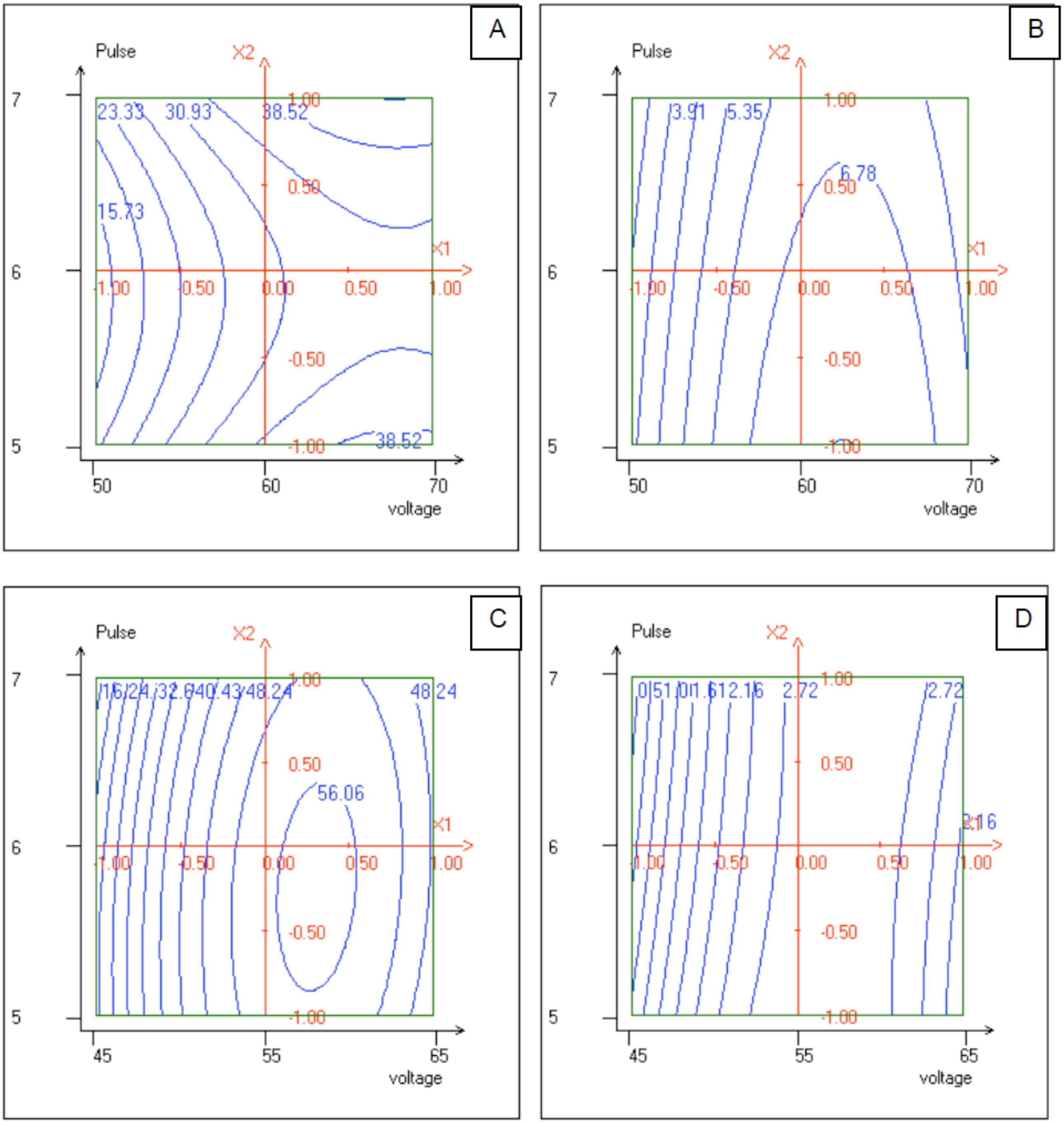

Figure 3: Isoresponse curves of rejection rates (**A**: stew male; **B**: steak male; **C**: stew female and **D**: steak female).

relationship between the factors of the electrical stunning and rejection rates can be established by examining the response surfaces generated by the voltage and the pulse. Surfaces confirm that voltage and pulse have a negative effect on rejection rates. The pulse has a negative effect on rejection rates but with a lower level than the voltage. Figure **2** shows that it is possible to obtain a low rejection rates (< 1% for the steak of male turkey) at a voltage of 50 Volts and pulse of 7. In comparison of pulse, voltage remains the most important factor. The optimum conditions for the rejection rates are: voltage: 50 volts; pulse: 7 for the male and voltage: 45 volts; pulse: 7 for the female. Under these experimental conditions, the rejection rates range from 0.23% to 0.80% and from 11.93% to 13.27% respectively for steak and stew. In this way it is possible to combine these parameters to achieve the desired objective.

Our results shows that voltage and therefore amperage applied should be different from the male (15.175 kg) to female (6.5 kg) because it depends on the electrical resistance of the body. So, ineffective stunning can be very painful and paralysis may occur without loss of consciousness [2].

4. CONCLUSION

We have applied response surface methodology for electrical stunning of turkey optimization. It was a useful tool to investigate the optimum operatory conditions of electrical stunning. It showed a good fit with the model of rejection rates. The rejection rates increased with decreasing voltage and increasing pulse. Voltage was the most significant factor affecting the rejection rates. The optimum conditions for rejection rates of stew and steak were as follows: voltage: 50 volts; pulse: 7 for the male and voltage: 45 volts; pulse: 7 for the female. The interactive effects between two factors were studied. The most important interactive effect between voltage and pulse was observed for stew and steak of male and stew of female turkey. However, the effect of each factor and their interactive effects on other anatomical parts of turkey and other birds can be studied and applied for a large scale.

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