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The Use of L-Carnitine and Oregano As Feed Additives in Alternative Forced Molting Programmes in Laying Hens[#]

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ABSTRACT

This research was carried out in order to determine the effects of forced molting programs induced by feeding the hens an alfalfa based and low Na-Ca induction feed as an alternative to forced molting program with feed withdrawal and the effect of L-carnitine and Oregano plant in powder form as feed additives on the induction period parameters. A total of 1170 birds were divided into 13 groups. The trial was continued for a total of 36 days, with the first 14 days of adaptation and 22 days of force molting. A negative control group (*K*) which was neither forced molted nor provided with any feed additive substances in their diets Moreover, other three treatment groups (*M*: Marbel powder, *Y*: Alfalfa, *F*: Low Ca-Na diet), were forced molted and one withhold feeding group was used for the study; the forced molted groups were supplied L-carnitine (*C*) (100 ppm) and dried oregano leaves (*O*) (5%) as a feed additive in designed individual diets (*MC, AC, FC, MO, AO, FO*) alone and in combination (50 ppm; 2.5%) in groups (*MCO, ACO, FCO*). In the light of this data it could be said that the live weight loss achieved with qualitative alfalfa meal feed restriction exceeds 30% and exceeds the live weight loss ensured by an 11 day feed withdrawal. Based on these parameters it is concluded that supplementing feed additives into alfalfa meal could be an alternative to the feed withdrawal method and alfalfa meal with a combination of L-carnitine and oregano plant has a positive impact on performance.
Keywords: Moulting, Laying Hen, L-Carnitine,Oregano, Egg Yield

Yumurtacı Tavuklarda Kekik (Oregano) ve L-karnitin'in Yem Katkısı Olarak Alternatif Zorlamalı Tüy Dökümü Programında Kullanılması

ÖZ

Bu araştırma, yem çekmeli zorlamalı tüy dökümü programına alternatif olabileceği düşünülen yonca unu esaslı ve düşük Na-Ca ihtiva eden zorlanım yemi kullanılarak yapılan zorlamalı tüy dökümü programlarının ve zorlanım periyodunda yem katkı maddesi olarak toz formda L-karnitin ve kekik bitkisinin, zorlanım dönemi parametreleri üzerine etkilerini belirlemek amacı ile gerçekleştirilmiştir. Deneme, zorlanım uygulanmayan ve yem katkı maddeleri kullanılmayan bir negatif kontrol grubu (*K*); biri yem çekmeli olmak üzere 3 adet zorlamalı tüy dökümü metodu uygulanan deneme grupları (*M*: Mermer tozu, *A*: Yonca, *F*: Düşük sodyum-kalsiyumlu yem), zorlanım uygulanan gruplara yem ilavesi olarak L-karnitin (*C*) (100 ppm) ve kurutulmuş kekik (*O*) (%5) tozunun belirlenen oranlarda ayrı ayrı (*MC, AC, FC, MO, AO, FO*) ve birlikte kullanıldığı (50 ppm; %2,5) gruplar (*MCO, ACO, FCO*,) olmak üzere, her grupta 6 tekerrür ve tekerrür gruplarında 15 adet hayvan olacak şekilde 13 grupta toplam 1170 tavuk üzerinde yürütülmüştür. Deneme ilk 14 gün alıştırma, 22 gün zorlanım dönemi olacak şekilde toplam 36 gün boyunca sürdürülmüştür. Araştırmada, yonca unu ile yapılan kalitatif yem kısıtlaması ile canlı ağırlık kaybının %30'u geçtiği ve 11 gün açlığın sağladığı canlı ağırlık kaybından daha fazla canlı ağırlık kaybına neden olduğu tesbit edilmiştir. Sonuç olarak, yonca ununun, yem çekme metoduna alternatif olabileceği, bilhassa yonca ununa L-karnitin ve kekik bitkisinin bir arada katılmasının performans üzerine olumlu etkiler gösterdiği kanısına varılmıştır.
Anahtar Kelimeler: Zorlamalı Tüy dökümü, Yumurta Tavuğu, L-Karnitin,Kekik, Yumurta Verimi

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INTRODUCTION

The increasing concern in the world for sustainable animal welfare has generated a need to develop alternative methods for the traditional forced moulting methods which are effective and economical. Moulting is a physiological event in poultry which incurs naturally before the days get shorter and the migration season. The procedure involving rapid moulting and the regeneration of feathers and subsequent stimulation of egg-laying within a program is known as "Forced Moulting" (Turkoglu et al. 1997). Forced moulting is a method used to prolong the productive life of broiler and laying hens or to interrupt egg laying temporarily when egg prices are very low. Various methods are used for this purpose, such as restrict feeding, water and light, providing feed with inadequate calcium or sodium, adding elements such as aluminium, zinc and iodine, feeding only grain and administering various drugs and hormones (Senkoylu 2001, Berry 2003, Webster 2003, Kakhki et al. 2018). It has been reported that instead of the traditional limitation of feed and water for laying hens, the application of alternative forced moulting methods without subjecting the hens to restricted feeding can provide better living conditions for the animals and facilitate the transition from the resting period to the production period (Koelkebeck and Anderson 2007). Minoura et al. (2005) reported that egg production is enhanced by the implementation of forced moulting by feed prepared with wheat bran or fat free rice bran. McReynolds et al. (2006) reported that the colonization of *Salmanella enteritidis* is restricted in forced moulting when clover is used together with layer feed. In a study in which forced methods were used as an alternative to the traditional methods, it was noted that the administeration of Vitamin E had a positive impact on the immune systems of layer hens (Gulhan et al, 2006). Increasing concerns for animal welfare emphasize the importance of other methods which provide better living conditions for poultry without keeping them on fasting. The alfalfa flour can be an alternative material for forced moulting like feed withdrawal method (Landers et al. 2005 ; Donalson et al. 2005; Kim et al.,2006; Landers et al. 2008). Kim et al. (2007) reported that the provision of alfalfa based rations during the moulting period produced effects which are similar to those generated by feed withdrawal and that the application of alfalfa flour may be beneficial to the sustenance of the mechanical properties of the bones. It has been reported that applications without feed withdrawal are less harmful in terms of bone mineral density and concentration compared to feed withdrawal (Mazzuco and Hester 2005). Szabo et al. (2005) reported that during a forced moulting applied for 12 days with classical feed withdrawal caused severe hepatic membrane degradation and increased lipid peroxizadion.

Carnitine (β-hydroxy γ-trimethylamine butyrate) is a vitamin-like substance that plays an important role in the anabolism and catabolism of lipids and is a cofactor involved in the passage of long chain fatty acids into the mitochondrial matrix (Zhai 2007). There are several studies which indicate that it increases egg production and their weight, decreases abdominal fat ratio and improves feed utilization in poultry (Ergün et al. 2004; Harmeyer 2002, Hrncar et al. 2015). It has also been used extensively in recent years as an alternative feed additive to support the immunity system (Rabie et al. 1997a). Studies have reported that the addition of L-Carnitine into balanced rations increases live body weight gain (Rabie et al. 1997a) and decreases abdominal fat ratio (Buyse et al. 2001; Xu et al. 2003) while other studies indicated that the addition of L-Carnitine has no impact on live weight, feed utilization (Buyse et al. 2001, Lien and Horng 2001) or abdominal fat percentage (Lien and Horng 2001).

Although there are numerous studies regarding the impact of Oregano leaves and its oil in particular in the strengthening of antibacterial, antioxidant properties, as an alternative growth factor, as an antiseptic, anticoccidial and immune system enhancer (Baratta et al.1998; Lambert et al. 2001; Si et al. 2008) but the number of studies dealing with the applicability of the oregano leaves powder and its products for forced moulting are limited.
Keeping in view the above facts, current study has been conducted to determine the impact of dried Oregano and L-Carnitine alone or in combination during the process of moulting when commercial laying hens fed alfalfa flour or a concentrated feed mix with low Na-Ca content as an alternative to the conventional method in terms of viability, stress, production parameters and metabolism.

MATERIAL and METHODS

1170 white laying hens of Lohmann LSL breed at the age of 72 weeks were used in this study and carried out in the poultry unit of Afyon Kocatepe University Livestock Research Center (KUHAM). The experimental protocols were approved by the Animal Care and Use Ethical Committee at Afyon Kocatepe University (147-2008). The concentrated feed mix used during the adaptation and egg laying periods and fed to the negative control group during the trial period in the study were formulated according to NRC (1994) (Table 1). The alfalfa and Oregano used in the study were the products of (İzmir Oregano; *Oreganum onites*) in powder form. L-Carnitine was used as a premix with 50.000 mg L-Carnitine HCL per one kilogram. The study

comprised of 13 groups, one was negative control group (*K*) and not subjected to forced moulting and received no additional additives; 3 treatment groups (Marbel:*M*, Alfalfa:*A*, Low Na-Ca: *F*) were subjected to forced moulting methods and one of which was subjected to feed withdrawal; the groups subjected to forced moulting were given L-carnitine (*C*) and dried oregano leaves powder (*O*) as a feed additive alone (*MC, AC, FC, MO, AO, FO*) and together (*MCO, ACO, FCO*,) with 6 replicates in each group and 15 animals in each replicate (Table 2). The birds were placed randomly into an apartment type cage system with 5 animals in each cage. During the forced moulting period the moulting groups were subjected to light restriction while the negative control group was kept under the same environmental conditions with no light restriction and ensured 16 hours of light in their cages within the unit. Water was provided *ad libitum* to all the groups during the trial. The first 2 weeks of the trial was used for adaptation and 22 days were used for the forced moulting period. The moulting groups were given 100 ppm of L-carnitine HCI and 5% dried oregano leaves powder separately and 50 ppm of L-carnitine HCl and 2.5% dried oregano leaves powder together. At the start of the study, all animals were immunized with an oil-adjuvanted inactivated vaccine against Newcastle Disease, Infectious Bronchitis and Egg Drop Syndrome Disease with a 0.5 cc intramuscular injection per animal. These vaccines were obtained from MSD-İntervet company.

Six animals from each subgroup were weighed on a digital scale at the start of the moulting period as well as 4 times during the trial and at the end of the period, in total 6 times to determine their live weights and the changes in live weight was calculated. Feed consumption was determined by weighing on the 11th and 22nd days of the moulting period. After the 11th day of the moulting period, the groups subjected to moulting with feed withdrawal, (*M* groups) were given 40 grams of barley per day, on the last day of the trial the remaining amount of barley in the group feeders were weighed to calculate barley consumption. Egg yield (including cracked, broken and abnormal eggs) was recorded daily during the moulting period. During the study, mortality was recorded on daily basis while moulting period viability and yield period viability were calculated as percentage.
Blood samples were collected from 6 hens in each group on the 11th and 22nd days of the moulting period. Malondialdehyde (MDA), Glutathione (GSH) and antioxidant activity (AOA) were determined for the collected samples according to the methods described by Draper et al. (1986), Beutler et al (1986) and Koracevic et al (2001) respectively. The antibody titers of Newcastle Disease were measured by Hemagglutination Inhibition (HI) test in the 2log, 8HA unit for the assessment of the impact of the humoral immunity induced by vaccination, moulting methods and the feed additives used and the results were recorded. At the end of the moulting period 6 animals from every group were euthanized by cervical dislocation and their weights were measured and recorded. The liver, spleen, full and empty stomach weights of the euthanized animals, digestive system were weighed with a digital scale and intestinal lengths were measured with the aid of a ruler. At the end of the moulting period, Ca and P analyzes were performed according to AOAC (1984) after removing the right tibia bones from the euthanized animals and removing the fat with ether extraction. The feed used in the trial was analyzed according to (AOAC 1984) (Table 2). Statistical analyzes of the collected data was performed using one-way analysis of variance in the J.M.PTM 5.0.1a (SAS Institute Inc., A Business Unit of SAS 1989-2002) package program. Tukey test was applied on the data to assess importance.

RESULTS

The amounts of consumed moulting feed by the trial groups (0-11. day;11-22. day) are indicated in Table 3. While all the groups had a homogenous distribution of live weight at the start of the trial, it is noted that the live weights of groups which were not subjected to moulting had not decreased at the end of the trial whereas live weight loss had incurred in the groups which were moulted (Table 4). Egg yield, cracked-broken eggs and abnormal egg percentage values throughout the moulting period (0-22. day) are shown in Table 5. When the serum ND titers measured with HI from the blood samples taken on day 11 and day 22 of the moulting period were assessed, it was noted that the antibody titers in the experimental groups were not different from the titers of the non-moulted hens (P> 0,05) (Table 6). The values for the organ weights and the live weights of the animals slaughtered at the end of the moulting period are shown in Table 7. When the results of MDA, GSH and AOA in the blood samples taken on the 11th and 22nd days of the moulting period were evaluated, it was discovered that there was no significant difference between the MDA and GSH values during both moulting periods and that in terms of AOA levels, the values had decreased in all moulting groups during both moulting periods (P<0,05) (Table 8). Significant differences were found between the percentages of Ca and P levels of the right tibia bone specimens extracted from poultry slaughtered on day 22 (P <0,05) (Table 9). At the end of the twenty-two days moulting period, differences between the mortality rates of the groups were observed and the mortality rates were

noted to be between 0% and 15.56% (P<0,05) (Table 10).

The nutrient contents of the alfalfa flour used in the experiment were 17.07% Crude Protein (CP), 19.79% Crude Cellusose (CS), 6.38% Crude Ash (CA), 2.09% Crude Fat (CF) and 89.44% Dry Matter (DM). The nutrient quantities of barley were 13.78% CP, 4.46% CS, 3.15% CA, 3.13% CF and 92.08% DM respectively.

Table 1. Composition of the experimental diets, kg/ton

Feedstuffs	Control group (*K*)&Production period diets	Low Na-Ca Diets (*F* groups)
Corn	371,81	381,33
Wheat	174,00	220,00
Sunflower meal % 36 CP	140,00	0,00
Sunflower meal %28 CP	0,00	150,00
Barley	0,00	30,00
Rasmol	30,00	90,00
Soybean meal %48 CP	34,00	94,00
Full fat soya	44,00	0,00
Canola meal	30,00	0,00
Corn bran	30,00	0,00
DDGS	30,00	0,00
Vegetable oil	10,00	0,00
DCP 18	6,74	7,50
Marbel Powder	89,00	20,00
Salt	3,50	0,00
L-Lysine HCl	1,10	1,15
DL-Methionine	0,70	0,92
Safizyme XP 20*	1,00	1,00
Karzyme P 500**	0,65	0,60
Kavimix 23 15/5***	2,50	2,50
Kavimix M 1****	1,00	1,00
Analysed values		
Crude protein%	16,47	16,88
Crude cellulose %	5,01	6,06
Crude ash %	12,54	6,93
Crude fat %	4,27	3,14
Dry Matter %	88,58	88,53
Calculated values		
ME kcal/kg	2700,00	2700,00
Methionine%	0,39	0,39
Lysine%	0,74	0,74
Ca%	*3,66*	*1,10*
Av. P%	0,35	0,37
Na%	*0,19*	*0,06*
Cl%	*0,24*	*0,04*
Linoleic acid%	2,23	1,29

* Endo-1,4-beta-xylanase, Kartal Kimya, Turkey: 1.400.000 U

** Fitaz, Kartal Kimya, Turkey: 500.000 FTU/g

***Vitamin Premiks, Kartal Kimya, Turkey: Each 2,5 kg premix contains: Vitamin A: 12.000.000 IU, Vitamin D_3: 2.400.000 IU, Vitamin E: 30.000 mg, Vitamin K_3: 2.500 mg, Vitamin B_1: 3.000 mg, Vitamin B_2: 7.000 mg, Vitamin B_6: 4.000 mg, Vitamin B_{12} :15 mg, Niasin: 40.000 mg, Ca-D-Pantothenate: 8.000 mg, Folic Acid: 1.000 mg, D-Biotine: 45 mg, Vitamin C :50.000 mg, Choline Cl. : 125.000 mg, Canthaxantin: 1.500 mg, Apo-Carotenoic acid ester: 500 mg

****Mineral Premiks, Kartal Kimya, Turkey: Each 1 kg premix contains: Manganese: 80.000 mg, Iron: 40.000 mg, Zinc: 60.000 mg, Cupper: 5000 mg, Iodine: 400 mg, Cobalt: 100 mg Selenium: 150 mg, include.

Table 2. Experimental Groups

Groups		Molting Method		Feed Additives		Method&Water	
Group	Group	0-11 day	11-22 day	L-Carnitin HCl	Oregano	Method	Water
01	*K*	Negatif Control		No	No	No	Ad-libitum
02	*M*	Marbel powder	Limited Barley	No	No	withdrawal	Ad-libitum
03	*MC*	Marbel powder	Limited Barley	100 ppm	No	withdrawal	Ad-libitum
04	*MO*	Marbel powder	Limited Barley	No	%5	withdrawal	Ad-libitum
05	*MCO*	Marbel powder	Limited Barley	50 ppm	%2,5	withdrawal	Ad-libitum
06	*A*	Alfalfa powder	Alfalfa powder	No	No	No withdrawal	Ad-libitum
07	*AC*	Alfalfa powder	Alfalfa powder	100 ppm	No	No withdrawal	Ad-libitum
08	*AO*	Alfalfa powder	Alfalfa powder	No	%5	No withdrawal	Ad-libitum
09	*ACO*	Alfalfa powder	Alfalfa powder	50 ppm	%2,5	No withdrawal	Ad-libitum
10	*F*	Low Na, Ca	Low Na, Ca Feed	No	No	No withdrawal	Ad-libitum
11	*FC*	Low Na, Ca	Low Na, Ca Feed	100 ppm	No	No withdrawal	Ad-libitum
12	*FO*	Low Na, Ca	Low Na, Ca Feed	No	%5	No withdrawal	Ad-libitum
13	*FCO*	Low Na, Ca	Low Na, Ca Feed	50 ppm	%2,5	No withdrawal	Ad-libitum

Table 3. Feed Consumption of moult period (0-11 and 11-22. days)

		0-11. days		11-22.days	
Group	N	Mean	SEM	Mean	SEM
K	6	131,98[a]	1,29	105,71[a]	3,97
M	6	21,03[c]	0,99	37,97[d]	0,89
MC	6	26,85[c]	3,63	37,98[d]	1,52
MO	6	23,98[c]	1,76	33,99[de]	1,55
MCO	6	23,60[c]	1,47	35,06[d]	0,91
A	6	27,00[c]	3,06	29,03[de]	2,09
AC	6	23,27[c]	3,29	23,00[e]	3,31
AO	6	22,60[c]	2,49	22,51[e]	1,85
ACO	6	30,47[c]	3,94	27,55[de]	2,35
F	6	107,67[b]	4,69	64,15[c]	3,81
FC	6	108,32[b]	2,91	69,61[bc]	2,72
FO	6	104,08[b]	4,12	79,21[b]	1,85
FCO	6	108,49[b]	4,19	77,59[b]	1,57
P			*0,000*		

a,b,c: Means with different superscripts in each row are significantly different

Table 4. Body weight changes (g,%)

Group	N	Day 1	Day 11	Day 22	0-11. % .	0-22. %
K	36	1532,50	1663,06[a]	1622,78[a]	+8,52	+5,89
M	36	1547,92	1180,69[c]	1282,64[ef]	-23,44	- 17,14
MC	36	1488,47	1120,69[c]	1259,44[ef]	-24,71	-15,38
MO	36	1544,72	1194,31[c]	1271,00[ef]	-22.68	-14,61
MCO	36	1533,19	1162,50[c]	1214,72[f]	-24,17	-20,77
A	36	1540,28	1200,14[c]	1020,64[g]	-22,08	-33,74
AC	36	1499,44	1180,28[c]	1027,50[g]	-21,29	-31,47
AO	36	1495,97	1193,75[c]	1007,08[g]	-20,20	-32,68
ACO	36	1487,92	1217,78[c]	1024,58[g]	-18,16	-31,14
F	36	1506,39	1440,69[b]	1345,00[cde]	-4,36	-10,71
FC	36	1557,50	1481,39[b]	1397,36[bcd]	-4,90	-10,29
FO	36	1523,89	1480,97[b]	1513,75[ab]	-2,81	-0,66
FCO	36	1517,78	1481,81[b]	1434,86[bc]	-2.37	-5,46
P		*0,487*	*0,000*	*0,000*		

a,b,c,d,e,f,g: Means with different superscripts in each row are significantly different.
K:Control, M: Marbel powder, MC: Marbel powder +Carnitine, MO: Marbel powder +Oregano, MCO: Marbel powder +Carnitine+Oregano A:Alfalfa, AC: Alfalfa+Carnitine, AO: Alfalfa+Oregano, ACO: Alfalfa+Oregano+ Carnitine F:Low Na-Ca, FC: Low Na-Ca+ Carnitine, FO: Low Na-Ca+Oregano, FCO: Low Na-Ca+ Oregano+ Carnitine

Table 5: Egg production of moulting period (0-11 and 11-22.day)

	0-11.day			11-22.day		
Group	Egg production %	Cracked and broken egg %	Abnormal egg %	Egg production %	Cracked and broken egg %	Abnormal egg %
K	64,44[a]	21,32[a]	4,75	65,98[a]	19,62[a]	0,92[a]
M	12,53[c]	6,77[b]	3,13	0,00[d]	0,00[c]	0,00[b]
MC	12,73[c]	7,07[b]	3,23	0,00[d]	0,00[c]	0,00[b]
MO	12,83[c]	7,47[b]	3,54	0,00[d]	0,00[c]	0,00[b]
MCO	10,93[c]	6,17[b]	2,94	0,00[d]	0,00[c]	0,00[b]
A	9,29[c]	5,96[b]	2,93	0,00[d]	0,00[c]	0,00[b]
AC	8,49[c]	6,07[b]	2,53	0,00[d]	0,00[c]	0,00[b]
AO	10,50[c]	6,77[b]	3,13	0,00[d]	0,00[c]	0,00[b]
ACO	9,19[c]	6,57[b]	2,62	0,00[d]	0,00[c]	0,00[b]
F	49,73[b]	22,71[a]	2,32	34,06[bc]	16,42[ab]	0,83[a]
FC	47,48[b]	21,54[a]	2,83	32,81[c]	16,13[b]	0,61[ab]
FO	42,83[b]	18,38[a]	2,43	37,68[b]	16,97[ab]	0,51[ab]
FCO	44,86[b]	18,64[a]	3,14	34,08[bc]	14,99[b]	0,30[ab]
P	*0,000*	*0,000*	*0.980*	*0,000*	*0,000*	*0,000*

a,b,c: Means with different superscripts in each row are significantly different

Table 6. Immune response after vaccination – ND titre values (HI; 2Log)

		11.day		22.day	
Group	N	Titre	SEM	Titre	SEM
K	6	14,67	0,42	14,00	0,45
M	6	15,33	0,42	13,67	0,21
MC	6	16,00	0,32	14,40	0,25
MO	6	15,57	0,20	14,33	0,42
MCO	6	15,33	0,49	14,33	0,62
A	6	15,83	0,40	14,60	0,51
AC	6	14,66	0,84	13,83	0,54
AO	6	14,57	0,43	14,00	0,52
ACO	6	13,80	0,58	13,67	0,67
F	6	14,50	0,67	13,67	0,42
FC	6	14,66	0,56	13,17	0,40
FO	6	14,00	0,73	14,17	0,40
FCO	6	15,00	0,26	13,17	0,48
P		*0,117*		*0,562*	

Table 7. End of moulting period (22. day) some organ parameters (% Body weight)

		Live weight (g)		**% Liver weight**		**%Spleen weight**		**%Digestive sys.weight**		**%Full of stomach**		**%Empty stomach**		**%intestine lenght**	
Group	**N**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
K	6	1595,00^{a}	87,54	2,35^{ab}	0,12	0,12	0,01	9,19	0,11	2,94^{d}	0,14	2,46^{d}	0,11	11,10^{cd}	0,26
M	6	1320,00^{abc}	31,09	2,01^{ab}	0,10	0,14	0,01	11,19	0,68	4,71^{abc}	0,18	3,49^{abc}	0,10	11,72^{abcd}	0,70
MC	6	1165,00^{cde}	55,38	2,06^{ab}	0,12	0,15	0,01	12,92	0,42	4,95^{ab}	0,11	3,65^{a}	0,04	13,42^{abcd}	0,50
MO	6	1231,67^{bcd}	53,85	2,10^{ab}	0,06	0,15	0,01	12,63	0,62	5,11^{ab}	0,15	3,78^{a}	0,11	12,39^{abcd}	0,71
MCO	6	1251,67^{bcd}	104,76	2,06^{ab}	0,11	0,13	0,01	11,50	1,30	4,21^{abcd}	0,43	3,18^{abcd}	0,26	11,78^{abcd}	0,61
A	6	968,33^{de}	84,59	2,13^{ab}	0,12	0,27	0,15	12,71	1,74	3,65^{abcd}	0,78	3,55^{ab}	0,12	15,43^{a}	1,83
AC	6	944,17^{de}	63,05	2,17^{ab}	0,18	0,12	0,01	11,77	1,26	4,39^{abcd}	0,25	3,63^{a}	0,12	15,25^{ab}	1,03
AO	6	902,50^{e}	91,54	1,79^{b}	0,14	0,10	0,01	11,06	0,92	4,32^{abcd}	0,21	3,65^{a}	0,16	14,86^{abc}	1,09
ACO	6	970,32^{de}	49,90	2,30^{ab}	0,16	0,10	0,01	13,68	1,45	4,58^{abc}	0,30	3,69^{a}	0,15	14,84^{abc}	0,93
F	6	1319,17^{abc}	35,60	2,78^{a}	0,15	0,12	0,01	10,95	0,65	3,66^{abcd}	0,15	2,70^{bcd}	0,11	12,62^{abcd}	0,77
FC	6	1426,67^{abc}	43,62	2,46^{ab}	0,13	0,12	0,01	9,22	1,21	3,48^{bcd}	0,26	2,35^{d}	0,48	11,17^{bcd}	0,490
FO	6	1590,83^{a}	57,31	2,71^{ab}	0,08	0,12	0,01	11,81	0,46	3,43^{cd}	0,21	2,61^{cd}	0,14	10,88^{cd}	0,47
FCO	6	1516,67^{ab}	60,13	2,64^{ab}	0,09	0,13	0,01	10,29	0,33	3,25^{cd}	0,05	2,52^{d}	0,06	10,55^{d}	0,54
P		*0,000*		*0,012*		*0,387*		*0,487*		*0,000*		*0,000*		*0,000*	

a,b,c,d,e:Means with different superscripts in each row are significantly different

Table 8. Oxidant- antioxidant parameters

11.Day	MDA (nmol/l)			GSH (µmol/l)		AoA(mmol/lt)	
Group	N	Mean	SEM	Mean	SEM	Mean	SEM
K	6	16,25	2,42	13,55	1,23	28,78[a]	6,50
M	6	17,59	1,92	17,09	1,75	7,15[b]	2,29
MC	6	18,64	1,85	17,73	1,80	4,87[b]	1,90
MO	6	17,26	1,33	15,63	1,28	8,47[b]	3,41
MCO	6	18,37	1,70	14,88	1,41	7,70[b]	2,61
A	6	18,35	1,75	14,37	1,42	5,15[b]	1,00
AC	6	17,18	1,51	12,45	1,04	7,16[b]	1,39
AO	6	19,03	2,35	15,03	2,06	7,92[b]	3,49
ACO	6	16,93	1,77	15,60	1,90	9,29[ab]	5,41
F	6	16,62	1,60	12,97	1,41	10,50[ab]	0,17
FC	6	15,88	1,73	16,61	1,94	23,25[ab]	5,48
FO	6	12,51	1,59	14,70	1,22	17,41[ab]	5,96
FCO	6	15,53	1,44	15,10	1,75	12,97[ab]	3,98
P		*0,547*		*0,555*		*0,000*	
22.Day	MDA (nmol/l)			GSH (µmol/l)		AoA(mmol/lt)	
Group	N	Mean	SEM	Mean	SEM	Mean	SEM
K	6	14,72	0,36	17,31	0,64	27,48[a]	5,37
M	6	14,23	0,76	20,70	1,95	9,11[b]	1,51
MC	6	15,04	1,39	19,98	1,04	6,79[b]	1,25
MO	6	15,52	1,35	23,23	1,37	8,75[b]	2,49
MCO	6	14,17	1,50	20,40	2,01	5,91[b]	0,69
A	6	16,29	3,35	23,79	1,97	8,96[b]	2,97
AC	6	15,62	2,33	24,79	2,03	5,57[b]	0,48
AO	6	15,14	0,97	23,20	1,60	7,29[b]	1,18
YCO	6	14,68	1,32	22,80	1,56	6,51[b]	1,25
F	6	15,44	1,01	22,79	2,12	9,77[b]	1,79
FC	6	15,14	0,87	20,88	1,27	11,49[b]	3,98
FO	6	12,96	0,87	21,47	2,56	11,46[b]	4,96
FCO	6	13,20	0,69	21,58	1,66	14,68[ab]	4,41
P		*0,956*		*0,268*		*0,000*	

a,b: Means with different superscripts in each row are significantly different.

Table 9. Ca- P Levels of Tibial Ash

22.Day		Ca %		P %	
Group	N	Mean	SEM	Mean	SEM
K	6	15,95[ab]	0,10	8,76[a]	0,11
M	6	14,18[abc]	0,08	6,54[cd]	0,15
MC	6	14,21[abc]	0,14	8,01[abc]	0,04
MO	6	15,91[ab]	0,15	6,62[cd]	0,22
MCO	6	13,27[bcd]	0,24	8,17[ab]	0,00
A	6	14,30[abc]	0,70	8,39[a]	0,65
AC	6	11,21[d]	0,27	8,24[ab]	0,27
AO	6	12,13[cd]	0,48	8,15[ab]	0,08
ACO	6	12,91[cd]	0,71	8,81[a]	0,12
F	6	16,47[a]	0,24	6,09[d]	0,11
FC	6	14,91[abc]	0,86	6,90[bcd]	0,39
FO	6	14,73[abc]	0,33	7,50[abcd]	0,22
FCO	6	13,72[abcd]	1,34	7,70[abc]	0,49
P		*0,000*		*0,000*	

a,b,c,d: Means with different superscripts in each row are significantly different.

Table 10. Mortality rate of groups (%)

	(0-22 day)		
Group	N	Mean	SEM
K	6	2,22^{bc}	1,41
M	6	14,39^{ab}	3,62
MC	6	12,22^{abc}	2,05
MO	6	8,89^{abc}	2,81
MCO	6	10,00^{abc}	5,64
A	6	10,00^{abc}	1,49
AC	6	12,22^{abc}	1,11
AO	6	12,22^{abc}	2,05
ACO	6	15,56^{a}	4,44
F	6	3,34^{abc}	1,49
FC	6	1,11^{c}	1,11
FO	6	0,00^{c}	0,00
FCO	6	2,22^{bc}	1,41
P		*0,000*	

a,b,c,: Means with different superscripts in each row are significantly different.

DISCUSSION

Although literature content is available regarding the assessment of hybrid, age, environmental factors in forced moulting as well as yield parameters for alternative forced moulting methods and their impact on intestinal morphology and microbiology, studies dealing with feed additives in powder form supplemented in feed during moulting are practically non-existant. In a study in which feed containing 100%, 90% and 70% of alfalfa flour was used for nine days as moulting material (McReynolds et al.,2006) it was reported that the level of alfalfa flour consumption remained at a rather low level during the moulting period and in conclusion the poultry had consumed a limited amout of alfalfa flour. In a study carried out by Aygun and Yetisir (2014) was reported that moulting feed consumption by groups for which feed withdrawal was not applied was between 63.57-72.83 g. In the presented study it is evident that the feed consumption of the alfalfa flour groups is less than this value whereas the consumption of *F* groups is similar to the data of the researchers. It is reported that the targeted live weight loss of extensively used forced moulting methods with feed withdrawal is 25-30% (Bell 1987, Brake 1992, Brake 1993). Ruszler (1998) asserts that a successful moulting program must incur a live weight loss in the range of at least between 15-40%. The live weight loss of the groups with feed withdrawal in this study ensured during the 0-11 day moulting was recorded in the range of 22.68 - 24.71% while the values recorded for the groups with feed withdrawal and alfalfa groups were in the range of 14.61% - 33%. The acquired data indicates that the live weight loss recommended by the researchers has been realized in these groups. The values found in the groups which had been given moulting feed with a low Na-Ca content, namely 10.71%; 10.29%; 0.66% and 5.46% were less than the values suggested by the researchers. Bell and Kuney (1992) reported a live weight loss of 24.5%-28.8% with poultry which had been treated with feed withdrawal moulting. These values are similar to the values calculated for the *M* groups on the 11th day. It has been reported that providing water and organic acid to animals undergoing the feed withdrawal method did not effect live weight loss and that during 9 days of

moulting a live weight loss of 25%-26% had been achieved in the groups (Kubena et al. 2005). In a study carried out by Kucukyilmaz et al. (2003) with white laying hens a live weight loss of 23.21% was reported at the end of 8 days of fasting, the percentage was 26.43% after 12 days of fasting and 32.66% at the end of 16 days of fasting. The live weight losses in our feed withdrawal groups in the level of 22.68%- 24.71% are commensurate with the 11 day fasting period values. These values are also similar to the research results of poultry moulted by starving (Mc Cormick and Cunningham 1984, Mc Cormick and Cunningham 1987, Hurwits et al. 1998). An assessment of the live weight losses for 0-22 days of the study reveal that the values for groups *M* and *A* were higher than the values of 8.20%-15.00 reported by Biggs et al (2003) for moulting groups based on maize and wheat bran, they were similar to groups *F* and *FC* and higher than the figures for groups *FO* and *FCO*. It can be said that the differences between the results are due to the different feeds used in the moulting and the duration of the application. During the 0-11 day period of the moulting in the present study, live weight losses in the alfalfa flour groups were found to be in the range of 18.16% - 22.08%. In a study in which alfalfa flour was used in the form of pellets and powder as moulting material (Landers et al. 2005b), a live weight loss on the level of 15.2%- 23.3% was reported. The difference is thought to be attributable to the particle size of the alfalfa flour, the duration of the moulting and environmental conditions. In a study carried out by Kim et al. (2007), moulting feed containing alfalfa at different rates was used in a feed withdrawal method and live weight losses of 28.2% was reported for the feed withdrawal group while the other groups achieved a live weight loss of between 18.8% - 25.4%. These results are similar to the values obtained on the 11th day for groups *M* and *Y* in our study. At the end of the twenty-second day it is concluded that our values for groups *Y* were higher than the study data of the aforementioned values while the values of groups *F* were less. It can be constructeded that the difference manifested at the end of the moulting on the 22nd day is due to the duration of the moulting, moulting feed consumption and the difference in the raw nutrient substance composition of the moulting feed in terms of groups F. The presented study results are commensurate with the study findings in a moulting study carried out using maize without salt, P and added vitamins (Bell, 2005) in which rather low live weight losses compared to the losses incurred by classical feed withdrawal method were reported. Although the live weight losses incurring on the 22nd day in groups *F* and *FC* and the values indicated for the 3rd and 4th weekends of the same study are similar, the values recorded for groups supplemented with thyme display a difference. Based on these data, it is considered that the negative effect of a salt-free diet on live weight is eliminated with the addition of Oregano.

When the percentage of liver weight in the study is evaluated, it is evident that the percentage of liver weight in group *AO* is significantly lower. This result is consistent with the results reported by Donalson et al. (2005) that the percentage of liver weight reported in groups consuming alfalfa flour was significantly lower than the percentage of liver weight noted in the control group in studies in which alfalfa was used as a moulting material on various levels. The findings of the researchers that a difference is not formed between the groups in terms of the percentage of spleen weight are similar to those of the researchers.

An examination of the egg yield during the moulting period of the study revealed that egg yields were similar between the alfalfa groups and the feed withdrawal groups and significantly lower than the figures for groups *F*. The results for groups *F* are consistent with the yields for groups without feed withdrawal reported by Aygun and Yetisir (2014) while the results for groups *A* were different. It is believed that the diffeent egg yield values manifested in the trial are due to the variety of the moulting feed and difference between consumption. Szabo et al. (2005) reported that relative liver weight decreased strikingly at the end of 12 days of fasting while tissue MDA levels increased significantly due to membrane lipid degradation. Sandhu et al. (2007) carried out a study which assessed the immunological impact of feed withdrawal moulting and Zn induced moulting programs and reported that Zn supplementation enhances the humoral immune response. In another study conducted by Alodan and Mashaly (1999), in which the immunological effects of moulting were evaluated, it was concluded that using alternative methods and feed additives did not affect the immunological response induced by vaccination and that similar titers were observed in non-moulted animals.

It has been concluded that alternative moulting applications have a lesser negative impact on bone mineral concentration and density compared to starving methods (Mazzucco and Hester 2005). Ca and P percentages for the tibia have been assessed in the present study and it is evident that the moulting method and feed supplements used have had an impact on the amount of these minerals. When a comparison of the bone Ca amounts of the feed withdrawal groups and the alfalfa flour groups during the moulting period, in view of the findings of the trial, it is evident that the deposits in group *AC* are lower than the amounts of the feed withdrawal groups and groups *A,AO* and *ACO*. In view of these data it can be asserted that the addition of L-Carnitine alone to alfalfa flour groups

had a decreasing impact on Ca deposits. Furthermore, bone Ca deposits were found to be similar in the groups with feed withdrawal and low Na-Ca ration consuming groups. In terms of phosphorus deposition, it was concluded that the value measured in the L-Carnitine supplemented group (*MC*) is similar to the alfalfa flour groups and control group. The addition of Oregano alone seems to have significantly reduced the P deposition in the feed withdrawal group (*MO*) and in the feed withdrawal group without feed supplementation (*M*). Hence, it can be concluded that the addition of L-Carnitine in group *M* has increased the P deposit.

At the end of the 22-day moulting period, differences between mortality rates of the groups were observed, mortality rates were between 0% and 15.56%. When these rates are compared with the mortality rates reported by Biggs et al. (2004) for programs without feed withdrawal (0-2.4%) it is evident that this value range is commensurate with the results for groups *FC, FO* and *FCO* and considerably less than the results for group *F* and the alfalfa flour groups. When the results are compared with the mortality values reported by Petek (2001) for groups without feed withdrawal it is evident that the results for groups *FC, FO* and *FCO* are low whereas the values for group *F* and the alfalfa flour groups are higher than this value. The results of the study are not commensurate with those of Aygun and Yetisir (2014), which indicated that there was no difference between the mortality rates of the groups without feed withdrawal. This result suggests that the group feed consumptions during the moulting period could have been insufficient. Kucukyilmaz et al. (2003) reported that the viability of white laying hens which had been subjected to different fasting periods was 90.68% in group *K* during the yield period and between 92.29% and 94.73% in the experimental groups. These values are less than the values reached in the study. This may be due to the fact that the poultry used in the study have a lower yield age.

The study concluded that the feed consumption of *M* groups in the study were similar to group *K* and differed from the report that manifested increased feed consumption of white layer hens which had been moulted by the application of different periods of fasting (Kucukyilmaz et al. 2003). Molino et al. (2009) carried out a study comparing the efficiencies of feed withdrawal programs and feed restriction programs and concluded that the group averages were similar in terms of feed consumption.

CONCLUSION

In the light of this data, it can be said that qualitative feed restriction with alfalfa flour causes more than 30% of live weight loss and causes more weight loss than the live weight loss obatined with the 11-day conventional feed withdrawal method. It is safe to say that because of ongoing egg-laying, a 22 day moulting period is not adequate when feed with low Na-Ca is used as a moulting material. In this application, it can be said that the addition of Oregano inhibits the loss of live weight and it may be more beneficial not to use it. A review of the viability during the moulting period indicates that feed supplements had a positive impact on the viability of *M* groups and that the application of alfalfa flour and feed withdrawal method had a similar mortality. The forced moulting methods used in the trial did not have an impact on the immune response induced with vaccination against Newcastle disease. As a result it is concluded that using alfalfa flour supplemented with various additives as an alternative to the feed withdrawal method and that adding L-Carnitine and Oregano together into alfalfa flour has a positive impact on performance in terms of animal welfare.

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