

Growth Promoting Effects of Dried Nettle
Extracts and its Impact on Hematology and
Antibody Titer in Broiler ChickensSeyed Mohammad Hashemi^{1*}, Ali Soleimanifar¹, Seyed Davood Sharifi² and
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Abstract

The aim of this research is to find out the influence of Nettle Leaves Extract (NLE) powder on the growth performance and immune response in broiler chickens. A total of 400 day old chicks (ROOS-308) were used. Treatments included basal diet (control), control+0.15% NLE, control+0.20% NLE, control+0.25% NLE and control+0.6% flavomycin (positive control) replicated 4 times each. Body weight gain, feed intake and feed conversion ratio were recorded weekly. Blood samples were collected from 4 birds in each treatment at the age of 36 day (a week after last Newcastle vaccination) for antibody determination and hematological parameters and white blood cell differential count. Cutaneous Basophil Hypersensitivity (CBH) response was used to measure the cell mediated immunity at the age of 28 days. Results showed that feed conversion ratio were significantly better in NLE treated diets in any of rearing periods and less feed intake particularly in younger age (1-14 d). No positive immune response was seen by NLE levels except the basophil ratio increased by NLE diets. Red blood cells concentration increased significantly by NLE fortification as compared with control. Different levels of NLE caused significantly different hemoglobin and hematocrit values. Finally it is concluded that NLE has a strong growth promoting ability and has potential to impact on the hemoglobin and hematocrit.

Introduction

Historically, bioactive compounds extracted from medicinal plants are used for health benefits in human and animal life. Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardiac glycoside are some chemicals from medicinal plants which have health benefits [1]. These phytochemicals hold disease-preventing properties and may be considered as antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents [2].

In old civilizations like India, China and Iran, there is a wide range of medicinal plant usage. Nowadays, many countries tend to use plants to treat human or animal disorders because herbal remedies has no or very low side effects and low expenses. In poultry nutrition herbs are needed mostly as antibiotic replacements [3,4], growth promoter [5], feed additives [6,7] and immunity booster [8].

Urticadioica, often called as nettle is a perennial plant with medicinal characteristics. It is reported that nettle can enhance immune system in the fish [9], defending endo- and ectoparasites of rabbit and poultry [10] and influencing the blood lipid profile [11]. Nettle leaves contain carotenoids, essential fatty acids, vitamins, minerals, phytosterols, glycosides and proteins, with most promising application in the feed, therapeutic and cosmetic industries [12]. In broiler diet the substitution of nettle leaves for soybean meal up to 9% is suggested as a feeding strategy [13]. Even though, the nettle leaves can be considered for anemia treatment because of high iron content and is good source of calcium and vitamin A [14]. By the way the antibacterial property of nettle extracts has been demonstrated [15]. However the chemicals used for nettle extraction is very effective on the final product's antibacterial property [16].

It is reported that nettle leaves improved broiler body weight gain and feed conversion ratio at the age of 21-42 [17]. In the meantime, it is reported that nettle leaves could not improve oxidative index in the body of broiler [18]. This information implies that nettle can be a good supplement for growth promotion in poultry nutrition. Dried Nettle Leaves Extracts (NLE) is a product of nettle. While it is reported that NLE can be used against fungi [19], the effects of NLE on microbes need further investigation [20]. Nowadays many countries banned using antibiotics in poultry diets as growth promoter because of microbial resistance made by antibiotics. The extraction method is very effective on the quality of NLE [21].

Table 1: Basal diets composition and analysis.

Ingredients %	Starter	Growth	Finisher
corn	55.06	60.86	64.56
wheat	5	5	5
soybean meal	34.5	29	25.5
soy oil	1.41	1.31	1.26
MCP	1.25	1.1	1
calcium carbonate	1.6	1.5	1.4
Min. permix&Vit	0.5	0.5	0.5
sodium bicarbonate	0.1	0.15	0.15
salt	0.2	0.15	0.15
Met	0.19	0.21	0.24
Lys	0.11	0.14	0.16
Tre	0.08	0.08	0.08
Calculated analysis			
Metabolisable energy (Kcal/Kg %)	2970	3050	3070
Protein	21.2	19.3	18
Ca	1	0.94	0.9
avil. P	0.49	0.43	0.41
Na	0.15	0.14	0.14
Cl	0.16	0.15	0.15
Arg	1.12	1.03	0.96
Lys	1.1	1	0.9
Met	0.49	0.44	0.39
Met+ Cys	0.49	0.44	0.69
Tre	0.8	0.75	0.69
Val	0.74	0.68	0.63
Try	0.87	0.17	0.16
Iso	0.18	0.7	0.64
Lus	0.74	1.06	0.96

While there is a bunch of report on the using nettle leaves in the broiler chickens nutrition, there is very low information about the NLE effects on broiler growth. The aim of the current research is to investigate the effects of NLE as a growth promoter on broiler as well as their immunity responses. This research is carried out based on animal ethics regulation of Agricultural Research, Education and Extension Organization (AREO), Iran.

Material and Method

Experimental birds and diets

Table 2: Nettle leave extract characteristics.

Character	Accepted level	Results
color	brown	brown
smell	nettlesmell	nettlesmell
dry matter %	90	95.43
clorogenic acid%	at least 0.35	3.06

Table 3: The effects of Nettle Leave Extract (NLE) on chicks performance at the age of 1-14 d.

Treatments	Feed intake	Weight gain	Feed conversion
	g/c/d ¹	g/c/d	
control	51.5 ^a	30.6 ^b	1.62 ^a
0.15% NLE	39.6 ^d	34.3 ^a	1.21 ^c
0.20 NLE	42.5 ^c	35.4 ^a	1.25 ^c
0.25% NLE	42.3 ^c	35.4 ^a	1.26 ^c
Flavomycin	47.9 ^b	34.7 ^a	1.42 ^b
SE	0.76	0.82	0.02
P-value	0.001	0.005	0.001

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P < 0.05$)
¹gram/chick/day=g/c/d.

A total of 400 day old chicks (ROOS-308) were used and every 10 chicks were allocated to an experimental field. Mass chicks weight in each fields were the same to avoid differences at start. Chicks had ad-libitum access to feed and water. Five treatments were control (basal diet), control +0.15% NLE, control + 0.20% NLE, control + 0.25% NLE and control + 0.6% flavomycine (positive control). Each treatment was replicated 4 times. The basal diets are shown in table 1. According to local vaccination program chicks were immunized against common diseases. The powder of NLE was prepared by Barij Essence Pharmaceutical Company and contained 95% dry matter (Table 2).

Measured parameters

Growth performance were recorded weekly including body weight gain (WG) and feed intake (FI). Feed Conversion Ratio (FCR) was calculated accordingly.

In order to measure the humoral immunity, blood samples were collected from 4 birds in each treatment at the age of 36 day (a week after last Newcastle vaccination) for antibody determination and hematological parameters and white blood cell differential count. Cutaneous Basophil Hypersensitivity (CBH) response were used to measure the cell mediated immunity based on the method described previously [22]. For determining CBH, four chicks in each treatment were selected and injected with 0.25mL of 2,4-dinitrochlorobenzene (DNCB) solution (10 mg/mL acetone). DNCB was purchased from

Table 4: The effects of dried Nettle Extract (NLE) on chicks performance at the age of 15-28 d.

Treatments	Feed intake	Weight gain	Feed conversion
	g/c/d ¹	g/c/d	
control	124.8 ^a	68.9 ^{ab}	1.95 ^{ab}
0.15% NLE	116.7 ^{ab}	65.5 ^{bc}	1.86 ^{ab}
0.20 NLE	120.7 ^{ab}	68.6 ^{ab}	1.81 ^b
0.25% NLE	109.7 ^b	72.4 ^a	1.60 ^c
Flavomycin	120.9 ^{ab}	61.4 ^c	2.07 ^a
SE	3.66	1.48	0.06
P-value	0.009	0.001	0.002

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P < 0.05$)
¹gram/chick/day=g/c/d.

Table 5: The effects of nettle extract (NLE) on chicks performance at the age of 29-42 d.

Treatments	Feed intake	Weight gain	Feed conversion
	g/c/d ¹	g/c/d	
control	162.6	90.1 ^b	1.83 ^{ab}
0.15% NLE	162.6	94.1 ^a	1.72 ^b
0.20 NLE	164.9	94.1 ^a	1.73 ^b
0.25% NLE	160.4	94.8 ^a	1.59 ^c
Flavomycin	163.3	87.8 ^b	1.87 ^a
SE	3.44	1.24	0.04
P-value	0.13	0.001	0.001

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P<0.05$) 1 gram/chick/day=g/c/d.

Table 6: The effects of Nettle Leave Extract (NLE) on chicks performance at the age of 0-42 d.

Treatments	Feed intake	Weight gain	Feed conversion
	g/c/d ¹	g/c/d	
control	113.0 ^a	63.2 ^{bc}	1.80 ^a
0.15% NLE	106.7 ^{bc}	64.6 ^{bc}	1.60 ^b
0.20 NLE	109.4 ^{ab}	66.0 ^{ab}	1.59 ^b
0.25% NLE	101.4 ^c	86.2 ^a	1.49 ^c
Flavomycin	110.7 ^{ab}	61.3 ^d	1.78 ^a
SE	1.97	0.84	0.03
P-value	0.001	0.001	0.001

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P<0.05$) 1gram/chick/day=g/c/d

Table 7: The effects of Nettle Leave Extract (NLE) on carcass parts relative weight at the age of 42 d.

Treatments	Carcass percentage	Liver %	Heart %	Abdominal fat %	Gizzard %	Breast %	Drumstick %
control	72.75	2.57	0.45	1.57	2.12	31.62 ^b	33.1
0.15% NLE	72.76	2.65	0.52	1.62	2.07	32.27 ^{ab}	32.22
0.20 NLE	72.2	2.85	0.52	1.72	1.97	32.57 ^{ab}	33
0.25% NLE	70.2	2.72	0.5	1.92	1.87	30.97 ^b	31.99
Flavomycin	73.8	2.97	0.5	1.65	1.95	33.85 ^a	32.55
SE	1.13	0.17	0.03	0.17	0.11	0.6	0.17
P-value	0.27	0.52	0.62	0.67	0.56	0.04	0.52

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P<0.05$)

SIGMA Aldrich (2,4-nitrochlorobenzene,SKU 237329). DNCB solution is injected intradermal and in inter digital space between the third and fourth toes of right foot. Left foot of the same bird was injected by 0.25 mL acetone as control. Electronic caliper was used for measuring skin thickness at 24, 48, 72 h post-DNCB injection, the cell reaction produced by DNCB was assessed as CBH response.

Results

At the age of 1-14d, diets contained NLE improved FI and Feed Conversion Ratio (FCR) significantly ($P<0.05$) as compared to control diet and antibiotic diet (Table 3). Chicks received control diet had lowest weight gain as compared to all dietary groups indicating that

Table 8: The effects of Nettle Leave Extract (NLE) on Newcastle antibody titer and CBH index at the age of 28d.

Treatments	Antibody titer Nd	Cbh 24h post injection	Cbh ratio 48h post injection
control	5.00	0.39	0.29
0.15% NLE	5.63	0.48	0.30
0.20 NLE	5.88	0.67	0.45
0.25% NLE	6.13	0.29	0.51
Flavomycin	5.38	0.33	0.42
SE	0.45	0.13	60.1
P-value	0.46	0.32	0.43

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P<0.05$)

NLE can be considered as a replacer for antibiotic growth promoter especially in young chicks.

Growth performance results achieved in the age of 15-28d is shown in the table 4. The effects of 0.25% NLE on FCR was significantly ($P<0.05$) better than other groups. FI decreased significantly by 0.25% NLE as compared to control diet. In the meantime, the highest WG attained by the same dietary group which was significantly ($P<0.05$) higher than antibiotic treatment.

In the final period of rearing (29-42d) diets contained NLE caused improvement in WG significantly ($P<0.05$) as compared to control and antibiotic diets. Chicks received NLE had better FCR than antibiotic diet (Table 5).

In the whole period (1-42d), treatments improved FI and FCR significantly ($P<0.05$) and WG of 0.25% NLE was clearly superior to other groups (Table 6).

No effects of NLE were observed on carcass parts except breast meat which were decreased by NLE diets as compared to antibiotic diet group (Table 7). The relative weight is calculated as (organ weight/body weight) × 100.

At the age of 42 d 4 birds from each treatment were killed for measuring carcass parts relative weights. As shown in Table 8, there was no effect of NLE on immunity parameters (antibody titer and CBH index). By the way, meat quality and pH were not affected by the treatments (Table 9).

Among the blood indices, hematocrit and hemoglobin were affected by NLE significantly ($P<0.05$). NLE at the level of 0.15 and 0.25% increased red blood cells concentration. In the meantime

Table 9: The effects of Nettle Leaf Extract (NLE) on blood parameters at the age of 35d.

Treatments	Rbc ¹	Hb ²	Hct ³	Mcv	Mch	Mchc	Platelets	Tg ⁴	Chol ⁵
	cu.mm	mg.dl	%	FL	Pg/cell	%	Cu.mm	mg.dl	mg.dl
control	2.42 ^{bc}	11.88 ^{ab}	34.53 ^{ab}	142.6	49.03	34.38	1500	115	117.5
0.15% NLE	2.70 ^a	12.88 ^a	37.98 ^a	140.48	47.63	33.9	2750	142.25	122.5
0.20 NLE	2.40 ^c	11.53 ^b	33.75 ^b	140.55	47.98	34.15	2500	121.5	124.75
0.25% NLE	2.64 ^{ba}	12.70 ^{bc}	37.75 ^a	143.13	48.15	33.68	3500	125.75	116
Flavomycin	2.40 ^c	11.68 ^b	34.03 ^b	141.85	48.7	34.33	1666.67	127.25	143.33
SE	0.07	0.36	1.11	1.88	0.058	0.028	760.83	8.62	23.5
P-value	0.02	0.05	0.03	0.8	0.47	0.39	0.39	0.45	0.44

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P < 0.05$)

¹red blood cell ²hemoglobin ³hematocrit ⁴Triglycerides ⁵cholesterol

Table 10: The effects of nettle leaf extract (NLE) on white blood cells differential count at the age of 35d.

Treatments	Wbc ¹	Hetrophyl %	Leukocyte	Monocyte	Eosinophil	Basophil
control	67780	32.25	62.25	0.25	2.75	2.50 ^{bc}
0.15% NE	43500	35.00	55.75	1.50	3.25	5.00 ^a
0.20 NE	65277	34.5	56.25	2.75	2.50	4.00 ^{ab}
0.25% NE	57157	40.25	51.75	2.50	3.50	2.00 ^c
Flavomycin	44312	40.25	51.5	2.50	3.00	2.75 ^{bc}
SE	10079.22	3.46	3.72	0.89	1.06	0.57
P-value	0.36	0.39	0.21	0.3	0.96	0.01

Means in each column with different superscripts (^{a,b,c}) differ significantly ($P < 0.05$)

¹white blood cell

blood lipids including cholesterol and triglyceride were not affected by NLE ($P > 0.05$).

White blood cell differential count showed that only basophils were affected by NLE. Low level (0.15%) of NLE caused more basophil fraction while 0.25% NLE decreased it significantly ($P < 0.05$).

As it is shown in table 10 among lymphocytes, only basophils are affected by nettle extracts. This is indicating that there is some connection between immunity and nettle. In fact there is very little information about the nettle effects on White Blood Cell (WBC) differential count in broiler chickens.

Discussion

In the current experiment the promotion effects of NLE on body growth were seen clearly. It means the active biochemical compounds of NLE such as lectin [23], anti-inflammatory agents [24], fatty acids and carotenoids [25] have growth stimulatory effects. This effect is caused by whole NLE compounds all together. Consistently it has been reported that nettle have growth promoting effects [26,27], and have potential to improve immune system [9]. Moreover nettle extract is a good choice as antibacterial and antioxidant agent [28]. The results of current experiment are in agreement to these findings about growth but antibody titer showed no difference due to treatments. The immune system is under the effects of environmental stressors and micronutrients. Different environmental conditions and dietary compositions might be the cause for inconsistent result about the nettle impact on immune system. Moreover, the method of nettle compounds extraction might be the source of different results.

Like our result it has been demonstrated that oxidative stability were not affected by nettle extracts [18]. Anyway, we know that flavonoids are very powerful antioxidant and nettle biochemical content including many acids and phenols [29] are natural antioxidant. So, the lack of nettle extracts effects on oxidative indices may be due to destroying the effective materials in the nettle extracts.

The influence of nettle on blood lipids is already documented [30] but we did not observe the same effects. The reason for this observation again can be due to the changed biochemical content during the extraction procedure. Based on this, a standard method of extraction must be established to avoid the same inconsistencies. The hydroalcoholic extract of stinging nettle (*Urtica dioica*) has hypoglycemic effect [31]. This information indicates that nettle is connected with blood sugar and the effectiveness of nettle extracts on other nutritive agents in blood, like minerals and vitamins, worth further investigation.

There is not so much information regarding the impact of nettle compounds on blood parameters. Anyhow it is reported that an unusual lectin has been isolated from stinging nettle with high contents of glycine, cysteine and tryptophan showing very low specific agglutination activity [32]. In our experiment hematocrit concentration increased by nettle extracts. It is reported that nettle can increase WBC differential count and some hematology parameters like RBC, MCH, MCHC and Htc in fish [9] that is partly in agreement with our results. It is not clear whether this augmentation is made on production of red cells or blood plasma dehydrated. As previously reported it can be concluded that hematology and immunity has some connection with nettle extracts which need further investigation [33].

Conclusion

Nettle extracts surely is effective on growth performance of broilers and blood parameters are affected too, but its immune modulation or effects on blood chemicals need further investigation. The extraction method of nettle is a great source of variations. So it could be taken into account for interpreting inconsistent results.

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