



***Hypericum perforatum* and probiotic effects on performance, carcass characteristics and intestinal morphology in Japanese quails (*Coturnix japonica*)**

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ARTICLE INFO

Type: Original Research

Topic: Medicinal Plants

Received February 19th 2016

Accepted April 2th 2016

Key words:

- ✓ *Hypericum Perforatum*
- ✓ *Protexin*
- ✓ *Performance*
- ✓ *Intestinal morphology*
- ✓ *Japanese quails*

ABSTRACT

Background & Aim: *Hypericum perforatum* L. is native to Iran. This herb is one of the most important medicinal plants and had many medical effects. This experiment was conducted to investigate the effects of feeding *Hypericum perforatum* and protexin on performance, carcass characteristics and intestinal morphology Japanese quails.

Experimental: A total of 240 ten days old quails were divided into eight treatments in a completely randomized design (three replicates per treatment). The treatments were divided as basal diet with no protexin and *Hypericum perforatum* kept as control, and 0.2% (H1), 0.4% (H2) and 0.6 % (H3) *Hypericum perforatum* with 0.02 % or without protexin as P0 and P1 were used respectively. The live body weight gains, feed intake and feed conversion ratio of quails were calculated weekly. At the end of the trial two birds (male) from each replicates were slaughtered and dressing percentage were calculated and some carcass traits were weighed separately as percentage of carcass weight.

Results: Data indicated that using of protexin and *Hypericum perforatum* increased feed intake (FI) in treatments compared to control group. Also body weight (BW) (g/d) and Pre-slaughter weight (g) were higher in protexin and *Hypericum perforatum* compared to the control. There were no significant differences ($p < 0.05$) for feed conversion ratio (FCR) among treatments.

Recommended applications/industries: Considering that body weight gain of Japanese quails can improve by increasing the level of *Hypericum perforatum* in diet, this herb is recommended for industrial poultry farm.

1. Introduction

The genus *Heracleum* with more than 120 species in the world is one of the largest genera of the Apiaceae family (Ghasemi Pirbalouti, *et al.*, 2013). The herb

Hypericum perforatum or St Jhon's Wort is one of the Hypericaceae family (Re *et al.*, 2003). *Hypericum perforatum* L. is a shrubby aromatic perennial herb native to Europe, Western Asia and North Africa (Burger and Wachter, 1998). Medical effects of the herb are antibiotic Mennini and Gobbi, (2004), antiviral

(Meruelo *et al.*, 1988), antioxidant (Benedi *et al.*, 2004), anti-stress (Franklin *et al.*, 2004), anticancer (Hostanska *et al.*, 2013), anti-depression, and some other effects on natural killer cells (Helgason *et al.*, 2000). It was found that Hypericin available in this herb has antiviral activity against several viruses (Jacobson *et al.*, 2001; Lau *et al.*, 1998). The use of probiotics in poultry was pioneered by Tortuero (1973), who reported an increase in growth rate in chicks given a *Lactobacillus acidophilus* culture in drinking water for 11 days from hatching. Several researchers (Kalbane *et al.*, 1992; Jin *et al.*, 1997) also reported similar results on the beneficial effects of *Lactobacillus cultures* on the growth of chickens. One of the probiotics used in poultry feed is Protexin. Protexin is a multi-strain probiotic containing live microbes to establish, enhance or reestablish essential microflora in the gut. Protexin is a highly concentrated premix containing seven strains of bacteria and two yeasts (*Lactobacillus plantarum* 1.89×10^{10} cfu/kg, *Lactobacillus delbrueckii* subsp. *Bulgaricus* 3.09×10^{10} cfu/kg, *Lactobacillus acidophilus* 3.09×10^{10} cfu/kg, *Lactobacillus rhamnosus* 3.09×10^{10} cfu/kg, *Bifidobacterium bifidum* 3.00×10^{10} cfu/kg, *Streptococcus salivarius* subsp. *Thermophilus* 6.15×10^{10} cfu/kg, *Enterococcus faecium* 8.85×10^{10} cfu/kg, *Aspergillus oryza* 7.98×10^9 cfu/kg, *Candida pintolopesii* 7.98×10^9 cfu/kg). Protexin can be used in a wide range of circumstances, either to improve the general health of animals, address specific problems or to maximize animal's performance. Under general conditions Protexin has been promoted to: improve health naturally, stimulate appetite, aid in establishment of gut flora in immature animals like day old chicks, calves, lambs, kids, kittens, re-establish gut microflora after antibiotic treatment, optimize digestion of feed and reduce stress (Rajmane, 1998; Cyberhorse, 1999; Panda *et al.*, 2000, Vali., 2009). Many studies have been conducted to test the efficacy of protexin on animal growth and performance. Because of the importance of birds as an economic and nutritious form of animal protein and the fast growing characteristics of this animal, research workers have devoted studies to the use of probiotics and some medical in poultry and quails.

The objective of this study was conducted to evaluate the effects of protexin and *Hypericum*

perforatum on performance, carcass characteristics and intestinal morphology in Japanese quails (*Coturnix japonica*).

2. Materials and Methods

This experiment was done at the Aviculture farm of Islamic Azad University, Shahrekord, Iran. A total of 240 seven days old quails chicks with an average weight of 19.00 ± 50 g were divided into 4×2 treatments and were further subdivided into three replicates with 10 quails on each. *Hypericum perforatum* was purchased from local market Shahrekord, Iran and the flower of it has been used. Corn and soybean meal were analyzed in the lab for determine the amount of dry matter, crude protein, calcium, phosphorus and its crude fiber with Association of Official Analytical Chemists (AOAC, 2000). The basal diet was balanced on the basis of corn and soybean meal as recommended by National Research Council (NRC, 1994). The treatments were divided as basal diet with no protexin and *Hypericum perforatum* kept as control, and 0.2% (E1), 0.4% (E2) and 0.6 % (E3) *Hypericum Perforatum* with (0.02%) or without protexin as P₀ and P₁, respectively. Diets and fresh water were provided *ad libitum* during this experiment. The live body weight gains, feed intake and feed conversion ratio of quails were calculated weekly. At the end of experimental period, two birds (male) from each replicates (totally 48 birds) were slaughtered and dressing percentage was calculated and some carcass traits were weighed separately as percentage of carcass weight. The histomorphometric examination of intestine was done by light microscopy, and the measurement was performed using public domain image analysis software (Image J, National Institute of Mental Health, Bethesda, MD, USA) (Rezaian, 2006). The statically model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Y_{ijk} = average effect observed,

μ = total average,

α_i = effect of *Hypericum perforatum*,

β_j = effect of protexin

$(\alpha\beta)_{ij}$ = interactions (*Hypericum perforatum* \times protexin),

e_{ijk} = effect of errors.

The GLM procedure of SAS software (SAS, 2001) was used for data analysis of variance as completely randomized design. The significant difference among

the means was calculated by Duncan's multiple range tests at probability level of 5%.

3. Results and discussion

Results of this study indicated that use of protexin and *Hypericum perforatum* had increased feed intake (FI) significantly ($p < 0.05$) in comparison to control (Table 1). Result showed that body weight BW (kg) was higher significantly when the birds fed by protexin and *Hypericum perforatum* compared to control. Although feed conversion ratio (FCR) were lesser in protexin and *Hypericum perforatum* group but there were no significant differences compared to the control.

Table 1. The effects of *Hypericum perforatum* and protexin on growth performance of Japanese quails.

Treatments*	FI (g day-1)**	BW (g day-1)	FCR
<i>(Hypericum perforatum)</i>			
Control	20.45 ^c	6.55 ^{ab}	3.20
H (1)	20.63 ^c	6.62 ^{ab}	3.28
H(2)	21.01 ^b	6.28 ^b	2.83
H (3)	21.48 ^a	7.42 ^a	2.89
p Value	0.007	0.010	0.652
<i>(Protexin)</i>			
P (0)	20.32 ^b	6.17 ^b	3.54
P (1)	21.06 ^a	6.38 ^a	3.20
p Value	0.008	0.006	0.453
<i>(Hypericum perforatum × Protexin)</i>			
Control × P (0)	20.41 ^b	7.36 ^b	3.76
H (1) × P (0)	21.07 ^{ab}	7.79 ^{ab}	3.3
H (2) × P (0)	21.27 ^{ab}	7.81 ^{ab}	3.24
H (3) × P (0)	21.54 ^{ab}	8.25 ^{ab}	3.11
Control × P (1)	20.59 ^b	7.33 ^b	3.45
H (1) × P (1)	21.67 ^b	8.51 ^a	3.02
H (2) × P (1)	22.42 ^a	8.67 ^a	2.93
H (3) × P (1)	22.87 ^a	8.89 ^a	2.80
p Value	0.003	0.001	0.921
SEM	0.918	0.310	0.055

*No protexin and *Hypericum perforatum* kept as control, and for others 0.2% (T1), 0.4% (T2) and 0.6% (T3) *Hypericum perforatum* without (P0) or with (P1) (0-0.02% g/kg) protexin. **Feed intake (FI), body weight (BW), feed coefficient (FCR). ***Means within row with no common on letter are significantly different ($p < 0.05$).

In the present study, protexin and *Hypericum perforatum* supplementation had significant effects on the measured values in growing *Japanese quails*. The usage of protexin and *Hypericum perforatum* was significant influences on FI, BW, FCR and carcass yield.

Table 2. The effects of *Hypericum perforatum* and protexin on some carcass traits *Japanese quails*.

Treatments*	Carcass %	Breast %	Drumstick %	Gizzard %	Intestine %
<i>(Hypericum perforatum)</i>					
Control	81.65	36.10	24.51	2.32	3.63
H (1)	82.12	36.49	25.36	2.51	3.65
H (2)	82.73	37.76	26.25	2.74	3.85
H (3)	83.10	37.89	26.61	2.89	3.99
P Value	0.817	0.802	0.713	0.430	0.523
<i>(Protexin)</i>					
P (0)	81.81	36.58	25.34	2.65	3.62
P (1)	82.35	37.02	26.26	2.41	3.81
P Value	0.742	0.412	0.914	0.512	0.471
<i>(Hypericum perforatum × Protexin)</i>					
Control × P (0)	81.82	36.12	25.22	2.25	3.32
H (1) × P (0)	82.13	36.29	26.14	2.26	3.65
H(2) × P (0)	82.25	36.67	27.02	2.43	3.55
H (3) × P (0)	82.66	36.83	27.49	2.55	3.61
Control × P (1)	82.55	36.99	26.55	2.30	4.00
H(1) × P (1)	82.96	37.11	27.67	2.44	4.02
H (2) × P (1)	83.11	37.28	27.88	2.69	4.36
H (3) × P (1)	84.41	37.65	28.11	2.65	4.52
P Value	0.810	0.902	0.961	0.246	0.743
SEM	4.65	2.87	2.01	1.25	0.76

*No protexin and *Hypericum perforatum* kept as control, and for others 0.2% (H), 0.4% (H2) and 0.6% (H3) *Hypericum perforatum* without (P0) or with (P1) (0-0.02% g/kg) protexin. **Means within row with no common on letter are significantly different ($p < 0.05$).

Many scientists showed that beneficial effects of herbal or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and

antibacterial, antiviral, antioxidant, and antihelminthic activities (Janssen, 1989; Manzanilla *et al.*, 2001; Jamroz *et al.*, 2003).

Table 3. The effects of *Hypericum perforatum* and protexin on intestinal morphology of *Japanese quails* (*Micron*)

Treatments*	Length	Width	Height	Mucosal Muscle
<i>(Hypericum perforatum)</i>				
Control	42.42 ^d	7.0 ^b	4.36 ^c	1.01
H (1)	48.37 ^c	7.13 ^b	5.15 ^b	1.18
H (2)	50.23 ^b	7.68 ^{ab}	5.36 ^{ab}	1.19
H (3)	54.31 ^a	8.32 ^a	6.17 ^a	1.25
P Value	0.002	0.009	0.006	0.91
<i>(Protexin)</i>				
P (0)	48.29 ^b	7.16	5.02	1.00
P (1)	52.11 ^a	7.68	5.55	1.19
P Value	0.012	0.110	0.411	0.501
<i>(Hypericum perforatum × Protexin)</i>				
Control× P (0)	45.45 ^d	7.14 ^c	4.10 ^c	1.00
H (1) × P (0)	49.14 ^b	8.19 ^b	5.11 ^b	1.08
H (2) × P (0)	50.38 ^{ab}	8.38 ^b	5.26 ^b	1.26
H (3) × P (0)	51.52 ^{ab}	8.65 ^{ab}	5.46 ^b	1.45
Control× P (1)	48.47 ^c	7.38 ^c	4.67 ^c	1.11
H (1) × P (1)	54.16 ^b	9.11 ^a	5.16 ^b	1.66
H (2) × P (1)	56.69 ^b	9.39 ^a	6.98 ^a	1.86
H (3) × P (1)	58.34 ^a	10.11 ^a	7.12 ^a	1.92
P Value	0.004	0.004	0.010	0.511
SEM	0.126	0.746	0.452	0.091

*No protexin and *Hypericum perforatum* kept as control, and for others 0. 2% (H), 0. 4% (H2) and 0. 6% (H3) *Hypericum perforatum* without (P0) or with (P1) (0-0.02% g/kg) protexin. **Means within row with no common on letter are significantly different (p<0.05).

It can be assumed that given at high dosages, plant secondary metabolites may have anti-nutritional effects, such as reduced feed intake and digestibility of the diet, and toxicity may arise when supplemented at very high concentrations. Results of a study by Parreira (1998) showed that dietary supplementation of protexin increased growth performance and decreased mortality in broilers. Rajmane *et al.* (1998) showed a significant improvement in body weight, improved feed conversion efficiency and reduction in mortality with the use of protexin as a growth promoter such as coneflower in broilers. In addition, Shabani *et al.* (2012) indicated that the chicken broilers feed with

protexin have the lowest feed conversion ratio and was the most favorable. Feizi and Nazeri (2011) demonstrated that use of *Hypericum perforatum* induces FCR improvement and mortality rate decrease, which is significant in comparison treatment groups with control group (P < 0.05). Kheiri *et al.* (2014) concluded that *Hypericum persicum* extract at 100, 150, and 200 mg/L levels can enhance body performance, blood cholesterol, and Newcastle vaccine disease titer in broiler chicks. Balevi *et al.* (2001) showed that diet supplementation with probiotic could improve FI and FCR. Franklin *et al.* (2004) found that the extract from *Hypericum perforatum* could reduce brain's cortisol and corticosterone. Result a previous study (Landy *et al.*, 2012) indicated that addition of *Hypericum perforatum* powder seem not to have a positive influence on growth performance. They showed that broilers receiving *Hypericum perforatum* (10 g/kg) had higher feed intake compared to other groups during starter period, but there was not significant (P > 0.05). They mentioned that lowest body weight observed in chicks receiving *Hypericum perforatum* could partially be due to the presence of some anti-nutritional factors in *Hypericum perforatum*, such as tannins. Tannins can bind proteins in digestive tracts and reduce protein absorption, thus resulting in decreased growth performance. This result is agreed with results reported by Kavyani *et al.* (2012), who indicated that carcass yield increased in broilers fed diets containing probiotic. Shang *et al.* (2012), reported that using *Hypericum perforatum*, especially at 1330 or 667.9 mg/kg BW per day has a certain therapeutic efficacy on infectious Bursal disease infected artificially with the virus and immune enhancement.

4. Conclusion

We could be explained by the facts that *Hypericum perforatum* and protexin could benefit acts on performance for broilers chicks. This improvement may be due to the biological functions of *Hypericum perforatum* and protexin to improve growth or that may be due to its role as stimulant, carminative, enhanced digestibility antimicrobial properties. However further studies are needed for more explanations.

5. Acknowledgments

The authors are grateful to agricultural Staff of Islamic Azad University, Shahrekord Branch for supporting this study and Dr. Abdollah Ghasemi Pirbalouti for excellent assistance.

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