



Short Communication

Effect of supplementation of Oregano (*Origanum onites*) Dried Leaves on the Intestinal Properties in Japanese Quails

Abdur Rahman^{1*}, Ibrahim Sadi Cetingul², Ismail Bayram², Cangir Uyarlar², Abdil Burhaneddin Akkaya², Eyup Eren Gultepe², Hikmet Keles³, Aykut Ulucan⁴ and Zafar Hayat¹

¹Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore (CVAS, Jhang), Pakistan

²Department of Animal Nutrition, Afyon Kocatepe University, Afyonkarahisar 03200, Turkey

³Department of Pathology, Afyon Kocatepe University, Afyonkarahisar 03200, Turkey

⁴Department of Medical Services and Techniques, Bingol University, Bingol 12000, Turkey

ABSTRACT

This study was designed to evaluate the effect of supplementation of Oregano dried leaves in the quail diet on the intestinal properties and gut morphology. Quails fed diets containing 0% (A), 1% (B), 2% (C), 3% (D), 4% (E) and 5% (F) dried ground Oregano leaves. Results revealed that villus height was significantly higher in group D compared to control group, whereas crypt depth was significantly higher in all supplemented groups specifically remarkable in group D. Thickness of the tunica muscularis was higher in group C while other groups like D, E and F showed reduction in thickness compared to control group. Overall, this study data suggest that group D, which was supplemented with 3% dried oregano leaves, showed better performance than control. We conclude here that Oregano dried leaves may have the potential activity to modify the gut morphology and can result in better performance of the quail by increasing the nutrient absorption from the intestine.

Article Information

Received 02 November 2017

Revised 30 December 2017

Accepted 10 January 2018

Available online 28 May 2018

Authors' Contributions

ISC and IB conceived the idea and supervised the study. CU, ABA and EEG executed the experimentation. HK and AU performed analytical examinations. AR and ZH wrote the manuscript.

Key words

Oregano, Dried leaves, Villus, Crypt, Quail.

Poultry farming is popular due to its efficient production and short time span. Poultry meat is contributing 50% share in the world meat production (USDA, 2015). Among the poultry, quail meat is getting popularity as it is delicious and has less cholesterol. Better feed utilization is the important factor for efficient growth rates. In feed low levels antibiotic are used to increase nutrient utilization by causing the thinning of intestinal lining and preventing or suppressing the infections. These antibiotics cause serious health problem which is widely known as drug resistance. To avoid drug resistance, alternative solutions are being explored, especially after the complete banned antibiotics in feed by European Union.

Herbal products, like leaf powders and extracts, are being used to evaluate their potential to be used in poultry nutrition to replace antibiotics (Nychas, 1995). Herbs are

natural products and posed no threat on the consumer health. The use of herbs is from the prehistoric times at home remedies for the cure of flu, and some digestive disorders (Dragland *et al.*, 2004). These products are showing potential effect on growth performance and egg production. Some studies have demonstrated the pronounced effects of natural herbal products on immunity enhancement, as bactericidal, fungicidal and antiviral effects (Runnie *et al.*, 2004). Phytogetic products are also known to possess good effect on the intestinal functions by improving the absorption of nutrients and production of mucous. Phytobiotics are also known to have effect on intestinal morphology by improving the villus height, crypt depth and tunica muscularis thickness as well as antioxidant properties (Awad *et al.*, 2008).

Among phytoGENICS, Oregano (*Origanum onites*) is also being investigating for its potential to be used in poultry rations. Oregano is commonly grown in Mediterranean region which is containing phenolic compounds and antioxidant in major proportion (Chun

* Corresponding author: abdurrehman@uvas.edu.pk
0030-9923/2018/0004-1577 \$ 9.00/0

Copyright 2018 Zoological Society of Pakistan

et al., 2005). Oregano is composed of more than 60 different compounds but major constituents are carvacrol and thymol (Teixeira *et al.*, 2013). In previous studies, it was found that oregano dried leaves supplementation has potential to improve egg taste and fertility without exerting any adverse effect in quails (Cetingul *et al.*, 2007, 2009). Oregano extract is known to possess improved impact on growth performance of quails (Yasilbag *et al.*, 2012) by improving the gut morphology and increasing the nutrient digestibility and absorption. In view of above facts, this study was designed to evaluate the effects of supplementation of oregano dried leaves powder in quail diet on the villus height, crypt depth and tunica muscularis thickness in the quail intestine, which directly influence the nutrient absorption and utilization leading to improved performance of broiler.

Materials and methods

Data was taken from the project No. 042VF11, which was conducted at the Animal Research Centre of Afyon Kocatepe University, Turkey. In this study, a total of 180 day old quail birds were procured from a local reputed hatchery. Birds were divided into 6 groups having 30 birds in each. Each group was further subdivided into 6 subgroups having 5 birds in each. The study was conducted for 70 days.

A total of 6 different diets were formulated containing 0%, 1%, 2%, 3%, 4%, and 5% dried oregano leaves and fed to groups A (Control), B, C, D, E and F, respectively. Dried oregano leaves were purchased from the reputed local market in Afyonkarahisar city. Diets were formulated isonitrogenic and isocaloric according to the recommendations of NRC (1994). Ingredient and chemical composition of diets mentioned in Table I.

Table I.- The ingredient and calculated nutrient/chemical composition of diets.

Ingredient/ nutrients	Group A (control)	Group B	Group C	Group D	Group E	Group F
Maize	34.70	37.00	34	36	35	34
Wheat	30.00	27.00	28	27	27	26
Full fat soya bean	9.40	10.00	14	10.50	13	17
Soybean meal	17.50	16.00	13	14	11	8
Oregano leaves	-	1.00	2	3	4	5
Fish meal	1.30	2.03	2	2.77	3.46	3.31
Lime stone	5.30	5.35	5.33	5.20	5.10	5.20
DCP	1.07	0.90	.94	0.80	0.70	0.75
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix*	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.13	0.12	0.13	0.13	0.14	0.14
Calculated composition						
ME (MJ/kg)	12.13	12.11	12.12	12.02	12.04	12.05
DM (g/kg)	89.10	89.20	89.20	89.10	89.10	89.20
CP (g/kg)	19.90	19.90	19.90	19.80	19.80	19.80
CF (g/kg)	2.60	2.60	2.70	2.50	2.50	2.60
Fat (g/kg)	3.33	3.46	4.10	3.54	3.97	4.61
Calcium (g/kg)	2.47	2.50	2.50	2.47	2.47	2.49
Available P (g/kg)	0.35	0.34	0.35	0.35	0.35	0.35
Methionine + cystine (g/kg)	0.71	0.70	0.71	0.71	0.71	0.70
Lysine (g/kg)	1.19	1.17	1.17	1.15	1.15	1.13
Linoleic acid	1.60	1.70	2.00	1.70	1.90	2.20

*Levels of vitamin per 2.5 kg and mineral supplements per 1 kg product: Vit. A, 12 000.000 UI; Vit. D3, 2 000.000 UI; Vit. E, 35.000 mg; Vit. K3, 4000 mg; Vit. B1, 3000 mg; Vit. B2, 7.000 mg; Vit. B6, 5.000 mg; Vit. B12, 15 mg; niacin, 20.000 mg; D-Biotin, 45 mg; Apo Carotenoid acid ester, 500 mg; Folik Asit, 1 000 mg; Kolin Klorid, 125 000 mg; Vit. C, 50 000 mg; Kal D-Pantothenate, 10.000 mg; Ksanthaxantine, 1500 mg; copper, 5.000mg; cobalt, 200 mg; selenium, 150 mg; manganese, 80.000 mg; zinc, 60.000 mg; 2odine, 1.000 mg; iron, 60.000 mg; DL-Methionine, 99% pure; DCP, Dicalcium phosphate; ME, Metabolisable energy; DM, Dry matter; CP, Crude protein; CF, Crude Fiber. Groups A,B,C,D,E and F supplemented with 0%, 1%, 2%, 3%, 4%, and 5% dried oregano leaves respectively.

Table II.- Effect of *Oregano onites* on the villus height, crypt depth and thickness of tunica muscularis in different experimental groups.

	Groups					
	A	B	C	D	E	F
Villus height	683.29±15.82 ^{bc}	519.56±25.81 ^a	491.05±27.72 ^a	761.99±24.35 ^d	732.05±25.40 ^{cd}	654.54±16.79 ^b
Crypt depth	40.30±1.26 ^a	50.91±2.16 ^b	50.48±1.96 ^b	73.25±3.06 ^c	64.01±2.23 ^d	57.14±1.78 ^c
Tunica muscularis thickness	61.51±1.21 ^c	57.38±2.02 ^{bc}	69.87±3.36 ^d	51.51±1.73 ^{ab}	49.75±1.89 ^a	51.79±1.75 ^{ab}

^{a, b, c, d}, Mean values of groups with different superscripts in same column differ significantly with each other (P<0.05).

For details of groups, see Table I.

A total of 36 birds were slaughtered, by picking one bird from each subgroup randomly, at the age of 70 days. Intestinal samples were collected for histopathological examination using previously described method by [Awad et al. \(2011\)](#). Different segments of intestine were collected for the measurement of villus height, crypt depth and tunica muscularis thickness. Collected samples were preserved for 48 h in 10% buffered formalin solution. Samples were cut and imbedded in paraffin for routine histological examinations. After imbedding, 4 micrometer section was cut from each block by microtome, mounted on slide and stained by hematoxylin and eosine (HE) as describe by [Sakamoto et al. \(2000\)](#) and [Santos et al. \(2005\)](#). Prepared slides were examined in a light microscope equipped with digital camera (Olympus CX41 attached Kameram R Digital Image Analyze System) from different 10 places for each parameters in each slide. The villus height was taken from top of the villus upto the lamina propria ([Sakamoto et al., 2000](#)). Crypt depth was analyzed between the crypt and villus while mucosa (Muscularis thickness) was measured from the top of villus to the base of the muscularis mucosa ([Aptekmann et al., 2001](#)).

Data was subjected to ANOVA for a completely randomized design ([Steel et al., 1997](#)). Duncan's multiple range test ([Duncan, 1955](#)) was used to check the mean differences.

Results and discussion

Gut morphology plays an important role in the proper digestion and utilization of feed nutrients. Better digestion will ultimately reduce the cost of production by improving the feed conversion ratio (FCR) ([Tarachai and Yamauchi, 2000](#)). In the current years, the use of natural herbal products in the poultry diet is being explored and evaluated to enhance the production performance of birds. Researchers have paid enormous efforts to investigate the potential of phytochemicals to be used as alternative of antibiotics ([Wang and Bourne, 1998](#); [Rafeeq et al., 2017](#)). The results of current study indicated that villus height was significantly lesser (P<0.05) in groups B, C, and F, which were supplemented with 1%, 2% and 5% oregano

leaves, respectively, however it was surprisingly observed higher (P<0.05) in group D, which was supplemented with 3% oregano leaves as compared to control group A as shown in Table II. In this study, oregano dried leaves have revealed that supplementation of feed with oregano leaves resulted in improved villus height. This result is supported by [Jamroz et al. \(2006\)](#) who showed that phytochemical extract supplementation has pronounced impact on the performance in improving villus height of intestine. It is noteworthy to mention that increased villus height is also responsible for the better performance by improving surface area and absorption of nutrients ([Caspary, 1992](#)).

Many intestinal parameters are involved in digestive processes which are correlated with each other to support and enhance the performance and better feed utilization of nutrients. Crypt depth is also playing a vital role in surface area and gut functioning. In the current study, crypt depth showed higher value (P<0.05) in all groups compared to control, having highest crypt depth in group D supplemented with 3% oregano leaves. Likewise, in previous studies ([Denli et al., 2004](#); [Parlat et al., 2005](#); [Çabuk et al., 2006](#); [Alçiçek et al., 2004](#)), it has been observed that feed conversion ratio (FCR) was improved in the oregano oil supplemented groups which favors the concept that improvement in FCR might have resulted from improved gut morphology.

Passage rate of digesta determines the rate of absorption of nutrients from the intestine into the blood. Tunica muscularis is also responsible for the integrity and normal passage rate of digesta. In this study, muscularis thickness was shown higher (P<0.05) in groups C, D, E and F, while group B demonstrated non-significant (P>0.05) difference as compared to group A. Similarly, in other studies it has been proved that the supplementation of herbal extract (mixture of oils and dried material) resulted in improved growth rate, FCR, feed intake and overall performance through improvement of gut morphology ([Lee et al., 2003](#); [Jang et al., 2004](#); [Jamroz et al., 2006](#)).

Conclusion

Oregano-supplementation has shown potential effect

on the gut morphology by improving the villus height, crypt depth and tunica muscularis thickness in the groups D, C and E, respectively. It is recommended to use upto 5% level of oregano dried leaves in the diet of quail to improve performance. It is suggested to investigate the potential of oregano extensively which could replace the use of antibiotics in poultry diets.

Acknowledgment

This Project was supported by the Scientific Research Committee of AKU (Project No: 042VF11).

Statement of conflict of interest

Authors have declared no conflict of interest.

References

- Alçiçek, A., Bozkurt, M. and Çabuk, M., 2004. *S. Afr. J. Anim. Sci.*, **34**: 217-222.
- Aptekmann, K.P., Baraldi-Arton, S.M., Stefanini, M.A. and Orsi, M.A., 2001. *Anat. Histol. Embryol.*, **30**: 277-280. <https://doi.org/10.1046/j.1439-0264.2001.00331.x>
- Awad, W., Ghareeb, K. and Böhm, J., 2008. *Int. J. mol. Sci.*, **9**: 2205-2216. <https://doi.org/10.3390/ijms9122505>
- USDA, 2015. <http://search.ers.usda.gov/search?affiliate=ers&query=total%20meat%20production>
- Awad, W.A., Ghareeb, K. and Böhm, J., 2011. *J. Anim. Physiol. Anim. Nutr.*, **95**: 65-72.
- Caspary, W.F., 1992. *Am. J. clin. Nutr.*, **55**: 299S-308S. <https://doi.org/10.1093/ajcn/55.1.299s>
- Chun, S.S., Dhiraj, A., Vattam, Y.L. and Kalidas, S., 2005. *Process Biochem.*, **40**: 809-816. <https://doi.org/10.1016/j.procbio.2004.02.018>
- Cabuk, M., Bozkurt, M., Alcicek, A., Akbas, Y. and Kucukyilmaz, K., 2006. *S. Afr. J. Anim. Sci.*, **36**: 135-141. <https://doi.org/10.4314/sajas.v36i2.3996>
- Cetingul, I.S., Bayram, I., Yardimci, M., Sahin, E.H., Sengor, E., Akkaya, A.B. and Uyarlar, C., 2007. *Arch. Zootech.*, **10**: 57-65.
- Cetingul, I.S., Bayram, I., Yardimci, M., Sahin, E.H., Sengor, E., Akkaya, A.B. and Uyarlar, C., 2009. *Ital. J. Anim. Sci.*, **8**: 131-142. <https://doi.org/10.4081/ijas.2009.131>
- Duncan, D.B., 1955. *Biometrics*, **11**: 1-1. <https://doi.org/10.2307/3001478>
- Dragland, S., Senoo, H., Wake, K., Holte, K. and Blomhoff, R., 2003. *Nutrition*, **133**: 1286-1290.
- Denli, M., Okan, F. and Uluocak, A.N., 2004. *S. Afr. J. Anim. Sci.*, **34**: 174-179. <https://doi.org/10.4314/sajas.v34i2.3812>
- Jamroz, D., Wertelecki, T., Houszka, M. and Kamel, C., 2006. *J. Anim. Physiol. Anim. Nutr.*, **90**: 255-268. <https://doi.org/10.1111/j.1439-0396.2005.00603.x>
- Jang, I.S., Ko, Y.H., Yang, H.Y., Ha, J.S., Kim, J.Y., Kang, S.Y., Yoo, D.H., Nam, D.S., Kim, D.H. and Lee, C.Y., 2004. *Asian-Australas. J. Anim. Sci.*, **17**: 394-400. <https://doi.org/10.5713/ajas.2004.394>
- Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R. and Beynen, A.C., 2003. *Br. Poult. Sci.*, **44**: 450-457. <https://doi.org/10.1080/0007166031000085508>
- NRC, 1994. *Nutrient requirements of poultry*, 9th ed. National Academy Press, Washington, DC, pp. 27-31.
- Nychas, G.J.E., 1995. In: *New methods of food preservation* (ed. G.W. Gould). Blackie Academic Professional, London, pp. 58-89. https://doi.org/10.1007/978-1-4615-2105-1_4
- Parlat, S.S., Yildiz, A.O., Olgun, O. and Cufadar, Y., 2005. *J. Selçuk Univ. Agric. Facul.*, **19**: 7-12.
- Rafeeq, M., Rashid, N., Tariq, M.M., Tareen, R.B., Ullah, A. and Mustafa, Z., 2017. *Pakistan J. Zool.*, **49**: 1063-1069. <http://dx.doi.org/10.17582/journal.pjz/2017.49.3.1063.1069>
- Runnie, I., Salleh, M.N., Mohameda, S., Headb, R.J. and Abeywardena, M.Y., 2004. *J. Ethnopharmacol.*, **92**: 311-316. <https://doi.org/10.1016/j.jep.2004.03.019>
- Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1997. *Principles and procedures of statistics: A biometrial approach*, 3rd Ed. McGraw Hill Book Co. Inc., New York, USA, pp. 481.
- Santos, F.S., Tellez, G., Farnell, M.B., Balog, J.M., Anthony, N.B., Pavlidis, H.O. and Donoghue, A.M., 2005. *Poult. Sci.*, **84**: 1495-1498. <https://doi.org/10.1093/ps/84.9.1495>
- Sakamoto, K., Hirose, H., Onizuka, A., Hayashi, M., Futamura, N., Kawamura, Y. and Ezaki, T., 2000. *J. Surg. Res.*, **94**: 99-106. <https://doi.org/10.1006/jsre.2000.5937>
- Tarachai, P. and Yamauchi, K., 2000. *Poult. Sci.*, **79**: 1578-1585. <https://doi.org/10.1093/ps/79.11.1578>
- Teixeira, B., António, M., Cristina, R., Carmo, S., Olívia, M., Nuno, N.R., José, N.M.F., Alexandre, S.J. and Leonor, N.M., 2013. *J. Sci. Fd. Agric.*, **93**: 2707-2714. <https://doi.org/10.1002/jsfa.6089>
- Yesilbag, S.S., Gezen, H., Biricik, H. and Bulbul, T., 2012. *Br. Poult. Sci.*, **53**: 89-97. <https://doi.org/10.1080/00071668.2012.654763>
- Wang, R.D. and Bourne, S., 1998. *Biotechnology in the feed industry*. In: Proc. Altech's 14th Annual Symposium Altech Technical Publications Nottingham University Press. Nicholasville, K.Y., pp. 273-292.