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Original article

Evaluating the impact of honey inclusion in drinking water on the semen quality, immunological response, and haematology of naked neck cocks

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Abstract

Honey is natural nectar that honey bees gather from a variety of flowers. Honey is made up of many nutrients that are essential for the growth and development of various tissues and organs in all kinds of living organisms. The goal of the current study was to determine whether adding honey to diets of naked neck cocks would have a good impact on their semen quality, haematological profile, and immune system. A total of 90 naked neck cocks that were 75 weeks old were gathered for this purpose in March and April 2021 from the University of Agriculture Birds Stock. The birds were divided into 3 equal treatment groups: control, A and B, which each received a dose of 5 and 10 g of honey in 1 litre of water (w/v), respectively. The control group received no treatment. Five weeks of treatment were given, with one week serving as an adaptation period. One-way analysis of variance was applied to the recorded data for statistical analysis. The addition of honey to drinking water at a dose of 10 g (w/v) increased ($p<0.05$) sperm motility, followed by a dose of 5 g (w/v) and the control group, but the mean values of non-motile sperms were lowest ($p<0.05$) at a dose of 10 g (w/v), followed by a dose of 5 g (w/v), and the control group. However, there were no significant differences in semen volume, pH, or concentration across the treatment groups. The haematological parameters, such as haemoglobin, total leukocyte count, red blood cells, and packed cell volume, were not significantly affected by the addition of honey to the drinking water, but the ND titer of naked neck cocks treated with 10 g (w/v) honey was significantly ($p<0.05$) higher than that of the cocks in the 5 g (w/v) and control group. In conclusion, the addition of honey to drinking water at a dose of 10 g (w/v) may have positive effects on sperm motility and antibody titer against Newcastle disease in aged naked neck cocks. Furthermore, these results also suggest that the addition of honey to drinking water at a dose of 10 g (w/v) may rejuvenate naked neck cocks even at old age (75 week age).

Keywords: honey, naked neck, semen quality, haematology, antibody titer

Introduction

The use of natural substances as feed additives in animal production is currently receiving attention on a global scale. Honey is a naturally occurring product made by honeybees from the nectar of flowers (Alvarez--Suarez et al. 2014). It has been documented in the literature that honey includes 38.2% fructose, 31.3% glucose, 7.1% maltose, 1.3% sucrose, 1.5% other sugars, 0.2% ash, and 17.2% water. Additionally, a considerable amount of proteins, dietary fibres, vitamins like vitamin A, vitamin B, and vitamin C, minerals like zinc, calcium, iron, and copper, as well as a number of other substances including flavonoids, polyphenols, and diterpene acids are also present in it (USDA 2014). Honey has specific ingredients that are similar to those found in certain fruits and turn into bases in the digestive tract (Bradley 2008). The numerous antimicrobial properties of honey can aid the body in the battle against infection (Tan et al. 2009). The body uses the fructose and glucose in honey as a source of energy, which improves virility and sexual performance (Bradley 2008). There have been previous studies on the effect of honey on the testes and the quality of the sperm produced by rats. These studies revealed no significant differences in the weight, width, or length of the testes between groups, but they did reveal a significant improvement in sperm motility and sperm count in the honey-treated groups compared to the control group (Syazana et al. 2011). It is anticipated that using honey may encourage less motile sperm, a major factor in the declining fertility trend (Igbokwe et al. 2013). Literature has also documented the physiological recoveries of the laying birds when given vitamin C and honey (Isaac and Igw 2015).

Honey contains several antioxidants, including phenolic and non-phenolic antioxidants, in addition to its antibacterial and anti-microbial properties; the quantity and variety of these types of antioxidants mostly depend on the flora from which the honey was produced (Khadr et al. 2015). By preventing and scavenging radicals that naturally develop in the body, these antioxidants play a significant part in preventing reactive oxygen species from damaging cells (Ajibola et al. 2012). Phytochemicals such as vitamin C, thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, phenolic compounds, and the enzymes glucose oxidase, catalase, and peroxidase are also present in honey (Oke et al. 2016). Additionally, honey has a long history of being used as a traditional food for healing (Memon et al. 2021). Due to honey's antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, and anti-inflammatory characteristics (Viuda-Martos et al. 2010, Alvarez-Suarez et al. 2014), modern herbalists advise using it in medicine to boost the body's natural resistance to illnesses (Viuda-Martos et al. 2010, Alvarez-Suarez et al. 2014). Honey's higher acidity makes it a powerful germ-fighter. The study was designed to investigate the effects of honey on many aspects of naked neck cock semen, haematology, and immune state while keeping in mind the favorable effects of honey.

Materials and Methods

Experimental site, birds, and housing

This study used various concentrations of honey supplementation dissolved in water to examine various semen, immunity, and blood parameters in naked neck cocks. The study was carried out at the Poultry Unit of the Department of Poultry Science at the University of Agriculture Peshawar (**UAP**), Pakistan. A total of 90 naked neck cocks that were about 75 weeks old were used for the experiment, which was carried out using a complete randomized design (**CRD**). Birds were housed in various cages in a setting resembling an open-sided chicken farm. The birds were divided into three equal groups: control, A, and B, each containing three replicates of 10 birds each. Birds received supplemental treatments of natural honey in drinking water at concentrations of 0 g/liter, 5 g/liter, and 10 g/liter. The honey used in this experiment was purchased from local vendors and proximate composition of the raw honey is given in Table 2. The birds received layer feed. Table 1 displays the nutritional makeup of the diet. The experiment was carried out for a total of five weeks, with one week serving as an adaptation phase. All experimental protocols, including the treatment and usage of birds, adhered to Pakistani laws and regulations and were authorized by the Ethical Review Committee for Biomedical Research, UAP (Letter No. 6585-A/UAP, Dated: 06/01/2021).

Data collection

Semen samples

Semen samples were obtained following the adaption phase by abdominal massage and squeezing through the copulatory organs (Hafez 1978). The semen samples were kept at 38-40°C in a thermos flask. Semen was gathered twice a week on Mondays and Wednesdays from 6:00 AM to 8:00 AM. According to Zemjanis (1970), the obtained semen was subjected to microscopic analysis and physical evaluation at Khyber Teaching Hospital in Peshawar. Semen was examined, and measurements were made of its pH, volume, concentration, and motility and non-motility of the sperm.

Table 1. Composition of the ration offered to the experimental birds (naked neck cocks).

Ingredient (%)	Value
Corn	62.30
Guar meal	3.00
Raw rice bran	4.00
Soybean meal 44%	1.31
Rape seed meal	2.00
DL-Methionine	0.23
L-threonine	0.08
Calcium carbonate	8.29
Salt	0.11
Corn glutten	1.00
Canola meal	8.00
Cotton seed meal	4.00
Lysine sulphate	0.36
Premix	0.30
L-Tryptophan	0.01
Fish meal 47%	1.00
Feather meal 54%	4.00
Quantum 600FTU	0.01
Total	100
Nutrient composition	
CP %	16.5%
ME, kcal/kg	2902

[1] Provided per kg of diet: vitamin A – 11,000 IU, vitamin D_3 – 2,560 IU, vitamin E – 44 IU, vitamin K – 4.2 mg, riboflavin – 8.5 mg, niacin – 48.5 mg, thiamine – 3.5 mg, d-pantothenic – 27 mg, choline – 150 mg, vitamin B_{12} – 33 µg, copper – 8 mg, zinc – 75 mg, manganese – 55 mg, iodine – 0.35 mg, selenium – 0.15 mg.

Table 2. Proximate composition of the raw honey used in this trial.

Sr. No	Parameter	Percentage
1	pH	3.28
2	Moisture	33.65
3	Ash	0.24
4	Protein	0.75
5	Fat	0.04
6	Fiber	0.01
7	Carbohydrate	56.24
8	Energy (kcal/g)	2.51

Haematological parameters

From each replicate, three naked neck cocks were chosen at random to collect blood samples. A sterile, 5-milliliter needle was used to aseptically draw three milliliters of blood from the birds' wing veins. Blood was transferred to vacutainer tubes containing the anti-coagulant ethylene diamine tetra acetic acid (EDTA) as soon as it was collected to avoid clotting. The collected blood samples were examined at the microbiology lab of the University of Agriculture, Peshawar, using a mechanical blood analyzer machine to determine the levels of haemoglobin (**Hb**), red blood cells (**RBC**), total leukocytes (**TLC**), and packed cell volume (**PCV**).

Immune response

One week before the end of the experiment, three birds per replicate were chosen to determine antibody titer against Newcastle disease virus. The chosen birds received an anti-Newcastle disease virus vaccination.

Table 3. Effect of varying honey inclusion levels on various semen parameters of naked neck cocks.[1]

Effects[3]	Parameters[2]				
	V (ml)	pH	C (10^9 sperms/ml)	M (%)	N (%)
C	0.26±0.03	7.05±0.003	1.17 ± 0.01	72.66±0.66[c]	27.33±0.66[a]
A	0.40±0.05	7.04 ±0.003	1.20 ± 0.01	78.00±1.15[b]	22.00±1.15[b]
B	0.46±0.03	6.97 ± 0.03	1.35 ± 0.02	80.66±0.33[a]	19.33±0.33[c]
p-value	0.111	0.4571	0.6035	0.0009	00.00754

[a-c] The means of columns without any shared superscripts differ significantly ($p<0.05$).
[1] Data are means ± SE representing 3 replicates (n=3).
[2] V – volume, C – concentration, M – motility, N – non-motile.
[3] C – control, A – honey at 5g/liter, B – honey at 10 g/liter.

Table 4. Effect of varying honey inclusion levels on the antibody titer in naked neck cocks against NDV[1].

Effects[2]	Parameters		
	Min	Max	Mean (HI titer, $\log_2$)
C	5.1	5.2	5.16±0.03[c]
A	5.5	5.6	5.56±0.03[b]
B	5.7	6	5.83 ±0.04[a]
p-value			0.0019

[a-c] The means of columns without any shared superscripts differ significantly ($p<0.05$).
[1] Data are means ± SE representing 3 replicates (n=3).
[2] C – control, A – honey at 5g/liter, B – honey at 10 g/liter.

Blood samples were taken in order to measure the antibody titer against ND at the end of the experiment. The serum was separated from the blood samples using a centrifuge machine operating at 3000 revolutions per minute for 15 minutes. After that, the serum was placed in plastic containers and kept at -20°C prior to further examination. The haemagglutination inhibition (HI) test was carried out to determine the antibody titer against Newcastle disease (Alexander et al. 1977). For this, a 96-well MPT (micro titer plate) was utilized, with all wells containing 25 microliters of serum before 25 microliters of Newcastle disease antigen was added to all except the last row of wells. The serum's dilution ranges from 1:2 to 2048. Then, it was incubated for 30 minutes at 37.1°C before 50 ul of a 0.5% erythrocyte solution was added to each well. It was incubated once more for 30 minutes at 37°C. The maximum dilution at which RBC agglutination occurred in the sample was used as the end point.

Statistical analysis

Through the use of CRD, the data were statistically examined using the analysis of variance (ANOVA) process. By applying the Steel and Torrie (1980) approach, the means were compared for significance using the least significant difference (LSD) test. For the aforementioned analysis, a statistical software was used (SAS 2002).

Results

Semen quality

Between the treatment groups, the naked neck cocks demonstrated a significant difference in the number of motile and non-motile sperm (Table 3). The addition of honey to drinking water at a dose of 10 g (w/v) improved sperm motility, with a dose of 5 g (w/v) and the control group coming in second and third, respectively. In contrast, the mean values of immobile sperms were lowest at a dose of 10 g (w/v), followed by a dose of 5 g (w/v), and the untreated group. Despite the addition of honey in various amounts to drinking water, no alterations that were significant ($p>0.05$) in semen volume, pH, or concentration were found.

Immune Response

In the current investigation, the ND titer in the 10 g (w/v) honey-treated naked neck cocks was substantially higher ($p<0.05$) than that in the 5 g (w/v) and control group cocks (Table 4).

Haematological Parameters

Hb, TLC, RBC, and PCV were not significantly affected by the addition of honey to the water the birds drank (Table 5). All experimental birds' PCV readings fell within the range of 24.9 to 45.2% that is considered

Table 5. Effect of varying honey inclusion levels on various haematological parameters in week 1 and week 2 of naked neck cocks[1].

Effects[3]	Parameters[2]							
	Week 1				Week 2			
	Hb/dl	TLC (10^9/L)	RBC (10^{12}/L)	PCV (%)	Hb/dl	TLC (10^9/L)	RBC (10^{12}/L)	PCV (%)
C	8.73±0.74	16.33±0.33	3.63±0.17	29.33±1.45	8.56±0.47	16.33±0.33	3.40±0.11	28.00±0.57
A	8.66±0.52	14.33±1.20	3.73±0.17	31.30±2.59	9.30±0.60	14.66±0.88	3.66±0.29	31.66±0.92
B	9.66±1.03	15.66±2.02	3.66±0.28	32.66±4.05	9.96±0.67	14.33±1.45	3.63±0.14	34.33±4.37
p-value	0.798	0.428	0.646	0.204	0.933	0.273	0.273	0.518

[a-c] The means of columns without any shared superscripts differ significantly ($p<0.05$).
[1] Data are means ± SE representing 3 replicates (n=3).
[2] Hb – hemoglobin, TLC – total leukocytes count, RBC – red blood cells, PCV – packed cell volume.
[3] C – control, A – honey at 5g/liter, B – honey at 10 g/liter.

typical for chickens (Flecknell 1979). Although the oral administration of honey at a dosage of 10 g (w/v) exhibited numerically improved values for Hb and PCV during the first week, statistical analysis revealed that these improvements were not significant ($p>0.05$). The oral administration of honey at a dose of 10 g (w/v) during the second week of the study similarly produced numerically larger values for PCV when compared to other groups, although this difference was also not statistically significant.

Discussion

According to published research, honey contains a wide range of chemicals of varying biological and therapeutic relevance, including flavonoids and phenolic compounds (Michalkiewicz et al. 2008). To protect the reproductive system from various compounds produced in the body during the oxidation process, flavonoids and phenolic acid play a significant role and exhibit antioxidant activity (Estevinho et al. 2008). Furthermore, Estevinho et al. (2008) observed that adding honey to drinking water increases the sexual drive and semen qualities of chickens while also preventing toxicity of the reproductive system (Elnagar 2010). Therefore, it is possible to assume that honey may have increased semen quality of naked neck cocks by reducing the negative effects of free radicals caused by metabolic activity. Additionally, the honey's unique properties of enhancing the environment of sperm storage tubules in the hen's oviduct, allowing sperm to live longer, and lengthening the time of sperm storage may be attributed to the improved sperm motility and decreased non-motile sperms as a result of its inclusion in water.

Similar findings were made by other researchers, who found that supplementing bee pollen or honey in various amounts significantly improved the reproductive efficiency of cocks (Abuoghaba and Ismail 2018). Our findings for sperm volume, pH, or concentration, however, disagree with those of other researchers who found that supplying cocks with honey or bee pollen had a substantial impact on those variables (Abuoghaba and Ismail 2018).

The most frequent source of financial losses in poultry is the Newcastle disease virus (NDV), hence vaccines that are both live and inactivated are frequently used to stop the spread of disease caused by this pathogen (Awad et al. 2015). In the host's fight against pathogens, serum antibody titer is crucial. An important method for evaluating hens' humoral immunity is the measurement of antibody titers or immunological responses (Khan et al. 2019). Increased antibody titers in honey-treated cocks as compared to control animals demonstrated honey's immunostimulatory effects (Memon et al. 2019). According to published research, natural honey contains high levels of polyphenols, such as flavonoids, flavonols, and flavones (20 mg/100 g), which have antibacterial, antioxidant, and immunomodulatory activities (Hegazi et al. 2013). Furthermore, it has been demonstrated in earlier studies (Viuda-Martos et al. 2010) that honey contains zinc (0.02 mg/100 g), selenium (0.06 mg/100 g), manganese (0.2-2 mg/100 g), and vitamin E (0.03). These microminerals interact with vitamin E to protect cells from free radicals since it is a component of glutathione peroxidase (Hegazi et al. 2013). Thus, it may be assumed that adding honey to the drinking water of naked neck cocks may have increased their ND titer by reducing the negative impacts of various toxic chemicals the body produces during the oxidation process.

These findings are in line with those of other researchers who discovered that chickens treated with honey or propolis extract had a significantly higher antibody response to NDV than chickens fed a control diet (Hegazi et al. 2013, Zafarnejad et al. 2017, Rabie et al. 2018, Memon et al. 2019). However, several researchers reported no differences in the immune-related parameters they evaluated, such as antibody titers against NDV, between birds treated with propolis

or bee pollen and those fed the control diet (Wang et al. 2005b, Freitas et al. 2011).

As stated above, various doses of honey did not affect hematological profile of naked neck cocks. These outcomes are consistent with other studies' findings that broilers' haematological parameters were unaffected by supplements of honey or propolis given to them (Oke et al. 2016, Osunkeye et al. 2016). Similar to this, Adekunle et al. (2017) evaluated the use of honey in laying pullets and discovered no change in PCV when honey was administered up to 20 ml per litre of water. On the other hand, it was also shown that supplying broilers with honey, bee pollen, or propolis had a significant impact on the haematological parameters (Obun et al. 2008, Attia et al. 2014, Farag and El-Rayes 2016, Shreif and El-Saadany 2017, Rabie et al. 2018, Abioja et al. 2019).

Conclusions

The results show that adding honey to drinking water at a dose of 10 g (w/v) increased ($p<0.05$) sperm motility, while the mean values of non-motile sperms were lowest ($p<0.05$) at a dose of 10 g (w/v), followed by a dose of 5 g (w/v), and the control group. However, there were no significant differences in semen volume, pH, or concentration across the treatment groups. The haematological parameters, such as haemoglobin, total leukocyte count, red blood cells, and packed cell volume, were not significantly affected by the addition of honey to the drinking water of birds, but the ND titer of naked neck cocks treated with 10 g (w/v) honey was significantly ($p<0.05$) higher than that of the cocks in the 5 g (w/v) and control group. These findings collectively imply that sperm motility, ND titer, and the number of non-motile sperm in naked neck cocks may all be improved by the addition of honey to drinking water at a level of 10 g (w/v). Furthermore, these results also suggest that the addition of honey to drinking water at a dose of 10 g (w/v) may rejuvenate naked neck cocks even at old age (75 week age).

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