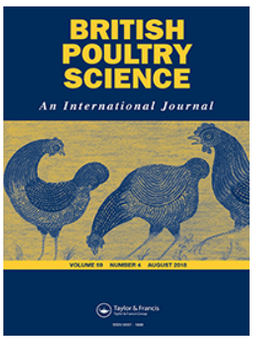

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Body growth, intestinal morphology and microflora of quail on diets supplemented with micronised wheat fibre

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ABSTRACT

1. Particle size reductions of fibre-rich materials alter structure, functional and digestive properties. To determine the effects of using fibre as an additive in Japanese quail rations on performance and gut physiology, a trial using micronised wheat fibre (MWF) at levels of 0.0, 5, 10 and 15 g/kg in feed was conducted.
2. Growth rate and feed efficiency were significantly improved when diets contained MWF while feed intake was not affected by levels of the fibre. As MWF content increased, the relative weight of gizzard and gastrointestinal tract (GIT) significantly increased whereas liver relative weight significantly decreased.
3. MWF inclusion significantly increased relative length of gut segments, villi height, villus thickness, the villi height to crypt depth proportion in jejunum and ileum and the number of goblet cells in different parts of intestine.
4. Tibia weight, length and ash content were increased linearly with rising MWF inclusion. Litter moisture was affected by MWF inclusions in a quadratic manner. The colony forming unit (CFU/g) of *Streptococci* spp. in ileal digesta was decreased with increasing MWF inclusion levels in the diet.
5. In conclusion, MWF can be used as a feed additive in quail diets and its inclusion in feed resulted in better performance, beneficial changes in intestinal microbial counts and improvements in small intestine morphology.

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Digestion; gut morphology; microflora; performance; wheat fibre

Introduction

Dietary fibre plays an important role in poultry diets and minimum levels are required to maintain physiological function in the digestive tract (Wenk 2001). A major concern when including fibre in diets for monogastric animals is that high levels can be associated with decreased nutrient utilisation and low net energy values (Noblet and Le Goff 2001). However, such negative impacts are determined by the fibre properties and may differ considerably between cereal sources (Lindberg 2014). For example, certain soluble non-starch polysaccharides (NSPs) present in cereals have been found to impair nutrient digestion and reduce the performance of poultry. The viscous nature of soluble NSPs increases the viscosity of intestinal contents, which in turn decreases the rate of diffusion of digestive enzymes into digesta and hinders their effective interaction at mucosal surfaces (Iji et al. 2001). However, insoluble fibre improves hydrochloric acid (HCl), bile acids and enzyme secretion, stimulates gizzard function in broilers and layers (Hetland et al. 2005; Jiménez-Moreno et al. 2009) and decreases transit time, enhances water holding capacity and assists faecal bulking in pigs (Souffrant 2001). Dietary fibre may have other benefits such as inducing increased satiety and reducing problem behaviour such as pecking, cannibalism, thereby improving poultry welfare (Hocking et al. 2004). The need for dietary fibre resulted in a minimum requirement level in published feeding standards for pigs (European Commission 2008). However, there is no similar requirement for poultry in requirements provided by the National Research Council (NRC 1994).

For a long time, antibiotics have been used as growth promoters in feed to control commonly occurring enteric diseases (de Lange et al. 2010). However, their regular use promotes bacterial resistance and tissue residues and may result in less efficient antibiotic treatments of diseases for human and animals (Pamer 2016), and this has led to limitations and bans on their use in various countries. One possible alternative control of enteric disease is by changing feed composition and uses various dietary interventions (Armstrong 2016). Many studies have been performed to change gut health and microbial populations by adding types of fibrous fractions into poultry diets (He et al. 2015).

Japanese quail is phylogenetically closely related to the broiler chickens (Stock and Bunch 1982) and both belong to the order *Galliformes* and the family *Phasianidae*. Both species have a karyotype chromosomes and similar genome length. Recent cytogenetic studies have confirmed highly conserved chromosome homology between Japanese quail and chickens, revealing only few chromosome rearrangements after divergence of the two species (Shibusawa et al. 2001). Thus, Japanese quail has been recommended as a model species for poultry (Mills et al. 1997) and it can be assumed that dietary treatments would have similar effects in Japanese quail and other poultry species.

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Micronised wheat fibre (MWF) is characterised by its small particle size and high content of insoluble dietary fibre. The MWF does not contain soluble fibre or antinutritional compounds such as phytate and chelating properties (Taylor-Pickard and Spring 2008). The fibre is insoluble, with a high swelling and water-binding capacity (McCleary and Prosky 2008), and could have potential as a beneficial dietary additive in poultry feed.

Positive effects on poultry digestion have been shown for various insoluble fibre products (Gonzalez Alvarado et al. 2010; Hetland and Svihus 2001; Sklan et al. 2003). Dietary fibre as a feed supplement in poultry diets has been studied recently by adding purified insoluble fibre commercial products such as Arbocel® and Vitacel® (Yokhana et al. 2015; Rahmatnejad and Saki 2016; Rezaei et al. 2011; Sarikhan et al. 2010); however, scientific data are still limited regarding levels of inclusion. The use of micronised fibre in quail diets and feed for other poultry species has, as far as published literature indicates, not been studied in detail. The aim of the present study was to study the effect of supplementing quail diets with increasing levels of MWF on feed efficiency, growth, small intestine morphology, digestive organ weights, tibial ash, ileal microflora counts and litter moisture.

Materials and methods

Birds, diets, performances and litter moisture

A total of 84, 1-d-old Japanese quail chickens were randomly allocated to 12 groups of 7 birds each, comprising three replicates per treatment. The birds were kept in stainless-steel wire cages 43 × 33 × 37 (h × w × d) with solid floor. The initial temperature was 34°C, which was reduced by 4°C each week until a final temperature of 22°C was obtained at week 4. The relative humidity was maintained at 55–65% throughout the rearing period. The basal diet (starter fed from 1 to 28 d) consisted of corn, soybean meal (440 g/kg CP) and fish meal (650 g/kg CP) and was formulated to meet or exceed NRC (1994) nutrient requirements for quail. Mash diets were supplemented with 5, 10 or 15 g/kg MWF (Vitacel® produced by the JRS Company, Rosenberg, Germany) in place of sand as an inert filler in the control feed (Table 1). According to our analyses, MWF contained 7144 g/kg insoluble and 0.0 g/kg soluble dietary fibres. The insoluble dietary fibre consisted of cellulose (~740 g/kg) and hemicellulose (~260 g/kg) and the average length and width of its particles were 250 and 25 µm, respectively (Market and Backers 2003).

Table 1. Composition of basal diets for quail.

Ingredient (g/kg as fed)	Starter (1–28 d)
Corn	414
Soybean meal (440 g/kg CP)	402
Vegetable oil (37.7 MJ/kg)	74.8
Fish meal (650 g/kg CP)	73
Calcium carbonate	12.1
Dicalcium phosphate	1
Sodium chloride	2.8
Source of vitamins and minerals permix[1]	5
DL-Methionine	0.3
Washed sand	15
Total	100.00
Calculated values	
Metabolisable energy (MJ/kg)	13.1
Crude protein (g/kg)	25.90
Lysine (g/kg)	1.4
Total sulphur amino acids	0.81
Calcium (g/kg)	0.86
Crude fibre (g/kg)	2.95
Non-phytate phosphorus (g/kg)	0.32

[1]Supplied the following per kilogram of diet: retinyl acetate, 2.7 mg; cholecalciferol, 0.05 mg; DL-α-tocopheryl acetate, 11.25 mg; menadione sodium bisulphite, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; calcium D-pantothenate, 6.4 mg; pyridoxine, 1.97 mg; nicotinic acid, 28 mg; cyanocobalamine, 0.01 mg; choline chloride, 320 mg; folic acid, 0.38 mg; $MnSO_4 \cdot H_2O$, 60 mg; $FeSO_4 \cdot 7H_2O$, 80 mg; ZnO, 51.74 mg; $CuSO_4 \cdot 5H_2O$, 8 mg; iodised NaCl, 0.8 mg; Na_2SeO_3, 0.2 mg.

During the experimental period (1–28 d of age), body weight (BW) and feed consumption were determined per cage every week and were used to calculate growth rate, feed intake and feed conversion rate (FCR). At the end of trial, litter samples were randomly collected from 4 points of each cage, and litter moisture contents were measured (AOAC method 934.01). Differences in water-holding capacities resulting from MWF supplementation were calculated for different diets based on the average water-holding capacity (5.01 g water/g MWF) based on the method described by Chau et al. (1997).

Sample collection and histology

At 28 d of age, two randomly selected birds from each cage were weighed and euthanised by cervical dislocation. The digestive tract, including contents, from the end of the crop to the cloaca, was removed and weighed. The carcass (without feathers, skin, intestines, heart, proventriculus, gizzard, liver and head), empty gizzard (without fat) and liver weights were recorded. The weights of the organs were expressed relative to live BW (g/kg) and, similarly, the relative lengths of small intestinal parts (duodenum, jejunum and ileum) were measured. The left tibia from each of the birds was excised and all cartilage and muscle were removed. The cleaned bone was weighed and total length and diameters were measured by means of a calliper (Mitutoyo, Japan) with an accuracy of 0.001 cm. The length was defined as the distance from the top of the tibia to the bottom of the groove between the outer and inner malleoli, while the width was defined as the distance between the outer and inner rims of the central shaft of the tibia, namely the central diaphysis. Dried tibias were ashed in a furnace at 600°C for 24 h and cooled in dessicator and ash weights were recorded.

Intestinal selected segments of approximately 2°cm were taken from the midpoint between the bile duct and Meckel's diverticulum (jejunum) and midway between Meckel's diverticulum and the ileo–caecal junction (ileum). These segments were immediately flushed twice with PBS to remove luminal digesta. Tissue samples were fixed in fresh 10% formalin buffer, dehydrated, cleared and embedded in paraffin. Eight sections were cut to a thickness of 5 µm, placed on glass slides, stained with haematoxylin–eosin and examined by light microscopy. All microscopy evaluations were conducted by the same person, with treatments blinded. Ten villi having a *lamina propria* were randomly selected on each slide. Villus height (VH) was defined as the distance from the tip to the base, excluding the intestinal crypt; villus thickness (VT) was calculated from villus at half height and crypt depth (CD) was defined as the distance from the villus base to the *muscularis* layer, not including the intestinal *muscularis*. The VH:CD ratio was calculated.

Mucin staining and goblet cells

Neutral mucin, as an indicator of goblet cells, was assessed by staining with periodic acid-Schiff reagent. Deparaffinised and rehydrated sections were incubated in 5 g/l of periodic acid (Merck, Darmstadt, Germany) for 15 min, washed and incubated with Schiff's reagent (Sigma Chemicals Co., St. Louis, MO) for 30 min. After washing in warm water, the slides were dehydrated and mounted. The density of goblet cells was calculated as the number per 100 µm of villus length and determined by light microscopy (standard 20, Carl Zeiss, Gottingen, Germany).

Bacteriological examinations

Ileal contents from the gut were collected and immediately put on ice, transferred to the lab and prepared for culture. To measure the microbial counts, 1 g of ileal contents was serially diluted, whereby 0.1 ml per dilution was inoculated on plate count agar for aerobes, MRS agar for *Lactobacilli* spp., MacConkay agar for coliforms and Kenner Faecal streptococcus agar for *Streptococci* spp. Total numbers of bacterial colonies were counted at 24 and 48 h incubation. Results were expressed as $\log_{10}$ colony forming units per gram of ileum digesta ($\log_{10}$ CFU/g).

Statistical analysis

The data were analysed as a randomised complete block design with analysis of variances performed using the general linear model procedure of SAS (version 9.3, SAS Institute). Two birds from each cage were sampled for digestive organs, tibial ash content, bacterial and morphological measurements and the average value of the selected birds were used as experimental unit for statistical analysis. Duncan's multiple range test was used to determine significant differences between treatments. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing inclusion level of MWF in diets on each measurement. Differences were considered significant using 95% confidence limits ($P < 0.05$).

The study protocol was conducted in accordance with the Animal Care and Use Review Committee guidelines of Tarbiat Modares University, Tehran, Iran.

Results

Performance

Fibre from MWF inclusion increased BW and daily body weight gain (BWG) and improved FCR of quail ($P < 0.001$) (Table 2). Quail fed diets containing 5 g/kg MWF showed better BWG than quail fed with 15 g/kg MWF ($P < 0.01$). There were linear and quadratic trends between fibre supplementation levels and improvements in both BWG and FCR ($P < 0.01$). MWF inclusion levels had no effect on feed intake (Table 2). There was a significant dose-dependent relationship between MWF concentrations and decreasing feed in the first week of the experiment (day 7; $P = 0.035$) but reached normal rates of feed intake 7 d later (day 14; $P = 0.381$).

Organ weights

Table 3 shows the results for organ weights and gut segments at the end of rearing period. Relative weights of carcass, gizzard, GIT and length of small intestine segments were significantly higher for quail fed diets containing 15 g/kg MWF compared with the other feed treatments. Inclusion of MWF in the diet decreased relative liver weight in quail ($P < 0.01$). There was a linear trend between increasing MWF inclusion level and higher relative gizzard ($P < 0.01$) and GIT weights ($P < 0.05$) and lower liver relative weight ($P < 0.05$). A linear relationship existed between MWF levels and relative length of small intestine segments. However, a quadratic relationship was observed between relative length of duodenum and MWF inclusion ($P < 0.05$) (Table 3).

Table 2. Effect of micronised wheat fibre inclusion in quail diet on growth and feed intake of quails on the different diets at 28 d.

	MWF inclusions (g/kg)				Contrast			
Growth performance	0	5	10	15	*P*	Linear	Quadratic	SEM
Growth (g/bird per d)	5.47^{c}	5.83^{a}	5.78^{ab}	5.75^{b}	<0.0001	<0.0001	<0.0001	0.043
Weight at the d28 (g/bird)	161.52^{c}	171.52^{a}	170.14^{ab}	169.62^{b}	<0.0001	<0.0001	<0.0001	1.190
Feed intake (g/bird per d)	15.45	15.48	15.48	15.46	0.9015	0.8111	0.5241	0.012
FCR (kg feed/kg weight gain)	2.75^{a}	2.57^{b}	2.59^{b}	2.60^{b}	<0.0001	<0.0001	<0.0001	0.022
Litter moisture (g/kg)	643.5^{a}	565.5^{b}	581.7^{b}	613.3^{ab}	0.0108	0.1521	0.0017	10.64

Mean, $N = 12$ pens, three pens per diet treatment. $^{a–c}$Means not sharing a common superscript are significantly different ($P \leq 0.05$).

Table 3. Effects of micronised wheat fibre inclusion in quail diet on digestive organs.

	MWF inclusions (g/kg)				Contrast			
Items	0	5	10	15	*P*	Linear	Quadratic	SEM
Carcass relative weight (g/kg)	610.5^{b}	621.3^{a}	618.2^{a}	618.7^{a}	0.0345	0.133	0.103	1.60
Liver relative weight (g/kg)	0.28^{a}	0.22^{b}	0.22^{b}	0.21^{b}	0.0041	0.023	0.220	0.110
Gizzard relative weight (g/kg)	24.5^{b}	24.7^{b}	27.5^{b}	33.8^{a}	0.0495	0.003	0.144	1.18
GIT relative weight (g/kg)	76.7^{b}	77.4^{b}	79.4^{b}	89.3^{a}	0.0026	0.024	0.232	1.90
Duodenum relative length (cm/kg)	50.5^{b}	51.5^{b}	52.6^{b}	62.4^{a}	0.0076	0.001	0.041	1.34
Jejunum relative length (cm/kg)	101.1^{c}	113.4^{b}	114.8^{b}	126.7^{a}	0.0008	0.001	0.091	2.38
Ileum relative length (cm/kg)	92.9^{b}	102.8^{b}	102.8^{b}	117.0^{a}	0.0164	0.013	0.071	2.68

$^{a–c}$Means not sharing a common superscript are significantly different ($P \leq 0.05$).

Table 4. Effect of levels of micronised wheat fibre in quail diets on small intestinal properties.

	MWF inclusions (g/kg)				Contrast			
	0	5	10	15	*P*	Linear	Quadratic	SEM
Jejunum villus height (mm)	0.45^{d}	0.47^{c}	0.51^{b}	0.53^{a}	<0.0001	<0.0001	0.275	0.006
Jejunum crypt depth (mm)	0.048	0.048	0.049	0.0501	0.454	0.0652	0.491	0.0005
Jejunum villus height to crypt depth ratio	9.48^{c}	9.87^{bc}	10.29^{ab}	10.53^{a}	0.019	0.0007	0.671	0.121
Jejunum villus thickness (mm)	0.117^{b}	0.124^{ab}	0.129^{a}	0.131^{a}	0.0070	0.0003	0.275	0.002
Jejunum goblet numbers	6.28^{b}	7.95^{a}	7.63^{a}	7.78^{a}	0.0029	0.0008	0.508	0.182
Ileum villus height (mm)	0.35^{c}	0.40^{b}	0.41^{ab}	0.42^{a}	<0.0001	<0.0001	0.001	0.006
Ileum crypt depth (mm)	0.051	0.051	0.051	0.051	0.9423	0.3815	0.734	0.0003
Ileum villus height-to-crypt-depth ratio	6.93^{b}	7.92^{a}	7.98^{a}	8.12^{a}	0.0002	<0.0001	0.007	0.116
Ileum villus thickness (mm)	0.110^{b}	0.112^{ab}	0.115^{ab}	0.118^{a}	0.0442	0.0161	0.775	0.001
Ileum goblet numbers	5.38^{b}	7.13^{a}	7.10^{a}	7.43^{a}	0.0028	0.0005	0.042	0.229

$^{a–c}$Means not sharing a common superscript are significantly different ($P \leq 0.05$).

Small intestine morphology

There were differences in the morphology of the intestinal mucosa of quail fed the experimental feeds at the end of the period of feeding (Table 4). In the jejunum and ileum, VH, VT, VH:CD and goblet numbers from samples from quail on the MWF-supplemented diets were higher ($P < 0.01$) than those observed in the unsupplemented group. There were no significant differences between MWF-supplemented and un-supplemented groups regarding to CD. Except for ileal CD, there was a linear relationship between increase in VH, VT, VH:CD and CD with higher inclusion of MWF of quail diets (Table 4).

Tibial ash content

Tibia dimensions and ash content were significantly influenced by MWF inclusion during the rearing period (Table 5). The weight of tibia ($P < 0.01$) and the ash content ($P < 0.05$) was higher for the 15-g/kg MWF supplemented group compared with those in the other groups. There was a linear relationship between fibre content and tibia weight, length and ash content ($P < 0.01$).

Intestinal microbial counts

The results for intestinal microbial counts are presented in Table 6. There were no significant differences in *Streptococci* spp., *Lactobacilli* spp., coliforms and total aerobic counts in the small intestine from quail fed diets with or without MWF supplementation (Table 6). However, the number of *Streptococci* spp. was linearly reduced ($P = 0.04$) as the concentration of MWF increased in the feed (Table 6).

Litter moisture

There was a significant difference in litter moisture ($P < 0.01$) between quail fed diets containing MWF at either 5 g/kg and the 0 g/kg control giving the lowest and highest litter moister, respectively. Moisture responded in a quadratic manner with MWF inclusion ($P < 0.01$; Table 2). The calculated increase in water-holding capacities attributed to MWF was 1.5, 2.0 and 2.6 g/kg for supplementation levels of 5, 10 and 15 g/kg, respectively.

Discussion

Performance

The inclusion of 5, 10 and 15 g/kg of MWF in place of sand improved BWG, BW at day 28 and FCR from 1 to 28 d of age, indicating that young quail require a certain amount of fibre in the diet to maximise growth performance. The results indicated that quails fed with diet supplemented with 15 g/kg MWF had 5% higher BWG and 5.5% lower FCR than those fed with 0 g/kg fibre-supplemented diet. This finding is consistent with Rezaei et al. (2011), where inclusion of 3, 4 and 5 g/kg insoluble fibre improved BWG and FCR linearly in broiler chickens by 4.5% and 5%, respectively. Sarikhan et al. (2010) reported that BWG was increased by the inclusion of insoluble fibre in broilers diet. Jiménez-Moreno et al. (2009) observed that the inclusion of 30 g/kg oat hulls and 30 g/kg sugar beet pulp as fibre sources in a rice–soybean meal diet improved broiler performance from 1 to 21 d of age. These findings are consistent with data reported by Gonzalez-Alvarado et al. (2007) using different fibre sources in broiler diets.

Broilers may compensate for decreased nutrient concentration associated with insoluble fibre inclusion by increasing feed intake (Mourao et al. 2008). Gonzalez Alvarado et al. (2010) reported that oat hull, containing higher lignin and cellulose, increased level of insoluble fibre resulting in a higher passage of digesta through the distal part of the GIT, leading to increased feed intake. Increased feed intake may be associated with an increased gut volume (Hetland and Svihus 2001). Interestingly, in this experiment, the quail fed 5, 10 and 15 g/kg MWF inclusion had almost the same feed

Table 5. Effect of micronised wheat fibre inclusion in quail diet on tibial ash content.

	MWF inclusions (g/kg)				Contrast			
	0	5	10	15	*P*	Linear	Quadratic	SEM
Tibia weight (g)	0.45^{c}	0.49^{bc}	0.55^{ab}	0.62^{a}	0.0142	0.0009	0.558	0.020
Tibia length (mm)	51.67	53.27	54.58	57.54	0.3017	0.0382	0.616	0.822
Tibia diameter (mm)	2.92	3.00	2.88	3.05	0.8025	0.5975	0.728	0.047
Tibia ash content (g/kg)	461.7^{b}	472.7^{b}	470.6^{b}	493.3^{a}	0.0152	0.0017	0.183	3.80

$^{a–c}$Means not sharing a common superscript are significantly different ($P \leq 0.05$).

Table 6. The effect of micronised wheat fibre inclusion on quail diet on intestinal microbial counts (log_{10} CFU/g).

	MWF inclusions (g/kg)				Contrast			
	0	5	10	15	*P*	Linear	Quadratic	SEM
Streptococci	7.98	7.12	7.16	6.61	0.284	0.040	0.463	0.247
Lactobacilli	9.48	8.50	7.86	8.52	0.358	0.146	0.134	0.255
Coliforms	9.22	7.90	8.03	8.10	0.245	0.133	0.145	0.242
Total aerobes	9.54	8.47	8.04	8.57	0.325	0.148	0.125	0.247

intake as chickens fed 0 g/kg MWF. This finding is somewhat in agreement with Gonzalez Alvarado et al. (2010) who reported that a moderate level of insoluble fibre, i.e. 30 g/kg, in broiler chickens feed had less effect on feed intake compared to 50 g/kg inclusion. Similarly, Sarikhan et al. (2010) concluded that feeding wheat insoluble fibre at less than 10 g/kg in broiler diets did not affect their feed intake. However, Pettersson and Razdan (1993) reported that the chickens fed the lowest inclusion of sugar beet pulp as a source of fibre (23 g/kg feed) had higher feed intake in comparison to 46 and 92 g/kg. Therefore, Gonzalez Alvarado et al. (2010) suggested that effects of dietary fibre on broiler chicken performance could be altered by type of fibre.

Insoluble dietary fibre in broiler diets increased starch digestibility, enhanced the gizzard activity (Rogel et al. 1987; Hetland and Svihus 2001) and increased gastro-duodenal reflux (Hetland et al. 2003). Hetland et al. (2003) found that the level of bile acid and digesta in the gizzard increased by the amount of the insoluble fibre in the broiler diets.

Feed particle size reduction is thought to improve the digestion by increasing the surface area available to digestive enzymes (Amerah et al. 2007), and this current experiment suggested that the distribution of MWF throughout the digesta could increase the efficiency of enzymatic degradation of feed ingredients by decreasing the bulking properties of feed, which implies increased penetration of digestive enzymes into the digesta (Amerah et al. 2007). However, the effect of fibre particle size is dependent on its source. Ricke et al. (1982) did not find any difference in performance in broilers fed diets containing 80 g/kg alfalfa with a particle size of less than 300 μm or more than 600 μm. In contrast, Hetland and Svihus (2001) observed that, from 8 to 17 d of age, FCR was impaired in broilers fed diets containing 100 g/kg coarsely ground oat hulls as compared with birds fed 100 g/kg finely ground oat hulls (547 vs.. 60 g/kg of particles with a geometric mean diameter of more than 600 μm). The better intestinal morphological conditions observed in birds fed a diet supplemented with MWF were evidenced by their higher BW and greater efficiency of feed utilisation. The positive effect of MWF on nutrient utilisation confirms findings of studies in which processed fibre was included in broiler diets (Sarikhan et al. 2010).

Quail fed the high-fibre diet showed reduced feed intake at the beginning of the feeding trial (7 d) but resumed normal rates of feed intake by day 14. This reduction of intake follows the same patterns reported in Starck (1999) and Starck and Rahmaan (2003), whereby adding fibre in diet reduced feed intake rate and increased gizzard and intestine size. Normal or elevated intake rates were re-established after 6 d, i.e. when organ and gut size had enlarged to accommodate the functional demands of the changed diet composition.

Organ weights from the current experiment indicated that MWF-inclusion significantly increased duodenal, ileal and jejunal length. Gastrointestinal length tended to increase with the diet supplemented with higher levels of MWF. In addition, MWF inclusion tended to increase relative weight of gizzard and GIT. Furthermore, the lengths of duodenum, jejunum and ileum were significantly increased by 15 g/kg MWF supplementation, compared to 5 and 10 g/kg MWF levels. The results in the present study corresponds with the study by Khempaka et al. (2009) who stated that ileal and jejunal length increased by insoluble fibre inclusion in broiler diets. In contrast, Rogel et al. (1987) observed that lengths and weights of segments of the small intestine of broilers decreased when the birds received 100 g/kg oat hulls in feed. Taylor and Jones (2004) reported that a gizzard enlargement may lead to a reduction of the relative weight of the small intestine, which in turn may reflect an adaptation of the gut to an increased availability of nutrients. Others have observed accumulations of gizzard contents, acidification of digesta in the gizzard and gizzard enlargement when feeding insoluble fibre (Gonzalez-Alvarado et al. 2007). This result was supported by Rogel et al. (1987) and Riddell (1976) who reported gizzard enlargement due to insoluble fibre inclusion in the feed. A more muscular and enlarged gizzard can improve digestion, as the feed is retained for a longer time in the upper digestive tract (proventriculus and gizzard). As suggested by Hetland et al. (2005), the gizzard plays a main role for digesta gastro-duodenal reflux and may be unable to affect the digesta movements when lacking feed stimuli. This suggests that birds need to have structural components such as dietary fibre for increasing the anterior digestive tract activity, including the gizzard.

In the current study, liver weight was affected considerably by MWF inclusion. Jiménez-Moreno et al. (2009) reported that liver weight was not affected by fibre inclusion. Liver size has been reported to decline in quail feed a high-fibre diet (Starck 1999). Analysis of liver composition permitted an assessment of whether these changes are based on lipid content, i.e. declining energy stores, or on changes in cell numbers, which would imply a difference in metabolic capacity of the liver. Akiba and Matsumoto (1982) showed that fibre may reduce liver lipid deposition and plasma lipid content in chickens fed *ad libitum*. Akiba and Matsumoto (1978) reported that plasma lipid was suppressed by cellulose inclusion in the diet and that feeding oat hulls led to a reduction in liver lipid in chickens. However, these findings did not reveal whether the lipid reduction in the liver was caused by fibre content or reduced energy intake by dilution (Akiba and Matsumoto 1978).

Shahin and Abd El Azeem (2006) demonstrated that high fibre in broilers' diet (80 g/kg feed) suppressed carcass weight in relation to live weight compared to low fibre

(40 g/kg) inclusion. Mourao et al. (2008) reported that broilers fed oat hulls had lower carcass weights relative to BW due to a more developed gastrointestinal tract. However, the results from the current trial were not in agreement with their findings, as the relative weight of carcass was improved by MWF inclusions in the quail diets.

Sklan et al. (2003) reported that total small intestinal length and surface area were increased by increasing dietary crude fibre intake in turkeys, which agree with the findings of the current trial. Although the inclusion of MWF in quail diets did not influence ileal CD, it significantly affected the VH, VT and VH:CD ratio owing to the gradual, cumulative effects of MWF on the VH and CD in different directions. Supplementation of MWF thus enhanced the mucosal absorptive surface area, as evidenced by increases in VH, and the VH:CD ratio and decreases in the CD (Dibner et al. 1996; Rezaei et al. 2011). Birds which develop greater villus surface area in the gut have greater nutrient absorption. Because of the lower crypt cell proliferation and greater absorptive villus surface area, the nutrients spared in birds supplemented with MWF resulted in a greater BWG and lower FCR. The MWF-induced increase in gut VH suggests that the fibre may exert some beneficial effects on intestinal mucosa. In agreement with this finding, Sarikhan et al. (2010) reported that inclusion of 5 and 7.5 g/kg insoluble fibre in the diet resulted in greater VH in broilers. Indeed, in other species, cellulose powder has been shown to enhance cell proliferation in the gastrointestinal tract (Dirks and Freeman 1987).

Several studies have indicated that insoluble sources such as wheat bran (Satchithanandam et al. 1989) and citrus fibre (Satchithanandam et al. 1990), and soluble fibres such as guar and oat gums (Begin et al. 1989), enhance mucin production. This is apparently due to an increase in the number of goblet cells (Schneeman et al. 1982), which are responsible for secretion of the mucin lining of the intestinal epithelium. These effects last even after discontinuing feeding fibre, for example rats maintained for 15 d on a diet containing oat gum had a higher viscosity of the intestinal contents after a 13-h fast than rats fed a fibre-free diet, although no β-glucan was detected in their intestinal content (Begin et al. 1989). In the current study, the numbers of goblet cells linearly increased with the levels of MWF in quail's diets. Rezaei et al. (2011) showed that the numbers of goblet cells in ileal villi of broiler linearly increased by increased levels of insoluble fibres in diets, which agrees the results in the present study.

Different mechanisms whereby indigestible dietary polysaccharides interfere with mineral balance of chicks have been suggested (Vanderaar et al. 1983). These have included chelating of cations by polysaccharides, retention of metal ions in pores of the fibre matrix, reduced transit time and increased endogenous minerals in microbial mass. In addition, dietary fibre may result in a decrease in concentration of cation-binding protein in the brush border of the small intestine, which inhibits mineral absorption (Vanderaar et al. 1983). Numerous negative effects of soluble dietary fibre on Ca, Zn, Cu and Fe balance have been reported. Hetland and Svihus (2001) reported that the apparent ileal uptake of ash was not modified when insoluble fibre was included in the broiler diets. Coarse feed particles remain longer in the upper part of the GIT, stimulating gizzard activity (Hetland et al. 2005) and increasing the production of HCl (Duke 1986). A low gizzard pH improves pepsin activity and nitrogen retention and increases the solubility of the mineral fraction of the feed (Guinotte et al. 1995), which in turn can favour its absorption. Souffrant (2001) and Hetland et al. (2005) reported that, because of its non-viscous nature, insoluble NSP may have beneficial effects on digestive processes. The current trial showed a linear relationship between MWF supplementation and increased tibia weight, length and ash content. This indicated a higher mineral uptake due to the effect of MWF on the mucosal layer of the intestine (Table 4), resulting in a higher calcium and phosphorus uptake from the digesta.

There was a quadratic relationship between MWF supplementation in feed and decreased litter moisture in the quail, a relationship not reported previously. Using insoluble fibre in broiler diets showed a dose-dependent relationship with litter moisture content (Rezaei et al. 2011). Rezaei et al. (2011) reported that the lower moisture, especially during the second half of the production period (days 22–42), may be indicative of the effect of insoluble fibre on the mucosal layer of the intestine, resulting from increased nutrient and water absorption from the digesta. Although Market and Backers (2003) attributed the decreased litter moisture observed in birds supplemented with micronised insoluble fibre to the high water-holding capacity of fibre source (up to 8 times its weight), the current calculations showed only slight differences in the water-holding capacity of the experimental diets.

Dietary fibre components are not digested by endogenous digestive enzymes and consequently are substrates for bacterial fermentation in the distal part of the gut (Montagne et al. 2003). The main products of fermentation are short chain fatty acids (SCFAs), predominantly lactate, acetate, propionate and butyrate. The SCFA may assist in developing the digestive tract by stimulating epithelium cell proliferation (Montagne et al. 2003). In an acidic environment, SFCA can inhibit the growth of enteric bacterial pathogens, such as *Salmonella, Escherichia coli* and *Clostridium* spp. (Montagne et al. 2003). The bacterial composition (quantity and proportion) is species specific (Moore et al. 1987) and varies depending on age, physiological state, gut region, as well as diet composition and the presence and nature of fibre. Quail harbour a permanent microflora in the digestive tract, mainly consisting of *Lactobacilli* spp. (Wilkinson et al. 2016).

Earlier studies demonstrated that inclusion of cellulose in a broiler diet can modify gut microflora by stimulating the growth of *Lactobacilli* and *Bifidobacteria* spp. (Cao et al. 2003). The effect of cellulose and lignin on ileal and caecal microflora is dose dependent. Cao et al. (2003) reported that feeding a high level of alpha-cellulose (100 g/kg) in chicken diets significantly enhanced caecal *Bifidobacteria* and *Lactobacilli* spp. compared to birds receiving diets containing 0 and 35 g/kg cellulose but did not affect the number of *Clostridia* and *Enterobacteriaceae* spp. Jiménez Moreno et al. (2011) studied the effects of including 50 g/kg oat hulls or sugar beet pulp in the diets of broilers, reared in floor pens with access to wood shavings litter, on *Lactobacilli* spp. counts in the crop and caeca and *Clostridium perfringens* and *Enterobacteriaceae* spp. in the caeca. *Lactobacilli* spp. in the crop increased with the inclusion

of sugar beet pulp, but not with oat hulls. However, no effects of dietary fibre on *Lactobacilli* spp. were detected in the caeca. On the other hand, the counts of *C. perfringens* and *Enterobacteriaceae* spp. in the caeca decreased significantly with oat hulls inclusion but were not affected by sugar beet pulp. The fibre levels in the diets in this study did not change *Lactobacilli* spp., coliforms and the total aerobes numbers in quail ileal contents while decreased the total number of *Streptococci* spp.

Quail grew faster when fed the low insoluble fibre-supplemented diet, indicating a difference in the response to fibre levels. Although the MWF supplemented birds were more efficient from a feed conversion point of view, the lower number of *Streptococci* spp. observed in the gut of fibre-supplemented group should be considered in relation to its prebiotic properties. Furthermore, higher level of tibia weight, length and ash content in the fibre-supplemented groups could be a good potential help to prevent growth-related leg problems during the late rearing period in meat type birds.

Disclosure statement

No potential conflict of interest was reported by the authors.

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