



## OPEN Implication of dietary barberry (*Berberis Vulgaris*) leaves inclusion on growth performance, nutrient digestibility, and carcass traits in ostriches

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This study determined production performance, nutrient intake, digestibility, and carcass traits from ostrich chicks fed with barberry (*Berberis Vulgaris*) leaves (BVL) as a replacement for alfalfa hay (AH). For 150 days, 30 male ostrich (*Struthio camelus*) (9.10 ± 0.89 kg live weight, mean ± SD) were randomly grouped into five dietary BVL inclusion levels to replace AH: a control diet based on 100% AH (CTRL), and four groups with BVL as a substitute for AH at varying levels including 25% (BVL25), 50% (BVL50), 75% (BVL75), and 100% (BVL100). The average daily feed intakes (ADFI) were recorded and birds were weighed monthly. The nutrient intake and digestibility were measured for the following final 5 days of the trial. The ADFI was higher ( $p = 0.025$ ) for BVL100 and BVL75 than for CTRL. Ostriches fed on BVL50 had the highest average daily gain (ADG,  $p = 0.025$ ) and the lowest feed conversion ratio (FCR,  $p = 0.0001$ ). Return per kg BW gain was also enhanced ( $p = 0.018$ ) with BVL50 feeding. Dietary BVL levels did not affect the digestibility of OM ( $p = 0.257$ ), CP ( $p = 0.260$ ), EE ( $p = 0.610$ ), CF ( $p = 0.427$ ), and Ash ( $p = 0.461$ ). Also, there were no changes in AME ( $p = 0.180$ ) or AMEn ( $p = 0.670$ ). The hot carcass weight (HCW,  $p = 0.331$ ) and dressing BW ( $p = 0.237$ ) were not affected. Overall, the results suggest that BVL can replace AH in ostrich diets without deleterious impact on performance, however, its partial substitution (for 50% AH of diet) has been associated with improved ADG, FCR, and reduced feeding costs.

**Keywords** *Berberis vulgaris*, Digestibility, Growth performance, Ostriches, Sustainability

The ever-increasing human population in the last decades has increased the demand for animal products worldwide, including meat<sup>1,2</sup>. The ostrich (*Struthio camelus var. domesticus*) meat has been recently introduced as a promising substitute for conventional red meat due to its low intramuscular fat content, desirable fatty acids (FAs) profile, and rich heme-iron that make it a healthy red meat appreciated by modern consumers<sup>3,4</sup>. The current world production of ostrich meat is 12,000 to 15,000 tons per year and Iran with over 60,000 ostriches per year contributes to 3000 to 3600 tons of produced meat, ranked the second biggest producer of ostrich meat after South Africa<sup>5,6</sup>. In modern farming systems, ostriches are reared mainly under intensive feedlots with formulated diets using both forages and concentrates<sup>7</sup>. As with other livestock, feed supply contributes to the highest percentage (for nearly 80%) of total ostriche production costs<sup>8–10</sup>. Therefore, appropriate and feasible feed resources are required to ensure the sustainability of production. Crop residues, agro-industrial by-products, shrubs, and tree foliage are usually considered alternate feeding resources that mainly contain bioactive profile with the potential favor effects on animal health and quality of products<sup>11–13</sup>.

Barberry (*Berberis vulgaris*) is a genus of spiny deciduous evergreen shrubs in the *Berberidaceae* family. The plant attains a height of 1–3 m with ovate-shaped leaves, bearing pendulous yellow flowers succeeded by

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oblong-shaped red fruits<sup>13,14</sup>. Iran is acknowledged as the greatest producer of barberry with a cultivation area exceeding 11,000 hectares yields more than 9200 tons of dried fruit annually<sup>14</sup>. Although *Berberis vulgaris* leaf (BVL) is a remarkable biomass production in Iran it mainly remains useless and the current methods of its disposal through landfill and burning are accompanied by environmental concerns<sup>15</sup>. Hence, the use of BVL as a low-cost feed for replacing conventional forages in animal diets appears to be a promising topic for sustainable livestock production. It has been well documented that BVL is a palatable and digestible (69.9%) feedstuff for herbivorous animals<sup>16</sup>. Moreover, the dietary inclusion of BVL for fattening lambs (at 7.5% and 15% dry matter [DM] of diet)<sup>16</sup>, and dairy goats (at 17.5% and 34% DM of diet)<sup>13</sup> have positive consequences on nutrient intake and the oxidative status of animal of without deleterious effects performance. Interestingly, BVL elevated antioxidants in muscle and enhanced the color stability of lamb meat<sup>16</sup>. Meanwhile, the anti-oxidative, anti-inflammatory, and immune-enhancing features of BVL and its main bioactive compound (berberine) have been reviewed by Ghavipanje et al.<sup>17</sup>. The therapeutic priorities of BVL have been attributed to its abundant bioactive phytochemicals, including flavonoids, sterols, vitamins, carotenoids, and especially alkaloids<sup>14,17</sup>. Thus, BVL can be used not only as a low-cost and eco-friendly but also as a biological activity and functional feed source in animal diets<sup>16</sup>.

To the best of our knowledge, no study investigated the effect of dietary BVL on the growing performance of ostrich chicks. We hypothesized that the dietary inclusion of BVL for ostrich seems likely to be a sustainable and economically viable alternative feed ingredient due to the unique digestive system in ostrich chicks enabling them to utilize high-fiber diets. Thus, the objective of this study was to investigate the possibility of using BVL as an alternative for alfalfa hay (AH) in diets fed to young ostriches and to determine the effects on growth performance, coefficients of total tract apparent digestibility of nutrients, and carcass traits. Results from this study will orientate farms for the possibility of dietary BVL use for growing ostrich.

## Results

### Growth performance

The BVL inclusion in diet as a substitute for AH altered the overall ADFI ( $p=0.007$ ; Table 1). The ADFI was higher for BVL100 and BVL75 than CTRL, with BVL50 being intermediate. In addition, the animals fed on BVL50 had the highest ADG compared to CTRL ( $p=0.025$ ), however, no differences were observed between the BVL25 and CTRL groups. The substituting of 50, 75, and 100% AH of diet by BVL (i.e. BVL50, BVL75, and BVL100) resulted in 15%, 4.6%, and 5.7% higher ADG compared to the CTRL, respectively. Furthermore, the lowest FCR values were found in BVL50 ( $p=0.0001$ ). Feed cost per kg BW gain decreased with BVL50, BVL75, and BVL100 (linear,  $p=0.012$ ; quadratic,  $p=0.048$ ) compare to CTRL. An increase (linear,  $p=0.022$ ) in return per kg BW gain was also observed with BVL50 feeding.

### Nutrient intake and digestibility

The intake of nutrients and ATTDC are presented in Table 2. There were no treatment effects for the intakes of OM ( $p=0.257$ ), CP ( $p=0.260$ ), EE ( $p=0.610$ ), CF ( $p=0.427$ ), and Ash ( $p=0.461$ ). Likewise, the different dietary BVL levels for ostriches did not affect the digestibility of OM ( $p=0.662$ ), CP ( $p=0.214$ ), EE ( $p=0.447$ ), CF ( $p=0.289$ ), and Ash ( $p=0.409$ ).

### Apparent metabolizable energy

No significant effects between treatments were observed regarding AME ( $p=0.180$ ) and AMEn ( $p=0.670$ ) (Table 3). Also, there were no changes in energy ( $p=0.660$ ) and protein ( $p=0.630$ ) efficiency in ostriches with BVL inclusion (Table 3).

### Carcass characteristics

The highest ( $p=0.028$ ) final body weight (FBW) was for the BVL50 group followed by BVL75 and BVL100 group (Table 4). The hot carcass weight (HCW,  $p=0.331$ ) and dressing BW ( $p=0.237$ ) were not altered with BVL feeding. Regarding the effect of diets on internal organs, there were no effects on weights heart ( $p=0.566$ ); liver ( $p=0.436$ ), visceral fat ( $p=0.257$ ), and gizzard ( $p=0.395$ ).

## Discussion

Globally, the number of people suffering from hunger rose to 828 million, an increase of about 46 million since 2020 and 150 million since the outbreak of the COVID-19 pandemic, hence, the world's demand for animal protein is increasing at an alarming rate<sup>18</sup>. However, the animal production systems are threatened with some occasional challenges including climatic changes, water crisis, land degradation, and food-feed competition making animal livestock unsustainable at global scales<sup>1</sup>. In this context, the use of agro-industrial by products can decrease the feeding cost and improve animal production in terms of quality and sustainability<sup>1,16</sup>. BVL has been proposed to be a great agricultural by-product for livestock feeding<sup>13,15,16,19</sup>, however, as we know, no data are available concerning BVL for ostriches. In the current study, AH was partially and totally replaced by BVL, and we found that substituting 50% AH of diet by BVL in growing ostriches improved nutrients intake and growth performance while reducing production costs without deleterious effects on digestibility and carcass traits.

### Growth performance

In the present research, ADFI, ADG, and FCR values were within the normal range suggested for growing ostriches in earlier reports<sup>10</sup>. The ADFI of growing ostriches in previous reports was between 1.5 and 2.1 kg/day and FCR between 5 and 10, which agreed with this study<sup>10,20</sup>. The ADG of birds at this stage of growth has been

| Parameters <sup>1</sup>                         | Time (month) | Diets <sup>6</sup>  |                      |                       |                      |                      | SEM <sup>7</sup> | p-values |        |           |
|---|--------------|---------------------|----------------------|-----------------------|----------------------|----------------------|------------------|----------|--------|-----------|
|   |              | CTRL                | BVL25                | BVL50                 | BVL75                | BVL100               |                  | Diet     | Linear | Quadratic |
| ADFI, g/day/bird                                | 1            | 719.6 <sup>a</sup>  | 782.9 <sup>b</sup>   | 773.5 <sup>b</sup>    | 882.8 <sup>ab</sup>  | 949.9 <sup>a</sup>   | 38.12            | 0.005    | 0.637  | 0.378     |
|   | 2            | 837.6 <sup>c</sup>  | 773.9 <sup>b</sup>   | 966.7 <sup>abc</sup>  | 1079.2 <sup>ab</sup> | 1238.0 <sup>a</sup>  | 65.62            | 0.001    | 0.730  | 0.155     |
|   | 3            | 1628.9 <sup>c</sup> | 1505.6 <sup>b</sup>  | 1845.6 <sup>ab</sup>  | 1825.6 <sup>ab</sup> | 1996.3 <sup>a</sup>  | 57.93            | 0.001    | 0.615  | 0.400     |
|   | 4            | 1978.1 <sup>c</sup> | 2002.9 <sup>bc</sup> | 2090.8 <sup>abc</sup> | 2169.4 <sup>ab</sup> | 2290.1 <sup>a</sup>  | 43.59            | 0.007    | 0.236  | 0.789     |
|   | 5            | 2168.1              | 2132.7               | 2174.2                | 2230.0               | 2190.59              | 68.62            | 0.889    | 0.927  | 0.917     |
|   | Overall      | 1566.5 <sup>b</sup> | 1539.6 <sup>b</sup>  | 1668.2 <sup>ab</sup>  | 1734.4 <sup>a</sup>  | 1816.8 <sup>a</sup>  | 38.89            | 0.007    | 0.557  | 0.309     |
| ADG, g/day/bird                                 | 1            | 344.2 <sup>c</sup>  | 353.3 <sup>b</sup>   | 415.0 <sup>a</sup>    | 396.7 <sup>ab</sup>  | 379.2 <sup>abc</sup> | 10.61            | 0.001    | 0.062  | 0.100     |
|   | 2            | 287.5               | 283.3                | 384.3                 | 326.7                | 340.0                | 33.33            | 0.239    | 0.210  | 0.353     |
|   | 3            | 479.2               | 455.8                | 538.3                 | 503.3                | 512.5                | 31.42            | 0.432    | 0.467  | 0.665     |
|   | 4            | 398.3               | 376.7                | 450.0                 | 386.7                | 448.3                | 65.71            | 0.886    | 0.870  | 0.901     |
|   | 5            | 440.0               | 426.7                | 455.8                 | 425.5                | 365.0                | 34.87            | 0.449    | 0.452  | 0.237     |
|   | Overall      | 389.8 <sup>b</sup>  | 379.1 <sup>b</sup>   | 448.7 <sup>a</sup>    | 407.7 <sup>ab</sup>  | 412.3 <sup>ab</sup>  | 13.66            | 0.025    | 0.110  | 0.213     |
| FCR, g/g  | 1            | 2.09 <sup>ab</sup>  | 2.24 <sup>ab</sup>   | 1.86 <sup>b</sup>     | 2.22 <sup>ab</sup>   | 2.51 <sup>a</sup>    | 0.130            | 0.040    | 0.214  | 0.077     |
|   | 2            | 2.93                | 2.86                 | 2.54                  | 3.37                 | 3.85                 | 0.380            | 0.180    | 0.340  | 0.120     |
|   | 3            | 3.43                | 3.32                 | 3.46                  | 3.65                 | 4.01                 | 0.270            | 0.443    | 0.651  | 0.320     |
|   | 4            | 5.27                | 5.94                 | 4.78                  | 5.97                 | 5.33                 | 0.790            | 0.805    | 0.831  | 0.920     |
|   | 5            | 5.01                | 5.08                 | 4.85                  | 5.33                 | 6.16                 | 0.450            | 0.320    | 0.441  | 0.189     |
|   | Overall      | 4.03 <sup>ab</sup>  | 4.07 <sup>ab</sup>   | 3.72 <sup>b</sup>     | 4.28 <sup>ab</sup>   | 4.40 <sup>a</sup>    | 0.130            | 0.020    | 0.069  | 0.216     |
| Feed cost per kg BW gain <sup>1,3,4</sup> , USD | 1            | 0.298               | 0.302                | 0.238                 | 0.270                | 0.288                | 0.025            | 0.112    | 0.077  | 0.105     |
|   | 2            | 0.418               | 0.386                | 0.324                 | 0.410                | 0.442                | 0.047            | 0.494    | 0.187  | 0.132     |
|   | 3            | 0.490               | 0.448                | 0.442                 | 0.443                | 0.460                | 0.034            | 0.857    | 0.263  | 0.316     |
|   | 4            | 0.752               | 0.802                | 0.609                 | 0.725                | 0.612                | 0.104            | 0.614    | 0.802  | 0.850     |
|   | 5            | 0.715               | 0.686                | 0.619                 | 0.647                | 0.706                | 0.057            | 0.738    | 0.187  | 0.202     |
|   | Overall      | 0.574 <sup>a</sup>  | 0.549 <sup>ab</sup>  | 0.475 <sup>c</sup>    | 0.520 <sup>bc</sup>  | 0.505 <sup>bc</sup>  | 0.018            | 0.012    | 0.012  | 0.048     |
| Return per kg BW gain <sup>2,3,5</sup> , USD    | 1            | 1.54                | 1.54                 | 1.61                  | 1.56                 | 1.55                 | 0.019            | 0.131    | 0.074  | 0.085     |
|   | 2            | 1.41                | 1.44                 | 1.52                  | 1.40                 | 1.38                 | 0.054            | 0.454    | 0.208  | 0.133     |
|   | 3            | 1.33                | 1.37                 | 1.38                  | 1.37                 | 1.36                 | 0.039            | 0.706    | 0.317  | 0.343     |
|   | 4            | 1.03                | 0.97                 | 1.18                  | 1.05                 | 1.17                 | 0.112            | 0.566    | 0.833  | 0.855     |
|   | 5            | 1.07                | 1.10                 | 1.18                  | 1.13                 | 1.07                 | 0.065            | 0.232    | 0.203  | 0.192     |
|   | Overall      | 1.23 <sup>b</sup>   | 1.24 <sup>b</sup>    | 1.34 <sup>a</sup>     | 1.28 <sup>ab</sup>   | 1.28 <sup>ab</sup>   | 0.010            | 0.018    | 0.022  | 0.630     |

**Table 1.** Growth performance and feeding cost in ostriches fed *Berberis vulgaris* leaf (BVL) included diets. Within row, different letters (a–c) indicate difference between diets ( $p \leq 0.05$ ). Values are least-square means. <sup>1</sup>IBW, initial body weight; FBW, final body weight; ADFI, average daily feed intake; ADG, average daily gain; FCR, Feed conversion ratio. <sup>2</sup>Each kilogram of AH and BVL was 0.270 and 0.200 USD. <sup>3</sup>Calculations are made with the following exchange: 1 USD = 274,000 IR Rials. <sup>4</sup>Total feed cost per kg BW. <sup>5</sup>Calculated as total feed cost per kg BW/total income per kg BW<sup>19</sup>. <sup>6</sup>Experimental diets consisting of a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100%. <sup>7</sup>SEM, Pooled standard error of the mean.

reported<sup>21</sup> to be 200–460 g/day/bird which is also in line with our results. The variation in the reports could be attributed to differences in the chemical and physical composition of diets, growing conditions, and genetics<sup>7</sup>.

Even though increasing the inclusion levels of BVL as a substitute for AH raised the ADFI compared to CTRL, the lower weight gain in BVL75 and BVL100 resulted in worsened FCR (6.2% and 9.2% increase in BVL75 and BVL100, respectively). Thus, the optimal growth performance of ostriches was achieved with BVL50 diets, those animals showed a 6.50% higher ADFI, 15.1% greater ADG, and 7.70% lower FCR compared to the CTRL group. Although there is no comparable report in the ostrich, however, in line with these results it has been recently shown the substituting AH of diet by BVL (at 7.5% and 15% DM on diet) resulted in higher feed intake in growing lambs<sup>19</sup>. Moreover, the dietary inclusion of BVL enhances dry matter intake (DMI) in dairy sheep<sup>15</sup> and dairy goat<sup>13</sup>. A previous report<sup>11</sup> showed that when the dietary AH was substituted by the great digestible leaves (grape, common fig, barberry, sweet cherry, apricot, peach, and oleaster) the animal feed intakes were increased. Additionally, using agro-industrial by products in ostrich diets led to enhanced ADG and FCR<sup>10</sup>.

The impacts of inclusion of unconventional forage sources on animal performance are associated with their nutritive value and digestibility as well as anti-nutritional factors<sup>22</sup>. In this context, BVL increased the ADFI of ostriches, in confirmation it has been reported that the BVL is suitable for animal feeding not only because of their great nutrient composition but also because of its low amounts of anti-nutrients<sup>13</sup>. However, in the current study, the high levels of BVL feeding as a replacement of 75 and 100% AH in ostrich diets with long-term exposure (210 d) led to lower ADG which is probably attributed to the plant secondary metabolites (PSMs) in

| Parameters <sup>1</sup> | Diets <sup>2</sup> |       |       |       |        | SEM <sup>3</sup> | p-values |        |           |
|-------------------------|--------------------|-------|-------|-------|--------|------------------|----------|--------|-----------|
|                         | CTRL               | BVL25 | BVL50 | BVL75 | BVL100 |                  | Diet     | Linear | Quadratic |
| Intake                  |                    |       |       |       |        |                  |          |        |           |
| OM intake, g/day        | 708.4              | 690.5 | 630.8 | 790.4 | 801.1  | 61.2             | 0.257    | 0.427  | 0.212     |
| CP intake, g/day        | 129.4              | 125.1 | 125.6 | 143.7 | 146.0  | 11.1             | 0.260    | 0.401  | 0.203     |
| EE intake, g/day        | 18.7               | 17.9  | 17.8  | 19.1  | 19.0   | 1.60             | 0.601    | 0.423  | 0.179     |
| CF intake, g/day        | 100.8              | 105.4 | 100.6 | 109.2 | 110.6  | 8.81             | 0.427    | 0.383  | 0.112     |
| Ash intake, g/day       | 68.9               | 66.4  | 65.1  | 70.1  | 69.8   | 5.99             | 0.461    | 0.552  | 0.410     |
| Digestibility           |                    |       |       |       |        |                  |          |        |           |
| OM, g/kg                | 74.5               | 71.2  | 77.3  | 70.1  | 70.5   | 7.47             | 0.662    | 0.787  | 0.841     |
| CP, g/kg                | 81.9               | 82.6  | 83.8  | 80.6  | 80.2   | 1.150            | 0.214    | 0.291  | 0.137     |
| EE, g/kg                | 88.3               | 88.8  | 89.2  | 87.8  | 87.3   | 0.750            | 0.447    | 0.373  | 0.195     |
| CF, g/kg                | 77.1               | 76.9  | 78.2  | 73.8  | 74.9   | 1.530            | 0.289    | 0.735  | 0.605     |
| Ash, g/kg               | 59.6               | 57.4  | 63.8  | 60.1  | 57.5   | 2.520            | 0.409    | 0.273  | 0.319     |

**Table 2.** Nutrient intake and apparent total tract digestibility coefficients (ATTDC) in ostriches fed *Berberis vulgaris* leaf (BVL) included diets. Within row, different letters (a, b) indicate difference between diets ( $p \leq 0.05$ ). Values are least-square means. <sup>1</sup>OM, organic matter; CP, crude protein; CF, crude fiber. <sup>2</sup>Experimental diets consisting of a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100%. <sup>3</sup>SEM, Pooled standard error of the mean.

| Parameters <sup>1</sup> | Diets <sup>2</sup> |        |        |        |         | SEM <sup>3</sup> | p-values |        |           |
|-------------------------|--------------------|--------|--------|--------|---------|------------------|----------|--------|-----------|
|                         | CTRL               | BVL25  | BVL50  | BVL75  | BVL100  |                  | Diet     | Linear | Quadratic |
| AME, kcal/kg            | 3493.4             | 3486.0 | 3567.3 | 3513.2 | 3427.55 | 37.50            | 0.180    | 0.810  | 0.597     |
| AMEn, kcal/kg           | 3229.1             | 3223.4 | 3296.0 | 3304.5 | 3209.5  | 55.80            | 0.670    | 0.841  | 0.546     |
| Energy efficiency       | 8.22               | 8.04   | 8.59   | 7.69   | 7.52    | 0.540            | 0.660    | 0.651  | 0.601     |
| Protein efficiency      | 1.43               | 1.39   | 1.48   | 1.32   | 1.29    | 0.090            | 0.630    | 0.770  | 0.632     |

**Table 3.** Apparent metabolizable energy (AME) in ostriches fed *Berberis vulgaris* leaf (BVL) included diets. Within row, different letters (a, b) indicate difference between diets ( $p \leq 0.05$ ). Values are least-square means. <sup>1</sup>AME, apparent metabolizable energy; AMEn, apparent metabolizable energy corrected to zero nitrogen balance. <sup>2</sup>Experimental diets consisting of a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100%. <sup>3</sup>SEM, Pooled standard error of the mean.

| Parameters <sup>1</sup> | Diets <sup>2</sup> |                    |                   |                    |                    | SEM <sup>3</sup> | p-values |        |           |
|-------------------------|--------------------|--------------------|-------------------|--------------------|--------------------|------------------|----------|--------|-----------|
|                         | CTRL               | BVL25              | BVL50             | BVL75              | BVL100             |                  | Diet     | Linear | Quadratic |
| FBW, kg                 | 66.3 <sup>b</sup>  | 69.0 <sup>ab</sup> | 78.2 <sup>a</sup> | 69.4 <sup>ab</sup> | 70.7 <sup>ab</sup> | 2.609            | 0.028    | 0.054  | 0.056     |
| H CW, kg                | 21.5               | 23.7               | 26.1              | 23.4               | 23.3               | 1.522            | 0.331    | 0.130  | 0.142     |
| Dressing BW, kg         | 14.5               | 15.8               | 17.6              | 12.1               | 15.4               | 1.387            | 0.237    | 0.228  | 0.234     |
| Heart, kg               | 0.490              | 0.521              | 0.533             | 0.494              | 0.515              | 0.320            | 0.566    | 0.112  | 0.107     |
| Liver, kg               | 1.57               | 1.48               | 1.60              | 1.45               | 1.50               | 0.076            | 0.436    | 0.121  | 0.162     |
| Visceral fat, kg        | 5.95               | 5.90               | 6.01              | 6.13               | 5.89               | 0.332            | 0.257    | 0.190  | 0.139     |
| Gizzard, kg             | 0.786              | 0.803              | 0.802             | 0.806              | 0.770              | 0.027            | 0.395    | 0.085  | 0.203     |

**Table 4.** Carcass characteristics in ostriches fed *Berberis vulgaris* leaf (BVL) included diets. Within row, different letters (a, b) indicate difference between diets ( $p \leq 0.05$ ). Values are least-square means. <sup>1</sup>H CW, hot carcass weight; CCW, cold carcass weight. <sup>2</sup>Experimental diets consisting of a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100%. <sup>3</sup>SEM, Pooled standard error of the mean.

BVL that altered digestion and nutrients utilization and further increases FCR. But, the better ADFI, ADG, and FCR in BVL50 provoke that the combination of forage feeds with different rates of digestion could modulate the efficiency of nutrient utilization and enhance growth performance<sup>23</sup>.

It has been also well documented that the dietary inclusion of agro-industrial feedstuffs rich in PSMs with antioxidative effects led to better growth performance in both monogastrics<sup>24</sup> and ruminants<sup>12</sup>. So, the benefits

derived from BVL feeding, on the other hand, have been linked to the presence of vitamins, carotenoids, flavonoids, and alkaloids<sup>13,15,19</sup> particularly berberine which has been shown to favor performance, immunity, health, and quality of animal products<sup>17</sup>. Altogether, present results showed a favorable effect of BVL inclusion on the ADFI of growing ostriches. Moreover, substituting 50% AH of diet by BVL is the optimal level that boosts ADG, FCR, and return per weight gain.

### Nutrient intake and digestibility

The availability of nutrients and energy from feed can be estimated by in-vivo digestibility assays<sup>8</sup>. Apparent digestibility investigations not only enable the formulation of cost-effective diets but also help in minimizing waste production. The digestibility of forage feed ingredients that contribute to higher NDF content is a significant concern for young monogastric animals<sup>25</sup>. However, the ostrich, a hindgut fermenter, boasts an impressive capacity for fiber digestion, thereby enabling them to extract a larger share of energy from its feed relative to poultry and swine<sup>21</sup>. Notably, Swart et al.<sup>26</sup> discovered that ostriches have the potential to absorb between 12% and 76% of their energy in the form of volatile fatty acids (VFAs), which are the main products of fiber digestion in the large intestine. Our results showed that the intake and digestibility of nutrients including OM, CP, EE, CF, and Ash were not altered by different inclusion levels of BVL in ostrich diets. It is well known that the digestion efficiency of diets in ostriches is largely influenced by the fermentability of NDF<sup>25,26</sup>. This factor plays a crucial role in determining the passage rate of the diet through the small intestine and the extent of the fermented diet in the hindgut. Therefore, forage feedstuffs are crucial for optimizing nutrient absorption and ensuring efficient digestion<sup>3</sup>. It has been shown that the long retention time of fibrous feed in the gastrointestinal tract of ostrich ensures exposure of feed particles to microbial fermentation and production of VFAs<sup>26</sup>. The larger particles of dietary fiber will help in the retention of bolus in the upper portion of the GIT, slowing down the passage rate and increasing the exposure of feed components to HCl and enzymes from the proventriculus<sup>3,25</sup>. In the present study, the similar NDF content and particle size of the forage component (i.e., AH and BVL) across diets may likely contribute to the absence of variations in the digestibility of the nutrients. In the world literature, we can find publications concerning BVL inclusion for ostriches, however, previous studies in ruminants showed that the addition of BVL to the diets had no negative effects on nutrient digestibility of fattening lambs<sup>15,19</sup>. Consistently, the in-vitro degradability coefficients of BVL were similar to those of AH<sup>13</sup>. Overall, we did not observe any determinant effects on nutrient digestibility following partial and total substitution of AH by BVL for growing ostriches, however, further studies are required to validate these data.

### Apparent metabolizable energy

Regarding using BVL for ostrich diet formulation this is a necessity to quantify the AME to ensure optimal economic viability. Our results indicated that feeding BVL as a partial and total substitute for AH affects neither AME nor AMEn nor the energy and protein efficiency of ostriches. The current AMEn values ranged from 3209 to 3296 kcal/kg which highlights BVL as an acceptable source of energy and nutrients for ostrich chicks. To our knowledge, there is currently no published data on the AME and AMEn content of BVL for ostriches. Mirbehbahani et al.<sup>20</sup> showed that the AME values in ostrich are greater than AMEn which is related to the high digestibility of protein in these birds. Therefore, it is necessary to correct the metabolizable energy based on zero nitrogen balance in the ostrich<sup>27</sup>. In ostrich, the AMEn value of whole date waste, an agro-industrial byproduct, was reported to be 3050 kcal/kg<sup>8</sup> which is in the range of observed values of BVL as a byproduct. It has been reported that the metabolizable energy value in ostrich chicks is affected by dietary factors (such as chemical composition, feed ingredients, or inclusion level) and/or animal factors (such as species, age, or type of feeding)<sup>8,21,25</sup>. The high AMEn value of BVL could be associated with its high contents of NDF which is similar to those in AH.

### Carcass characteristics

The slaughter weight, dressing percentage, and yield of the edible offal components were not affected by different levels of dietary replacement of AH by BVL to growing ostrich chicks. The observed hot carcass weight (HCW) in the current study was in line with the previous results with ostrich chicks<sup>25</sup>. Also, the mean dressing percentage fell within the range of 35.1–51.1% reported in previous studies<sup>9</sup>. Dressing percentage is a key factor in assessing meat production, as it affects the amount of meat obtained from the animal<sup>15</sup>. There is no comparable data regarding the effects of BVL on carcass characteristics either in ostriches or in any other avian species. However, in line with our results, a recent paper<sup>19</sup> reported that dietary inclusion of 7.5 and 15% BVL (DM basis) did not change the weights of carcass cuts and internal organs (including heart; liver, kidney, lungs, and testis) of fattening lamb. Similarly, Kardan Moghaddam et al.<sup>15</sup> suggested that the ingestion of barberry pomace, a by-product of barberry, at 7.5% and 15% DM of diet did not alter carcass weight, commercial cuts, and internal organs (including head, skin, feet, lung and trachea, heart, liver, spleen, gastro-intestinal, kidney, and bladder) of lambs. Nonetheless, it appears that BVL can replace AH in the diets of ostrich chicks without any detrimental effect on slaughter traits.

### Conclusions

Taking the results of this study into account, the inclusion of BVL in the growing ostrich diets, even as a total replacement for AH did not negatively affect the productive performance. However, the best growth performance, nutrient digestibility, and feeding costs were obtained when BVL was partially substituted for 50% AH of the diet. Altogether, BVL seems to be a viable and cost-effective alternative to AH in ostrich nutrition. Further trials should be conducted to verify the potential effects of BVL on microbiome, hematology, and quality of products in ostrich chicks.

## Materials and methods

### Ethical considerations and study site

The study was carried out at the experimental farm of the Faculty of Agriculture, University of Birjand, Iran located at 1491 m above sea level with the longitude and latitude, 37.42°N and 57.31°E from August 2020 to December 2020. The climate of the region is cold semi-arid with a mean annual precipitation and temperature of 171 mm and 18 °C, respectively. All experimental procedures and animal care were reviewed by the Animal Welfare and Ethical Review Board of the Department of Animal Science, University of Birjand (project ID: 9506) and were conducted in accordance with ARRIVE<sup>28</sup> guidelines and regulations.

### Forage collection and preparation

BVL was prepared manually from the plant of variety *Berberis vulgaris khorasanica* according to the dominant traditional harvesting method (cutting fruiting shoots) after fruit maturation from gardens around Birjand, South Khorasan, Iran, in November 2020, was sun-dried (35 °C and 0% humidity) to a constant weight. AH was also harvested at the bud stage, sun-dried in the field, and stored pending milling and incorporation in ostrich diets. Both BVL and AH were shredded using (1 mm) a threshing machine and incorporated into diets.

### Design, animal, and diet

Thirty healthy male ostrich (*Struthio camelus*) 2 months with an average initial body weight (BW) of  $9.10 \pm 0.89$  kg (mean  $\pm$  standard deviation [SD]) were purchased from a commercial farm in Birjand, South Khorasan, Iran. Animals were randomly assigned to the five experimental diets in a completely randomized design (CRD) with six animals per treatment, each kept in an individual in an outdoor shaded pen (2 m  $\times$  2.5 m) for 150 days (from 2 to 7 months of age) preceded by an adaptation period of 14 days. Husbandry practices were divided into two phases including starter (2–4 months), and grower (5–7 months). The isonitrogenous and isoenergetic diets (both starter and grower) included a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100% (Table 5). All birds fed twice daily *ad libitum* (0700 and 1700 h) and had free access to water during the experimental period.

### Sampling and measurements

The individual average daily feed intake (ADFI) by the ostriches was calculated using daily feed offered and feed refuse averaged over the interval of the trial period. All birds were weighed monthly after a 16-h fasting using a calibrated scale (ASA2200, Sepahan Towzin Co., Isfahan, Iran) to determine the average daily gain (ADG) by dividing the total weight gain by the number of days in the experimental period. The feed conversion ratio (FCR) was calculated as g ADG per g ADFI. An estimate was also made of the feeding cost per kg of BWG and return per kg BW gain for each dietary group<sup>19</sup>.

To determine the apparent metabolizable energy corrected to zero nitrogen balance (AMEn) and apparent total tract digestibility coefficients (ATTDC) in ostriches, the feed consumption and excreta output were accurately measured for the following final 5 days of the trial. Feed spilled on trays below the crate floors was also carefully collected to ensure the quantity of feed intake. To collect the daily fresh excreta, canvas bags with plastic linings were used and connected to a harness<sup>29</sup>. Bags were emptied 4 times a day during the squatting to prevent losses and immediately placed in a cold airtight container with ice cubes (around –4 °C) to prevent any potential microbial activity. They were then transferred to the laboratory and stored at –20 °C until further analysis. For AMEn determination, excreta samples were oven-dried at 65 °C for 72 h<sup>30</sup>, ground with a hammer mill (Arthur Hill Thomas Co., Philadelphia, PA) to pass through a 1-mm screen, and thoroughly mixed. Excreta samples from daily collections of individual ostriches were proportionally pooled over days and a representative subsample of the dry excreta (approximately 100 g) was then obtained for chemical analysis. The AME and AMEn values were calculated using the following formula<sup>27</sup>:

$$\begin{aligned} \text{AME (kcal/kg)} &= [(F_i \times \text{GE}_f) - (E \times \text{GE}_e)] / F_i \\ \text{AMEn (kcal/kg)} &= \{[(F_i \times \text{GE}_f) - (E \times \text{GE}_e)] - (N_r \times k)\} / F_i \\ \text{NR} &= (F_i \times N_f) - (E \times N_e) \end{aligned}$$

where AME is the apparent metabolizable energy; AMEn is the apparent metabolizable energy corrected to zero nitrogen balance;  $F_i$  is the feed intake, kg;  $E$  is excreta production, kg;  $\text{GE}_f$  is the gross energy intake, kcal/kg;  $\text{GE}_e$  is the gross energy of excreta, kcal/kg;  $N_f$  is the amount of nitrogen in feed, %;  $N_e$  is the amount of nitrogen in excreta, %; NR is the nitrogen retention;  $K$  is the 22.8 kcal/g of nitrogen (8220 kcal/kg).

For determination of ATTDC of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), and ash were determined by collecting the excreta and feed residues during the final 5 days of the trial. The amount of acid-insoluble ash (AIA) in the diet and excreta was determined according to McCarthy et al.<sup>31</sup>. The ATTDC of nutrients in the diets was calculated using the following equation:

$$\text{ATTDC} = 1 - [(AIA_{\text{diet}}/AIA_{\text{excreta}}) \times (\text{nutrient}_{\text{excreta}}/\text{nutrient}_{\text{diet}})]$$

where  $\text{nutrient}_{\text{diet}}$  and  $AIA_{\text{diet}}$  are the concentrations of nutrient and AIA in the diet (%) and  $\text{Nutrient}_{\text{excreta}}$  and  $AIA_{\text{excreta}}$  represent the concentrations of the same nutrient and AIA in the excreta (%).

At the end of 150-d trial period, ostriches were weighed after an overnight feed deprivation (where they had access to drinking water but no feed) and then were slaughtered. Hot carcass weight (HCW) was recorded immediately after slaughtering and cold carcass weight (CCW) was recorded after chilling (for 24 h at 0–4 °C).

| Item                        | Starter (2 to 4 months) |       |       |       |        | Grower (5 to 7 months) |       |       |       |        |
|-----------------------------|-------------------------|-------|-------|-------|--------|------------------------|-------|-------|-------|--------|
|                             | CTRL                    | BVL25 | BVL50 | BVL75 | BVL100 | CTRL                   | BVL25 | BVL50 | BVL75 | BVL100 |
| Ingredient (% of DM)        |                         |       |       |       |        |                        |       |       |       |        |
| Corn grain                  | 47.6                    | 46.8  | 46.1  | 44.9  | 43.5   | 43.6                   | 43.4  | 42.5  | 39.1  | 36.3   |
| Soybean meal                | 26.3                    | 26.8  | 27.1  | 27.7  | 28.5   | 10.8                   | 12.4  | 12.7  | 15.3  | 17.1   |
| Alfalfa hay <sup>a</sup>    | 22.0                    | 16.5  | 11.0  | 5.50  | 0.00   | 40.0                   | 30.0  | 20.0  | 10.0  | 0.00   |
| Barberry leave <sup>b</sup> | 0.00                    | 5.50  | 11.0  | 16.5  | 22.0   | 0.00                   | 10.0  | 20.0  | 30.0  | 40.0   |
| Di-Calcium phosphate        | 1.03                    | 1.01  | 1.18  | 1.29  | 1.44   | 1.83                   | 1.80  | 1.80  | 1.80  | 1.80   |
| Calcium carbonate           | 1.00                    | 1.00  | 1.02  | 1.10  | 1.15   | 1.21                   | 1.50  | 1.70  | 2.00  | 2.20   |
| Soybean Oil                 | 1.08                    | 1.32  | 1.57  | 1.89  | 2.22   | 0.20                   | 0.20  | 0.30  | 0.80  | 1.40   |
| Salt                        | 0.25                    | 0.25  | 0.25  | 0.25  | 0.25   | 0.25                   | 0.25  | 0.25  | 0.25  | 0.25   |
| Mineral premix <sup>c</sup> | 0.25                    | 0.25  | 0.25  | 0.25  | 0.25   | 0.25                   | 0.25  | 0.25  | 0.25  | 0.25   |
| Vitamin premix <sup>d</sup> | 0.25                    | 0.25  | 0.25  | 0.25  | 0.25   | 0.25                   | 0.25  | 0.25  | 0.25  | 0.25   |
| DL-Methionine               | 0.18                    | 0.21  | 0.20  | 0.20  | 0.21   | 0.06                   | 0.06  | 0.09  | 0.10  | 0.11   |
| L-Lysine HCl                | 0.08                    | 0.08  | 0.08  | 0.14  | 0.21   | 0.00                   | 0.00  | 0.00  | 0.00  | 0.00   |
| Chemical composition        |                         |       |       |       |        |                        |       |       |       |        |
| ME, Mcal/kg                 | 2.85                    | 2.85  | 2.85  | 2.85  | 2.85   | 2.79                   | 2.77  | 2.75  | 2.75  | 2.75   |
| Crude protein, %            | 19.3                    | 19.2  | 19.1  | 19.0  | 19.0   | 16.0                   | 16.0  | 16.0  | 16.0  | 16.0   |
| Ether extract, %            | 3.25                    | 3.46  | 3.67  | 3.93  | 4.22   | 2.32                   | 2.24  | 2.86  | 3.38  | 3.38   |
| NDF, %                      | 16.1                    | 16.1  | 16.1  | 16.1  | 16.1   | 19.4                   | 19.3  | 19.3  | 19.3  | 19.3   |
| Calcium, %                  | 0.85                    | 0.85  | 0.85  | 0.85  | 0.85   | 1.50                   | 1.50  | 1.50  | 1.50  | 1.50   |
| Available P, %              | 0.42                    | 0.42  | 0.42  | 0.42  | 0.42   | 0.60                   | 0.60  | 0.60  | 0.60  | 0.60   |
| Lys, %                      | 1.01                    | 1.00  | 0.99  | 1.03  | 1.07   | 0.76                   | 0.76  | 0.76  | 0.76  | 0.76   |
| Met + Cys, %                | 0.78                    | 0.77  | 0.76  | 0.76  | 0.65   | 0.65                   | 0.65  | 0.65  | 0.65  | 0.65   |

**Table 5.** Ingredients and nutrient contents (DM basis) of experimental diets. Experimental diets consisting a control with no BVL (CTRL) and four groups with BVL as a substitute for AH at varying levels including 25%, 50%, 75%, and 100%. <sup>a</sup>Contains 92.5% DM, 17.3% crude protein, 1.70% ether extract, 8.30% Ash, 32.6% NDF, 17.7% ADF (DM basis). <sup>b</sup>Contains 94.0% DM, 12.1% crude protein, 2.2% ether extract, 9.00% Ash, 31.7% NDF, 19.4% ADF (DM basis). <sup>c</sup>Containing 20 mg Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 15 mg Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 80mgMn (MnSO<sub>4</sub>H<sub>2</sub>O), 50 mg Zn (ZnO), 1.0 mg I (KI) and 0.15 mg Se (NaSeO<sub>3</sub>) per kilogram of diet. <sup>d</sup>Containing 3.6 mg alltransretinyl acetate, 0.055 mg cholecalciferol, 10 mg DL- $\alpha$ -tocopheryl acetate, 5 mg menadione dimethyl-pyrimidinol, 2.4 mg thiamin, 3.6 mg riboflavin, 35 mg nicotinic acid, 12 mg calcium pantothenate, 3.5 mg pyridoxine, 0.15 mg biotin, 1.4 mg folic acid and 0.03 mg cyanocobalamin per kilogram of diet.

Chilling losses were calculated as the difference between HCW and CCW expressed as a proportion of HCW. The weights of the liver, heart, visceral fat, and gizzard were measured using a calibrated scale (ASA2200, Sepahan Towzin Co., Isfahan, Iran).

### Laboratory analysis

The predried samples of ingredients, diets, refusals, and feces were ground in a Willey Mill (Arthur Hill Thomas Co., Philadelphia, PA) with a 1-mm mesh sieve and then analyzed according to AOAC<sup>32</sup> for DM (method no. 934.01), crude protein (CP; method no. 981.10), ash (method 94205), and ether extract (EE; method 920.29). The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined as described by Van Soest et al.<sup>33</sup>, with the modifications proposed in the Ankom device manual (Ankom Technology Corporation, Macedon, NY). Nonfibrous carbohydrates (NFC) were determined with the following equation:  $NFC = 100 - (CP + NDF + Ash + EE)^{32}$ .

### Statistical analysis

Statistical analysis of the data was first done by the GLM procedure for a completely randomized design (CRD) with five treatments (diets) and six replicates (ostriches) using a statistical software computer program (SAS Institute, version 9.0; SAS Institute Inc., Cary, NC, USA). The fixed effects in the model were: the dietary treatment (diet), the time of sampling (time), and their interaction (diet  $\times$  time). Normality and homogeneity of variances were evaluated by Shapiro–Wilk and Levene tests, respectively. Initial BW was included as a covariate in the model for ADG and ADFI. Least-square means (LSM) were obtained using the Tukey–Kramer test and results are presented with their corresponding SEs (SEM). A polynomial contrasts analysis was employed to determine the linear and quadratic effects of BVL levels. Differences in LSM were significant at  $p \leq 0.05$  and  $p \leq 0.10$  was considered as a tendency. The significance level was considered at a 5% confidence level.

### Data availability

All of the required data have been presented in our article.

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## References

1. -Georganas, A. et al. Utilization of agro-industrial by-products for sustainable poultry production. *Sustainability*. **15** (4), 3679. <https://doi.org/10.3390/su15043679> (2023).
2. -FAOSTAT, F. & License CC BY-NC-SA 3.0 IGO. <https://www.fao.org/faostat/en/data/QCL/visualize>. (accessed 13 May 2022).
3. -Abd El-Wahab, A. et al. Studies on the weight of the gastrointestinal tract, digesta composition and occurrence of gastro-and enteroliths in adult domesticated ostriches fed different diets. *Poult. Sci.* **100** (9), 101359. <https://doi.org/10.1016/j.psj.2021.101359> (2021).
4. -Polawska, E. et al. The ostrich meat—an updated review. *Anim. Sci. Pap Rep.* **29** (2), 89–97 (2011).
5. -Ministry of Agriculture Jihad (MAJ). Statistical Book of Agriculture of Iran. Iranian Statistical Centre (2020).
6. -Al-Khalifa, H. & Al-Naser, A. Ostrich meat: production, quality parameters, and nutritional comparison to other types of meats. *J. Appl. Poult. Res.* **23** (4), 784–790. <https://doi.org/10.3382/japr.2014-00962> (2014).
7. -Brassó, D. L., Béri, B. & Komlósi, I. Studies on ostrich (*Struthio Camelus*)-review. *Acta Agrar. Debr.* **1**, 15–22. <https://doi.org/10.34101/actaagrar/1/3772> (2020).
8. -Najafi, S., Ghasemi, H. A., Hajkhodadadi, I. & Khodaei-Motlagh, M. Nutritional value of whole date waste and evaluating its application in ostrich diets. *Animal*. **15** (3), 100165. <https://doi.org/10.1016/j.animal.2020.100165> (2021).
9. -Viviers, S. F. *Effect of dietary energy and protein on the production parameters of slaughter ostriches (Struthio camelus var. domesticus)* (Doctoral dissertation, Stellenbosch: Stellenbosch University). (2015).
10. -Dalle Zotte, A. et al. Effect of cottonseed oilcake inclusion on ostrich growth performance and meat chemical composition. *Meat Sci.* **93** (2), 194–200. <https://doi.org/10.1016/j.meatsci.2012.08.027> (2013).
11. -Kazemi, M. & Ghasemi Bezdi, K. The nutritional value of some fruit tree leaves for finishing lambs. *Anim. Biotechnol.* **33** (6), 1296–1307. <https://doi.org/10.1080/10495398.2021.1891929> (2022).
12. Correddu, F. et al. Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? *Animals*. **10** (1), 131. <https://doi.org/10.3390/ani10010131> (2020).
13. -Ghaviapanje, N., Nasri, M. F., Farhangfar, H. & Modaresi, J. In situ, in vitro and in vivo nutritive value assessment of Barberry leaf as a roughage for goat feeding. *Small Rumin Res.* **141**, 94–98. <https://doi.org/10.1016/j.smallrumres.2016.07.004> (2016).
14. -Imenshahidi, M. & Hosseinzadeh, H. Berberis vulgaris and berberine: an update review. *Phytother Res.* **30** (11), 1745–1764. <https://doi.org/10.1002/ptr.5693> (2016).
15. -Kardan Moghaddam, V. K. et al. Growth performance and carcass characteristics of finishing male lambs fed barberry pomace-containing diets. *Anim. Biotechnol.* **32** (2), 178–184. <https://doi.org/10.1080/10495398.2019.1674861> (2021).
16. -Vaghar Seyedin, S. M., Mojtahedi, M., Farhangfar, S. H. & Ghaviapanje, N. Partial substitution of alfalfa hay by Berberis vulgaris leaf modulated the growth performance, meat quality and antioxidant status of fattening lambs. *Vet. Med. Sci.* **8** (6), 2605–2615. <https://doi.org/10.1002/vms3.934> (2022).
17. -Ghaviapanje, N., Fathi Nasri, M. H. & Vargas-Bello-Pérez, E. An insight into the potential of berberine in animal nutrition: current knowledge and future perspectives. *J. Anim. Physiol. Anim. Nutr.* **107** (3), 808–829. <https://doi.org/10.1111/jpn.13769> (2023).
18. -WHO; FAO; IFAD & UNICEF; WFP. The State of Food Security and Nutrition in the World. Repurposing Food and Agricultural Policies to Make Healthy Diets More Affordable; FAO: Rome, Italy. <https://www.fao.org/3/cc0639en/cc0639en.pdf> (accessed 1 Dec 2022).
19. -Vaghar Seyedin, S. M., Ghaviapanje, N., Mojtahedi, M. & Farhangfar, S. H. Vargas-Bello-Pérez, E. Inclusion of Berberis vulgaris leaf in the diet of fattening lambs: effects on performance, nutrient intake, rumen fermentation, and carcass traits. *J. Anim. Sci.* **101**, skad131. <https://doi.org/10.1093/jas/skad131> (2023).
20. -Mirbehbahani, S. M., Hosseini-Vashan, S. J., Mojtahedi, M., Farhangfar, S. H. & Hosseini, S. A. Soluble and insoluble fibers in ostrich nutrition: influences on growth performance and blood biochemical indices during different ages. *Trop. Anim. Health Prod.* **52**, 3665–3674. <https://doi.org/10.1007/s11250-020-02403-6> (2020).
21. -Brand, T. & Olivier, A. Ostrich nutrition and welfare. In: (eds Glatz, P., Lunam, C. & Malecki, I.) *The Welfare of Farmed Ratites. Animal Welfare*, vol 11. Springer, Berlin. [https://doi.org/10.1007/978-3-642-19297-5\\_5](https://doi.org/10.1007/978-3-642-19297-5_5) (2011).
22. -Guil-Guerrero, J. L. et al. Antimicrobial activity of plant-food by-products: a review focusing on the tropics. *Livest. Sci.* **189**, 32–49. <https://doi.org/10.1016/j.livsci.2016.04.021> (2016).
23. -Soltan, Y. et al. Replacing maize with low tannin sorghum grains: lamb growth performance, microbial protein synthesis and enteric methane production. *Anim. Prod. Sci.* **61** (13), 1348–1355. <https://doi.org/10.1071/AN20605> (2021).
24. -Su, B. & Chen, X. Current status and potential of *Moringa oleifera* leaf as an alternative protein source for animal feeds. *Front. Vet. Sci.* **7**, 53. <https://doi.org/10.3389/fvets.2020.00053> (2020).
25. -Engelbrecht, J. A. The evaluation of lupins (*Lupinus angustifolius*) as alternative protein source to soybean oilcake meal in ostrich (*Struthio camelus var. domesticus*) diets. *MSc (Agric) thesis, University of Stellenbosch, South Africa.* (2016).
26. -Swart, D., Mackie, R. I. & Hayes, J. P. Fermentative digestion in the ostrich (*Struthio camelus var. Domesticus*), a large avian species that utilizes cellulose1. *S Afr. J. Anim. Sci.* **23** (5), 127–135 (1993).
27. -Cilliers, S. C., Hayes, J. P., Sales, J., Chwalibog, A. & Du Preez, J. J. The additivity of TMEn values of various ingredients in a complete diet for ostriches and adult roosters. *Anim. Feed Sci. Technol.* **71** (3–4), 369–373. [https://doi.org/10.1016/S0377-8401\(97\)00165-X](https://doi.org/10.1016/S0377-8401(97)00165-X) (1998).
28. Sert, P. et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol.* **18**, 1769–1777. <https://doi.org/10.1371/journal.pbio.3000410> (2020).
29. -Seyfori, H., Ghasemi, H. A., Hajkhodadadi, I. & Hafizi, M. Effects of water supplementation of anorganic acid-trace mineral complex on production and slaughter parameters, intestinal histomorphology, and macronutrient digestibility in growing ostriches. *Poult. Sci.* **98** (10), 4860–4867. <https://doi.org/10.3382/ps/pez221> (2019).
30. -Valdes, E. V. & Leeson, S. Measurement of metabolizable energy, gross energy, and moisture in feed grade fats by near infrared reflectance spectroscopy. *Poult. Sci.* **73** (1), 163–171. <https://doi.org/10.3382/ps.0730163> (1994).
31. -McCarthy, J. F., Aherne, F. X. & Okai, D. B. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. *Can. J. Anim. Sci.* **54** (1), 107–109. <https://doi.org/10.4141/cjas74-016> (1974).
32. -Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis* 18th edn (AOAC, 2015).
33. -Van Soest, P. V., Robertson, J. B. & Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74** (10), 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2) (1991).

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### Author contributions

Mojtaba Afshin: Conceptualization, data curation, formal analysis, investigation, methodology, farm sampling. Nazar Afzali: Conceptualization, supervision. Seyyd Javad Hosseini-Vashan: Methodology, supervision, project administration. Ali Hajibabaei: data curation, writing–review and editing. Navid Ghavipanje: Data curation, methodology, software, formal analysis, writing–original draft, writing–review and editing. Einar Vargas-Bello-Pérez: Writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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### Declarations

### Competing interests

The authors declare no competing interests.

### Ethics statement

All procedures were reviewed by the Animal Welfare and Ethical Review Board of department of animal science, University of Birjand (project ID: 9506) and were conducted in accordance with ARRIVE<sup>28</sup> guidelines and regulations on the protection of animals used for scientific purposes.

### Additional information

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